pH responsive polymer brushes grafted from the surface of intravaginal ring for on-demand drug delivery

By

Sedigheh Nazaripour

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Chemistry

University of Manitoba

Winnipeg, Manitoba, Canada

“Things don’t go wrong and break your heart so you can become bitter and give up. They happen to break you down and build you up so you can be all that you were intended to be.”

Charlie Jones

Abstract:

Stimuli responsive drug delivery systems demonstrate one of the most promising areas in the drug delivery sector due to their numerous advantages. Compared with traditional drug delivery systems, they show an improvement in their efficacy, reduced side effects of toxicity and the emergence of drug resistance in long term. Tethering of stimuli responsive polymer brushes on the surface of drug delivery systems can be an effective method to functionalize the system with desired features for on-demand drug release.

In this project, a new pH sensitive polyurethane intravaginal ring reservoir (PU Res IVR) drug delivery system was developed using a novel and versatile method. A combination of surface-initiated activator regenerated by electron transfer atom transfer radical polymerization (SI-ARGET-ATRP) and alkyne-azide click reaction was applied to graft pH sensitive polymers with different functional groups from the surface of PU Res IVR segments. First, poly(propargyl acrylate) (PPA) brushes as an alkyne polymer were grafted from the surface of PU Res IVR segments via SI-ARGET-ATRP. Secondly, pH sensitive monomers such as butyric acid (BAA) and 4-vinyl pyridine (4VP) were bonded onto the surface of PU-PPA substrate via alkyne-azide click reaction. Monomers with pH sensitive functional groups were synthesized in their azide forms. Proton NMR and Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) results indicate that azide monomers were synthesized successfully. XPS and ATR-FTIR confirmed that polymer brushes were successfully grafted from the PU surface. Grafting density of poly(propargyl acrylate-co-butyric acid) PPA-BAA and poly(propargyl acrylate-co-pyridine) PPA-PY brushes were determined to be 3.05±0.021 μmol/cm^2 and 2.02±0.044 μmol/cm^2 using a

colorimetric method. The pH-responsive behaviour of untreated PU Res IVR segments and functionalized segments with pH-responsive polymer brushes were studied in PBS buffer solution at pH 4 and pH 7 over a minimum of 5 cycles using hydroxychloroquine (HCQ) as a model drug. The untreated PU Res IVR does not show any pH responsiveness upon pH changes with average release of 9.75±1.11 µg/ml. The average release of HCQ from PU-PPA-BAA Res IVR was 65.67 ±3.83 µg/ml at pH 4 and 40.86±1.31 µg/ml at pH 7 between pH 4 and pH 7 cycles. On the other hand, when the average release of HCQ from PU-PPA-PY Res IVR was determined to be 11.90±0.72 µg/ml at pH 7 and decreased to 3.87±1.29 µg/ml at pH 4 between cycles. Scanning electron microscopy (SEM) images show that, the average size of pores decreased from 459±24 nm at pH 7 to 149±26 nm at pH 4 for PU-PPA-PY Res IVR segments. On the contrary, when PPA-BAA brushes are grafted from the surface of PU Res IVR segments, pores are almost closed at pH 7 and they are open at pH 4 with the average size of 576± 87 nm in length and 124±51 nm in width.

Acknowledgment

I would like to express my deepest gratitude to my advisor, Dr. Song Liu, for supporting me throughout the program. Without his supervision, and constant help this dissertation would not have been possible.

I would like to thank my committee members, Prof Joe O'Neil and Prof Emmanuel Ho for their insightful inputs to my research.

I wish to thank my colleagues and friends in Biomaterial Synthesis and Surface Engineering Lab and the department of chemistry especially Sadegh Ghanbar for his scientific consultation and suggestions in this project. I also would like to thank Dr.HO's research group especially Yufei Chen for their cooperation.

Table of Contents

List of Schemes and tables:

List of Figures:

List of abbreviations:

AFM	Atomic Force Microscopy
AIDS	Acquired immune deficiency syndrome
AO	Acid Orange II
ARGET-ATRP	Activators regenerated by electron transfer for atom transfer radical polymerization
ATR-FTIR	Attenuated Total Reflectance Fourier-Transform Infrared
BIBB	2-bromoisobutyrylbromide
DA	Dopamine hydrochloride
DMF	N,N-Dimethylmethanamide
HCQ	Hydroxychloroquine
HIV	Human immunodeficiency virus
HPMC	Methocel K100M premium (hydroxypropyl)methyl cellulose
NaMA	Sodium methacrylate
NNRTIs	Nucleoside/Nucleotide Reverse Transcriptase Inhibitors
NRTIs	Nucleoside/Nucleotide Reverse Transcriptase Inhibitors
P4VP	Poly (4-vyinil-pyridine)
PAA	Poly (acrylic acid)
PBAA	Poly (butyric acid)
PBS	Phosphate Buffered Saline
PDA	Polydopamine
PHEMA	Poly(2-hydroxyethyl methacrylate)
PIs	Protease Inhibitors
PMAA	Poly (methacrylic acid)
PMDTEA	N,N,N,N,N-pentamethyldiethylenetriamine
PP	Polypropylene
PPA	Poly(propargyl acrylate)
PU 60D 35	Polyether urethane Teco-philic HP -60D-35

PU Res IVR	Polyurethane intravaginal ring reservoir
PY	Pyridine
QCM	Quartz Crystal Microbalance
Res IVR	Intravaginal Ring Reservoir
SEM	Scanning Electron Microscope
SI-CRP	Surface-Initiated Control Radical Polymerization
SRPBs	Stimulus-responsive polymer brushes
STIs	Sexual Transmission Infections
TBO	Toluidine blue
XPS	X-ray Photoelectron Spectroscopy

Chapter 1
Introduction

Chapter 1. Introduction

1.1 Overview:

According to the data published at the end of 2013, by the Global Health Observatory (GHO), 78 million people have been infected with the human immunodeficiency virus (HIV) and nearly 39 million people died of HIV since the start of HIV epidemic.[1,2] In 2014, approximately, 36.9 million people were living with HIV, mostly in countries with low or middle-income economies. The vast majority of people with HIV live in Sub-Saharan Africa: almost 71% of people infected with the virus live in this region. Only 54% of people living with HIV know that they are infected with this virus.[3]

In the first years of HIV prevalence, HIV infection was predominantly among men. However, at the end of 2002, women accounted for nearly 60% of the infected population in low and middle-class countries. Among young women in these countries, the infection is spreading at a rate of one woman per minute. The majority of HIV infections are transmitted through sexual interactions.[4–8]

Biologically, women are more vulnerable to HIV infection than men. This can be due to the following reasons: larger surface area of the vagina and cervix in women, being exposed to high amount of seminal fluid, vulnerability to invasion by bacteria, viruses and other germs. Also, the level of the hormone estrogen can determine the risk of transmission of HIV, because when the level of estrogen is low, the vaginal wall becomes thinner, therefore it would be easier for HIV to pass through the wall.[9] The vaginal pH of a healthy women is between 3.8 and 5.[10] Lactic acid produced from glycogen in the vagina keeps the pH of vagina in acidic state. This pH protects the area against a variety of bacteria and viruses, including sexual transmission infections (STIs) such as HIV.[7,10] During sexual intercourse, the pH in the vagina changes to 7.2 due to the alkaline nature

of semen (pH 7.2-8), which prepares the environment for different bacteria and viruses to grow.[11] Unfortunately, in many countries women are not able to communicate about protective sex such as using condoms and sometimes they are more likely to be subjected to non-consensual sex. [9,12,13] Hence, there is a need to develop methods that can help women to protect themselves from HIV infection.

1.2 Human immunodeficiency virus (HIV)

Despite effective prevention approaches, such as using condoms, monogamy and abstention, HIV is considered as one of the major public health problems. [5] Acquired immune deficiency syndrome (AIDS) is the cause of HIV. The human body is able to get rid of most viruses, however, as for HIV, the human body is not capable of getting rid of it.

The virus can infect multiple cells in the body, such as brain cells, however the main target for HIV is the CD4 lymphocyte which is called T-cell or CD4 cells. HIV infects the CD4 cells and goes through multiple steps to reproduce itself and produce more virus particles. The dramatic loss of T cells accounts for many of the manifestations of HIV disease.[14]

1.2.1 Transmission ways:

It is found that HIV can be transmitted through blood, semen, and vaginal secretions. Also HIV has been detected in saliva, tears and urine. However, the concentration of HIV in these fluids is very low.[13,15]

The majority of HIV infections are transmitted sexually. In 2008, approximately 44% of new HIV infections belonged to men having sex with men (MSM) and 36% were through heterosexual

intercourse. Most of the transmission occurs through the genital and reproductive systems of men and women. The risk of HIV transmission from vaginal intercourse range from 0.05% to 0.19%. Higher rate of transmission has been reported for male-to-female sexual transmission compared with female-to-male sexual transmission. The virus can be transmitted via oral sex; however, the risk of infection is less than for sexual intercourse.[15] Therefore, addressing the crisis of HIV globally requires both prevention strategies and treatment methods.

1.2.2 Anti-HIV drugs and treatments:

There is no functional remedy for HIV or AIDS which means there is no procedure or medication that has been proven to eliminate the virus. Nowadays there have been many advances in HIV treatment that considerably increase the quality of life of the people infected with HIV. Behavioural alterations influenced by education, using condoms and other protective methods, along with male circumcision are crucial to prevent HIV transmission. In absence of a suitable vaccine, microbicides as antiretroviral drugs (ARVs) become one of the most effective strategies to slow down the spread of HVI. An ARV can be used in a dosage that hampers the establishment of HIV infection. Microbicides are inhibitory compounds that can be used in vaginal and rectal applications to impede infection in early stages of sexual transmission.[16]

In many countries, women are less likely to negotiate a protective sex, therefore, microbicides are considered as a preventive option that enables women to control the situation without cooperation, contest or even knowledge of their partner [17].

A valuable microbicide should be safe, effective, user-friendly and cost-effective. It should not destroy natural defensive mechanism against HIV.[16] Also it should not induce inflammation,

because any stimuli that activate immune system cells in the vicinity of the virus could promote the transmission of HIV. [17] Also the product should be satisfactory to both partners. Any product with an unusual property such as smell, colour, high or low viscosity or complexity to apply can be rejected by women.[18] Nowadays, there are four common antiretroviral drug categories used for treatment of HIV. These drugs target different stages of the HIV infection cycle. These classes are listed as below:

1.2.2.1.1 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)

NRTIs or sometimes called nucleotide or "nukes" were discovered in the 1980s when the AIDS epidemic increased in Western societies. The first NRTI was zidovudine, which was the first step towards the treatment of HIV. Among all NRTIs tested by the Food and Drug Administration (FDA), the only NRTI approved is tenofovir.[19] These drugs work to block a very important step in HIV's production process. They inhibit HIV to use a special type of enzyme named reverse transcriptase RT to correctly build a new DNA that the virus needs to make copies of itself.[14]

1.2.2.1.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are another category of antiretroviral drugs used to combat HIV. NNRTIS, non-nukes, bind to reverse transcriptase (RT) and cause a conformational change in the enzyme. Therefore, the enzyme cannot convert RNA into DNA. As a result, new virus cannot be produced.[19] 1-(2-2-hydroxyethoxymethyl)-6-(phenylthio) thymine (HEPT) and tetrahydroimidazo[4,5,1-jkj][1,4]benzodiazepin-2(1H)-one (TIBO) are the first two compounds of this category that were unexpectedly discovered to inhibit HIV activity.[20]

After this discovery, three other compounds of this generation known as nevirapine, delavirdine, and eavirenz were approved by the FDA and introduced to the market. Nowadays, nevirapine and efavirenz are commonly used in the market, however, the last one is hardly used because of complexity in its structure and consequently in its synthesis.[21]

1.2.2.1.3 Protease Inhibitors (PIs)

Protease inhibitors (PIs) are third class of antiretroviral drug which targets a protein in HIV named protease. This protein is responsible for cutting proteins and making functional new HIV particles.[14] Nowadays ten PIs are available in the market for the treatment of HIV infection including tipranavir, indinavir and saquinavir.

Most PIs have shown high efficacy in treatment when they are used in a combination with another PI drug by speeding up or slowing down their processing in the body. For instance, ritonavir is a strong PI and it slows down the processing of other PIs in the body. It has been shown that a small amount of ritonavir in combination with other PIs can boost blood level of other PIs and extend dosing intervals.[21–23]

1.2.2.1.4 Entry/Fusion Inhibitors

Entry inhibitors are another class of antiretroviral drugs for the treatment of HIV infection. In order to enter to a host cell, first glycoprotein gp120 on the surface of HIV interacts with the CD4 receptor of the host cell.[22] When this step is achieved, gp120 binds to a second co-receptor, CCR5 or CXXR4. Once gp120 binds to both CD4 and a co-receptor, fusion to the host cell can happen

and then viral components can release in to the cell. Entry inhibitors act at different stages of HIV entry including fusion and co-receptor attachments.[23,24]

Entry inhibitors can be divided in two groups based on their functions: fusion and co-receptor inhibitors. Enfuvirtide (Fuzeon) is the only fusion inhibitor that is available in the market for the treatment of HIV infection. Maraviroc (Selzentry or Celsentri) is a co-receptor inhibitor came to the market in 2007 for use in treatment of patients with HIV infection.[25]

1.2.2.2 Alternative drugs with Anti-HIV properties:

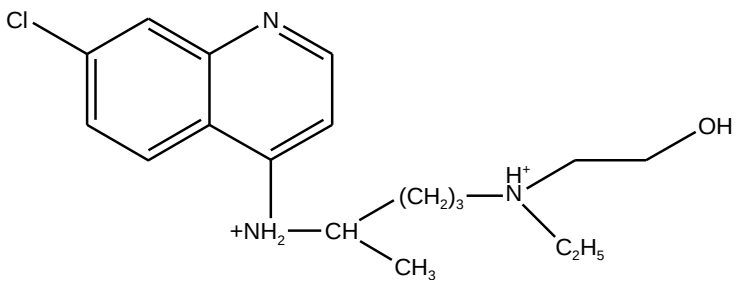
As mentioned, microbicides have a significant influence on the HIV prevention. The affordability of a microbicide, along with other factors such as its safety, acceptability and efficacy will determine its popularity in developing countries. For instance, some microbicides show high efficiency in preclinical studies, however, their high manufacturing cost might affect their acceptance.[16] Therefore, these days, available and inexpensive antiretroviral drugs to combat HIV are desirable.

1.2.2.3 Hydroxychloroquine (HCQ)

Hydroxychloroquine (HCQ) has been used for more than 70 years for the treatment of malaria. It was synthesized in 1946 and proposed as a safer alternative to chloroquine. In World War II, its anti-inflammatory properties were discovered when soldiers who took chloroquine and its derivatives for malaria treatment, experienced an improvement in their auto-immune condition. Since then, they have been used widely for the cure of autoimmune diseases including rheumatoid arthritis, because of its anti-inflammatory properties.[26]

Recently it has been shown that HCQ has anti-HIV activity through the changes in glycoprotein 120 (gp 120) in T cells and monocytes. It is presumed that HCQ inhibits the gp120 production by increasing endosomal pH and changing the enzyme required for gp120 production. The risk of HIV infection caused by inflammation in the genital track can also be reduced by the anti-inflammatory properties of HCQ. [27,28]

The parent molecule for this antimalarial agent is quinine. Hydroxychloroquine ($C_{18}H_{26}ClN_3O$) is an alkylated 4-aminoquinoline, a hydroxyl derivative of chloroquine with a molecular weight of 336 g/mol. HCQ is considered as an amphiphilic weak base. It contains two aromatic rings with conjugated double bonds, the 4-aminoquinoline nucleus. It also contains two basic groups: a quinolone-ring nitrogen and diethyl amino side-chain nitrogen with pKa values of 8.3 and 9.7 respectively, which means the majority of these nitrogen groups are ionized at pH 7.4 (Scheme 1-1).[29,30]



Scheme 1-1. Hydroxychloroquine structure.

1.3 Drug delivery systems

There has been a significant progression in the drug delivery sector over the past three decades. A decline in the development and design of new drugs, has encouraged scientists with the same interest to put effort in this field to develop novel drug delivery systems.[31,32]

Due to the fact that the process of design and development of new drug molecules requires lots of money and time, significant research has been done to existing drugs to design them in a new structure or new forms of delivery.[31] New strategies or devices have been developed in the field of drug delivery considering the carrier, the route and the target, to improve the adequacy of the controlled drug release.[33]

As mentioned, the highest rate of HIV transmission is through sexual intercourse, especially through the vagina. A study showed that in the case of using a condom as an effective way to prevent from HIV infection, 67% of participants did not use condoms frequently and 31% never considered using condom for protection. Therefore, development a female-controlled device such as intravaginal rings (IVRs) that can deliver anti-HIV drugs to protect women against HIV infection is needed.[13,34]

1.3.1 Vaginal route:

It has been proved, that the vagina can be considered as a route for drug delivery. Vaginal route is better than oral routes, since it shows less gastrointestinal side effects.[1] Its large surface area with dense blood networks and high permeability to drugs, not only makes it a favourable route for local-acting drugs, but also a great site for systematic delivery.[10] The physiological properties of the vagina cavity such as pH, vaginal secretions and enzyme activity can impact the efficacy of delivery systems for drug release.[35,36] Also, the physiochemical properties of the drug will influence the release of drugs from delivery systems and their adsorption into the vaginal cavity.

1.3.2 Vaginal drug delivery systems for preventing the spread of STIs and HIV

In the absence of an efficient HIV vaccine, developing new strategies to prevent STIs and HIV infection is needed. Intravaginal delivery systems for anti-HIV drugs are promising methods to deliver drugs in an efficient way with minimum toxic side-effects and virus resistance.[1,13,17,35,37]

Microbicides and anti-HIV drugs can be delivered with different types of delivery systems such as semi-solids, tablets, foams, capsules, films, tampons and vaginal rings based on the type of drug, drug adsorption, release duration and distribution of the drug in the vaginal cavity.[38] For instance, for rapid release, vaginal gels and tables are efficient. The duration of drug release from these systems is between 6 to 8 hours [39,40] while this time can increase to 71 days for vaginal rings.[41]

A perfect vaginal drug delivery system allows the drugs to spread evenly and not concentrate in one part of the vaginal cavity.[13] From this point of view, solutions, foams and suspensions are better than tablets.[38]

1.3.2.1 Intra-vaginal ring drug delivery systems (IVRs) for prevention of STIs and HIV transmission:

With the introduction of vaginal drug delivery technology, intravaginal ring drug delivery systems (IVRs) drew considerable attention in 1966, when for the first time, silicon elastomers were used to deliver steroids at a constant rate for a period of time.[2,42] At the beginning, IVRs were designed to deliver contraceptive agents such as progesterone and norethisteron through the vaginal track.[2] However, with an increase in STIs and HIV infection among women over the past decades, there is an interest to develop multipurpose prevention technology (MPT) IVRs which can be used for both contraceptive and therapeutic purposes.[2]

The IVR is a ring-shaped device designed to provide a long-term, sustained or controlled release of therapeutic agents to the vagina.[2] IVRs are superior to other intravaginal drug delivery systems since they can be inserted or taken out by women and they do not cause any problems during sexual intercourse. Also, they provide a constant release of drugs in low dosage and also a daily intake of drug is not required.[13,35,42]

Each IVR system can contain one or more microbicides that are designed to be delivered into the vaginal track at a high concentration.[43] Design of polymeric IVRs for a desirable drug delivery contains several parameters such as solubility, diffusion coefficient, and initial loading of drug in the polymer. The flexural/compressive characteristics, as well as the geometry and dimension of IVRs are also important for the ease of insertion, comfort of fit and prevention of tissue damage. The acceptance durometer for all rings according to ASTM D2240-05 is 40±5 D.[44] Consistency in durometer is critical since it determines the amount of tension caused by a compressed ring, and how comfortable and how well the ring fits? in the vaginal track. [45] Also it is reported that the size of the ring has a significant effect on the flexibility. In one study, IVRs

with different sizes (30×5, 25×5 and 20×5 mm) were inserted in two different macaques species ranging from 5 to 12 kg in weight for up to one month. The results indicate that the 25×5-mm rings would work well for both macaque species based on how comfortable they are during insertion and how well they are held in their place.[34]

The hot-melt extrusion or injection molding methods are usually used to fabricate IVR devices. Drug can be distributed totally or partially in the IVR systems during the fabrication process. Matrix and reservoir systems are two major types of IVR devices available on the market.[46]

The matrix system is a homogeneous ring, where the drug is dispersed throughout the entire polymer. The rate of drug release in these systems is dependent on (1) the solubility of drug in the polymer, (2) the diffusion of the drug in the polymer (3) the solubility of the drug in vaginal fluid and (4) the partition coefficient of the drug between the IVR and the vaginal fluid [42,43]. In this system the drug located near the surface is released first. Therefore, the daily amount of released drug decreases.

The reservoir IVR is a core-type ring that contains drugs in one or more central cores that are encapsulated by a drug-free layer of polymer membrane. The reservoir type removes the time-dependent release rate observed in matrix rings by slowing down the diffusion of the drug from the device. In this type of device, the drug concentration remains uniform in the core to provide a driving force for diffusion though the membrane that is almost constant with time which in turn results in a zero-order release. Compared with the matrix system, the release of drug from a reservoir-type is lower but it can be constant for several months. Also the manufacture of reservoir type is more complicated because of the multistep process in their manufacturing. [42,43,47].

Materials used in the manufacture of vaginal rings should be biocompatible, they should be flexible enough to deform during insertion, also they should be permeable to drugs.[42] Silicone

elastomers, especially polydimethylsiloxane (PDMS), were the first materials used for manufacturing IVRs due to their excellent biocompatibility and ease of fabrication.[42]

Thermoplastic elastomers are another class of materials which are used in IVRs. The common thermoplastic polymers used to fabricate IVRs are poly(ethylene vinyl acetate) (PEVA) and polyurethane (PU). They are particularly used for some of the polar anti-HIV drugs such as tenofovir (TVF) which are not released efficiently from silicone elastomers due to their low solubility in silicone.[48] Nowadays, thermoplastic polyether urethanes are used for fabrication of IVRs, because of their biocompatibility and favourable mechanical properties.[46,49]

These polymers are made of a polymeric diol (soft segment) like polyethylene oxide (PEO), a chain extender and an aliphatic isocyanate (hard segment). Aromatic isocyanates are commonly used to prepare medical devices made of PU because of their superior physical properties and reasonable cost. However, aromatic isocyanate can breakdown and convert to aniline derivatives under indecent conditions. Due to the concern about the toxicity of aniline compounds, aliphatic isocyanates are preferable to be used for synthesis of PUs.[48] Due to the fact that polyurethanes are made of two soft and one hard segment, by changing the ratio of the components, physical and mechanical properties of PU such as crystallinity and hydrophilicity can be altered. These changes in turn can affect the drug release in the final product.[42,48]

1.4 Stimuli responsive drug delivery systems:

1.4.1 A need for controlled release systems:

Stimuli-responsive controlled drug delivery technology is one the most promising areas in the field of drug delivery and health care. These types of systems have drawn attention of many pharmaceutical scientists, chemists and materials scientists, since they offer various advantages compared to the sustained-released systems. Stimuli-responsive systems are highly efficient in drug delivery with a precise amount of dosage. They also show less toxicity and they are more convenient to the patients.[50,51]

1.4.2 Polymers in controlled drug release:

Synthetic polymers are promising materials, in delivery of drug due to their physical and chemical properties. These polymers can provide a passive function as a drug delivery system or they can adopt a more active role for releasing a drug upon an external stimulus such as temperature and pH, which in this case they are called stimuli-responsive polymers.[52]

Preparation of drug delivery systems using pH-responsive polymers is an important approach in the design of stimuli-responsive drug delivery systems. In this method, stimuli-responsive polymers can be synthesized and directly used as a drug delivery system.[53] Surface modification of existing drug delivery systems with responsive polymer brushes is also an important alternative approach, as in this way, responsive properties can be introduced to the surface of a drug delivery systems by grafting so-called “smart” polymers. In this approach, the surface of a drug delivery system becomes responsive, while the bulk properties remain unchanged.[54]

1.4.2.1 Stimuli-responsive polymer brushes for controlled drug delivery systems:

Polymer brushes are described as polymer chains grafted form a surface, to an interface, or to a backbone of other polymer molecules with high grafting density; therefore, the crowded polymer chains stretch away from the surface in a "brush-like" conformation to avoid overlap.[55]

Depending on the grafting density known as the number of polymer chains per unit area, three different regimes are distinguished for polymer brushes. If the distance between grafting points is larger than the size of the molecules and there is a strong interaction between polymer chains and the substrate, the polymer chains have a flat "pancake-like" conformation.[56] However, if there is a repulsion force between polymer chains and substrate in a low grafting density regime a "mushroom-like" conformation is observed.[57] And finally, in high grafting density the polymer chains are in their "brush-like" regime to avoid overlap among polymer chains and minimize the excluded volume interaction.[58,59]

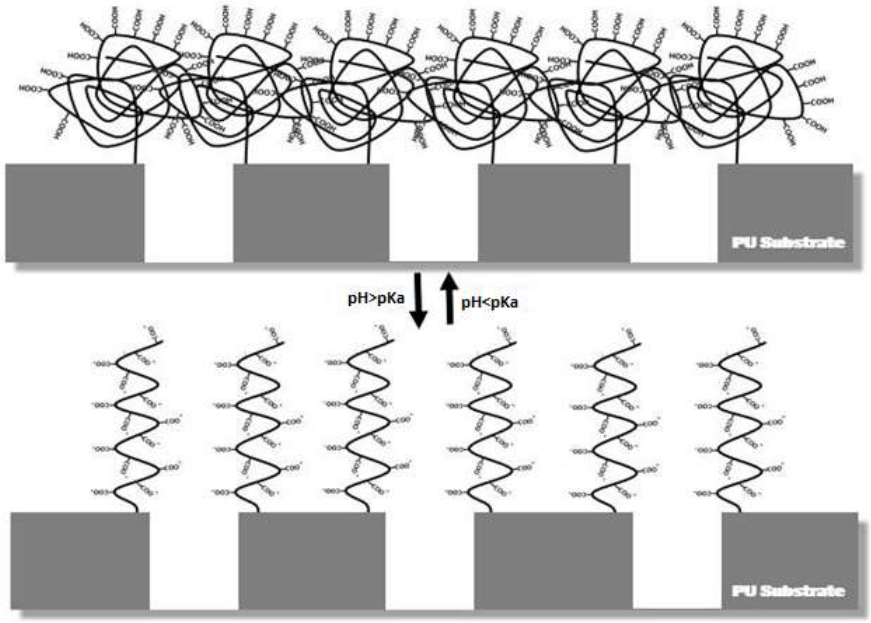
1.4.2.2 Mechanism of drug release from pH sensitive polymer brushes grafted substrate:

Stimulus-responsive polymer brushes (SRPBs) show changes in their physical and mechanical properties such as conformation, surface energy or charge state in response to stimulus including temperature, pH,[52] ionic strength and light.[60] For instance, poly(N-isopropylacrylamide) (PNIAM) with lower critical solution temperature 32 ˚C (LCST) shows changes in their surface wettability with change in temperature.[61] Diblock copolymer brushes of polystyrene-b-poly(4,5-dimethoxy-2-nitobenzyl methacrylate) were synthesized and their photo-controlled release was achieved by using a photo-cleavage process in which o-nitrobenzyl groups were converted to hydrophilic PMAA.[62]

Among SRPBs, polyelectrolyte brushes have drawn much attention due to their properties and applications in the biomedical and drug delivery fields.[62,63] External pH and ionic strength can

change the ionization state and ionic strength in weak anionic and cationic polymers. Poly cationic brushes extend with a decrease in pH, while, polyanionic polyelectrolyte brushes extend with an increase in pH. The conformational changes because of association and disassociation of polymer chains upon pH changes can be used for stimuli-responsive drug release. Lay et al. studied the release of encapsulated dyes in poly(methyacrylic acid) (PMAA) grafted fro the surface of hollow silica at different pH values. It was observed that the release of calcein blue from PMAA-g-hollow silica is very low at pH 2.0, but increased dramatically when the pH increased to 7.4.[64] A pH-sensitive nano-shell containing poly(methacrylic acid-co-vinyl triethoxylsialne) (PMV) was grafted from the surface of mesoporous silica nanoparticles (MMS) using reversible addition-fragmentation chain-transfer (RAFT) polymerization. A cumulative release study of Ibuprofen (IBU) from PMV/MMS showed that the IBU release was very fast at pH 7.5 with 85% release within 1hr, while the release decreased at pH 4 and pH 5 with an amount of less than 15% in 8 hr at pH 5.[65,66]

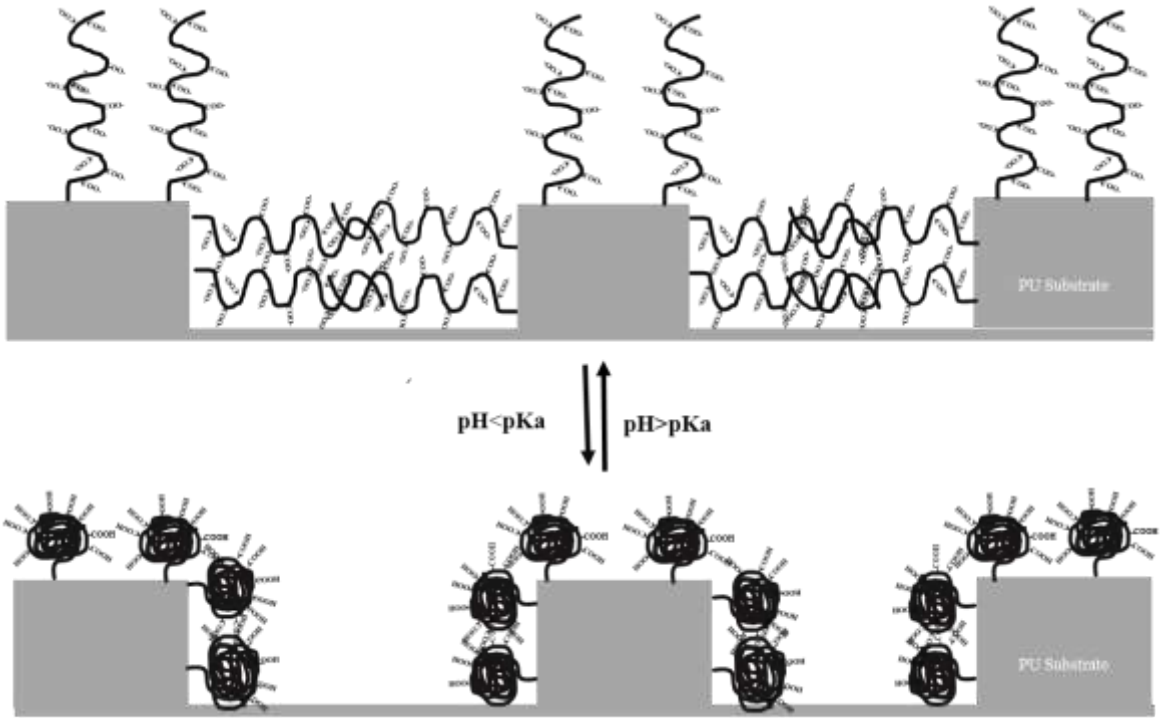
This can be explained by conformation changes of PMAA brushes on the surface. At pH values lower than the pKa of PMAA (pKa 6.5), PMAA brushes are protonated; the neutralized polymer brushes shrink and adopt a "mushroom" conformation. Therefore, pores on the surface of hollow silica shells are covered with shrunken polymer brushes. When the pH increased to values higher than the pKa of the PMAA brushes, deprotonated polymer chains adopt a "brush-like" regime because of repulsion between negatively charged polymer chains. Therefore, the pores in the hollow silica are opened and encapsulated species can be released.[64–67] (Scheme 1-2)



Scheme 1-2. Response of anionic polymer brushes grafted from the surface of PU Res IVR upon pH changes.

Wang et al.[68] grafted poly (acrylic acid) (PAA) brushes onto the surface of polypropylene (PP) membranes in supercritical CO_2. They observed that water permeation of the unmodified membranes did not change with changes in pH, however, when the pH increased from 3 to 6 water permeation of the PAA grafted membranes declined due to the changes in conformation of the PAA chains. The same behavior was observed when pH-sensitive polypeptide brushes were grafted on the surface of a gold nanoporous membrane. The rate of water permeation was high at low pH values but low at high pH values.[69] This behaviour can be explained by the mechanism called the "through-pore mechanism".[70–72] For the first time, this mechanism was proposed by Isreals et al. using a two-dimensional self-consistent mean-field (SCF) theory.[72] According to this mechanism, at pH values above the pKa of an anionic polymer brush such as PAA, polymer chains are deprotonated and extended due to repulsive forces between the negatively charged groups. The chains cover the pores and water permeation decreases. However, at pH values less than pKa,

polymer chains contract and go to their mushroom conformation. The steric obstruction of the pores is decreased and water permeation rate increases(scheme 1-3). [2–75]



Scheme 1-3. Response of anionic polymer brushes grafted onto the walls of pores of PU Res IVR substrate.

1.5 Surface-initiated Activators regenerated by electron transfer atom transfer radical polymerization (SI-ARGET-ATRP)

The two most commonly employed techniques to impart polymer brushes onto the surface are: the "grafting to" and "grafting from" methods.

In the "grafting to" approach, an end-functionalized polymer can be grafted onto the reactive sites on the surface of a substrate. This grafting technique can be performed physically or chemically. Controlled living radical polymerization techniques (CRP) can be applied to introduce polymers with a narrow molecular weight distribution. Functional groups can be attached to the surface of a substrate using coupling agents or self-assembled monolayers (SAM).[55] "Grafting to" methods, provide a way in which a free polymer with desirable properties can be synthesized before attachment, therefore, a layer of polymers with well-defined structure will be built up in this method. However, in this method, since the polymer chains should diffuse through polymer layers so that they can attach to the active sites on the surface, only a small amount of polymer brushes can be grated onto the surface and grafting density is relatively low. As the thickness of the polymer brushes increases, the hindrance from polymer film also increases.[55,76]

In recent years, the "grafting from" method has been widely used to synthesize grafted polymers onto the surface of solid substrates. This technique includes two steps: in the first step an initiator is immobilized onto the surface of a substrate. In the second step, monomers add to the immobilized initiator sites and polymer chains grow from those sites. This method can lead to a stronger structure. Well-controlled polymer structures with high grafting densities can be prepared since diffusing small monomers have less steric hindrance than diffusing polymer chains. [55,76–81]

Surface initiated controlled radical polymerization (SI-CRP) such as atom transfer radical polymerization (ATRP) is a suitable technique to synthesize polymer brushes through the grafting

from technique. ATRP can be considered as one of the most robust and powerful CRP techniques which have been used to produce polymers on a surface because it can offer efficient technique to synthesize polymer brushes with well-controlled molecular weight and novel structures.[68,82] Indeed, ATRP is a repeated activation/deactivation of alkyl halide, in which a vast majority of the polymer chains are allowed to grow with the same rate.[83] A functional nylon membrane was prepared with SI-ATRP by the Xu et al. group.[84]

SI-ATRP has been successfully used to graft functional polymer brushes from the surface of different substrates such as cotton[85], polymer microspheres, [86] cellulose, and chitosan beads.[87] However, the main drawback of SI-ATRP is that a large amount of a transition metal catalyst, usually copper-based, is necessary for this reaction. This copper-based catalyst is expensive and harmful to biological systems. Another disadvantage associated with SI-ATRP is that this reaction is very sensitive to the air, therefore a completely inert atmosphere is needed for this reaction, which makes the process complicated.[68]

More recently, Matyjaszewski and his group developed an improved version of the ATRP technique which overcomes problems associated with this technique. This modified version is called "activators regenerated by electron transfer" (ARGET) ATRP.[88,89] In this method, a Cu (II) compound, which is more stable than Cu(I), is used instead of a mixture of Cu(II) and Cu(I). Also, a reducing agent such as ascorbic acid is used in this reaction in order to convert dormant Cu(II) compounds to active Cu(I) compounds. Therefore, the concentration of Cu can be reduced to ppm level in this reaction.[83,88,89]

1.5.1 Immobilization of ATRP initiator on the surface:

The most important step in SI-polymerization is the immobilization of initiator. Various methods and approaches have been developed to immobilize the ATRP initiator on different substrates. Zhu and Edmondson developed a versatile method using a polydopamine (PDA) coating to immobilize ATRP initiator on the surface of a variety of different substrates.[90] In this method a thin layer of PDA containing ATRP initiator is coated onto the surface of the substrates.

Xu et al. applied a two-step approach to immobilize ATRP initiator onto the surface of the nylon membrane.[84] First formaldehyde was used to produce N-methylol polyamide membrane. In the second step, hydroxyl groups of the nylon-OH membrane react with ATRP initiators to immobilize the initiator on the surface of the nylon membrane. In this study both mentioned methods were used to immobilize ATRP initiators on the surface of PU Res IVR segments.

1.6 Combination of SI-ARGET-ATRP and alkyne-azide click reaction

The alkyne-azide click reaction, or the Sharpless click reaction, is a new method of reaction where an aryl/alkyl azide reacts with a strong active alkyne. Tetrazoles, 1,2,3 triazoles or 1,2-oxazoles are outcomes of this reaction.[91] It is a simple, fast reaction with high yield and selectivity.

A combination of click chemistry reaction with controlled radical polymerization (CRP) has been extensively used for the development of new materials with new architecture and new functionalities.[91,92] Among controlled radical polymerization methods, ATRP has been used widely in conjunction with the azide/alkyne click reaction.[93,94] A well-defined homo- and copolymer of acrylonitrile (AN) was prepared first by ATRP and modified with a click reaction with sodium azide and zinc azide to obtain copolymers with 5-vinyltetrazole.[95]

A combination of ATRP and alkyne/azide click reaction was employed to prepare well-defined (meth)acryloyl macromonomers.[94] Molecular brushes of poly(2-hydroxyethyl methacrylate) (PHEMA) with alkynyl side groups were synthesized using a "grafting onto" method through a combination of ATRP and click reaction. In this method, developed by Matyjaszewski's group, first PHEMA brushes were synthesized by ATRP. Afterwards, PHEMA polymers with alkynyl side groups were obtained via esterification between pentynoic acid and hydroxyl groups of PHEMA. At the end, click reaction between alkyne-containing PHEMA and azido-terminated polymeric side chains such as poly(n-butyl acrylate)-N3 were applied to make molecular brushes. This study demonstrated that several parameters such as chemical structure, molecular weight of azido-end polymer and initial ratio of linear chains to alkyne groups affect the grafting density of molecular brushes.[96]

In this project, an alkyne containing monomer such as poly(propargyl acrylate) (PPA) brushes were grafted onto the surface of PU Res IVR via SI-ARGET ATRP. Then an alkyne-azide reaction was carried out between PPA brushes with anionic and cationic monomers with azide groups to produce polyelectrolyte brushes on the surface of PU Res IVR segments. In fact, PPA brushes were used as a platform in which various different functional groups can be conjugated on the surface of PU Res IVR for different applications.

Chapter 2
Hypothesis and Objectives

Chapter 2. Hypothesis and objectives

In order to decrease side effects related to long-term exposure to anti-HIV drugs and the emergence of drug resistance in the virus, vaginal track stimuli-responsive drug delivery systems such as stimuli-responsive IVRs, have attracted significant interest in the drug delivery sector. Compared to the sustained release systems, stimuli-responsive release systems can improve therapeutic efficacy through selective release. They reduce toxicity related to long-term release of drugs and improve patient compliance and convenience.[50,97] To achieve a stimuli responsive drug delivery system, functional and stimuli-responsive groups can be incorporated into polymers with good biocompatibility.

As mentioned above, the choice of polymer material to fabricate IVRs is for biocompatibility, the ease of insertion, comfort of fit and prevention of tissue damage. For instance, it is reported that an ideal ring should have a durometer measure of 40±5 D according to ASTM D2240-05[44] with a size of 25×5 mm (overall diameter and cross-sectional diameter respectively).[34]

Polyurethanes PUs as a class of biocompatible polymer, have been used to fabricate IVRs, because of their excellent physical and mechanical properties as well as their biocompatibility. Chen et al., developed a PU-based IVR matrix and reservoir to deliver HCQ through the vaginal track for more than 14 days.[7]

In general, PUs are thermoplastic polymers, synthesized by step reaction between diisocyanates and polyols. The structure of the diisocyanates and polyols, as well as the structure and the length of the chain extenders play an important role in the final properties of the polymer.

For instance, the choice of polyols can affect the physio-chemical and mechanical properties of PUs. It is reported that polyester based PUs show more sensitivity to hydrolytic cleavage than polyether-based ones. Also PUs made of aliphatic diisocyanates show more durability to ultraviolet irradiation while PUs based on aromatic diisocyanates undergo photo-degradation when they are exposed to UV light.[98] Moreover, the choice of reactants can also affect biocompatibility of PUs. Aromatic-based PUs are known to be less biocompatible compared with aliphatic-based PUs.[99,100] This can be related to the fact that toxic products can be produced from degradation of aromatic compounds. In addition, the molecular weight of the polymer and the interaction of a drug with the PU matrix also affect the drug release from PU-based drug delivery systems. [100–102]

Therefore, synthesis of stimuli-responsive PUs that can be used for fabricating IVRs is very challenging due to the fact that besides being stimuli-responsive, the new polymer structure should provide enough flexibility and biocompatibility required for IVRs, and it should be easily manufactured through hot-melt extrusion or injection molding methods used for fabricating IVRs.

Hence, surface modification of existing PU-based IVRs with pH sensitive polymer brushes seems to be an attractive approach to functionalize the PU-based IVR without negatively changing the desirable bulk properties.

The purpose of this thesis is to graft pH-sensitive polymer brushes onto? the surface of intravaginal ring (Res IVR) made of polyurethanes (PUs) to achieve a switchable rapid release of the immunomodulatory drug hydroxychloroquine (HCQ), as a potential drug for prevention of HIV at basic pH (pH 7) and zero or close to zero drug release at acidic pH (pH 4).

We hypothesize that by chemically grafting a pH-sensitive polymer brush from the surface of PU IVR Res, an on-off release of drugs can be achieved.

The specific objectives of this project are:

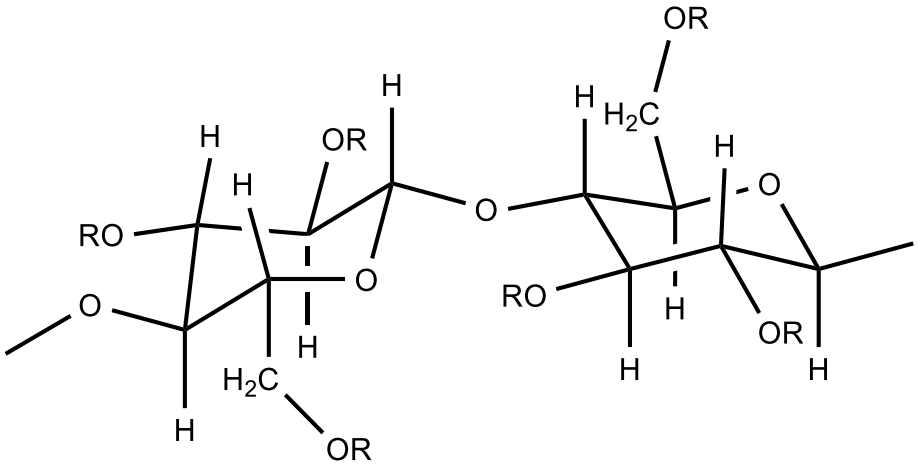
1. To identify feasible surface priming and grafting techniques for imparting PU-based reservoir IVR with the property of tuning the release of encapsulated model drug HCQ in response to changes of environmental pH.
2. To study and obtain a clear understanding of the correlation between specific pH-responsive functional polymers and the pattern of pH responsive drug release, given a chosen surface engineering technique.
3. To achieve the pattern of pH-responsive drug release desirable for the end-use: heterosexual intercourse-triggered drug release from reservoir-type IVRs.

Chapter 3
Materials and Experiments

Chapter 3. Materials and Experiments

3.1 Materials

All reagents and solvents were purchased from Sigma-Aldrich or Fisher Scientific and used as received. Methocel K100M premium hydroxypropyl methylcellulose (HPMC) (**Error! Reference s ource not found.**) and intravaginal ring reservoir (Res IVR) segments were kindly supplied by Dr. Emmanuel Ho's lab at the Department of Pharmacy, the University of Manitoba. A non-biodegradable medical-grade aliphatic polyether-based polyurethane Teco-philic HP-60D-35 (referred to here as (PU 60D 35) was used for fabrication of Res IVR segments. Shore hardness of this polyurethane is 42 D and it can absorb 35% water. The cross-sectional diameter, the wall thickness and the height of the Res IVR segment was determined to be 5 mm, 0.75 mm and 16 mm respectively. A digital caliper was used for the measurements. Ultra-pure water (18.2 MΩ) was obtained from a Millipore water purification system.



Scheme 3-1. Methocel K100M premium hydroxypropyl methylcellulose (HPMC) structure.

3.2 Experimental section

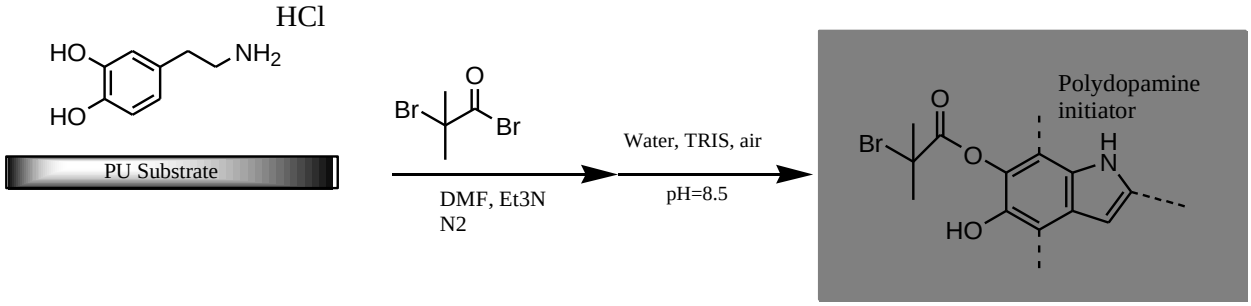
3.2.1 Immobilization of ATRP initiator

ATRP initiator, 2-bromoisobutyrylbromide (BIBB) was immobilized using two different methods:

3.2.1.1 Immobilization of ATRP initiator using poly(dopamine) PDA coating:

In order to immobilize the ATRP initiator on the surface of PU Res IVR segments, a method proposed by Zhu and Edmondson was applied. [90] In brief, dopamine hydrochloride (400 mg, 2.1 mmol) was dissolved in 20 mL dimethylformamide DMF under N_2 purge for 10 min. Then BIBB (0.13 mL, 1.05 mmol) and triethylamine (0.15 mL, 1.05 mmol) was added to the reaction mixture. After 3 hr stirring under N_2 at a round bottom flask (RT), the reaction mixture was transferred to a conical flask containing 100 mL Tris(hydroxymethyl)aminomethane (TRIS) buffer (10 mM).

In this condition, the pH decreases to acidic values because of the presence of carboxylic acid groups produced by acid bromide). Hence to adjust pH to basic pH (pH 8.5), 1 M TRIS was added to the solution. Finally, PU 60D 35 Res IVR was immersed in this reaction mixture and continuously shaken for 24 hr in the air. After that the PDA initiator-coated PU Res IVR (referred to as PU-PDA-BIBB) was removed from the solution, rinsed with deionized water and shaken in deionized water for overnight and dried with compressed air. (Scheme 3-2)

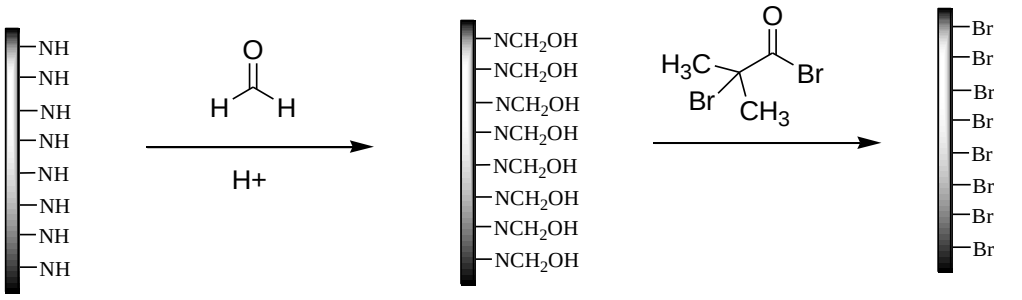


Scheme 3-2. Immobilization of ATRP initiator on the surface of PU 60D 35 Res IVR segments using poly(dopamine) PDA coating.

3.2.1.2 Immobilization of ATRP initiator using formaldehyde activation.

In this method, a two-step procedure was applied to immobilize ATRP initiator on the surface of PU IVR Res.[84] Briefly, in a 100 mL round bottom flask (RB) containing 10 PU Res IVR segments, formaldehyde solution (50 mL) was mixed with 1 mL of 85% phosphoric acid. The reaction mixture was kept at 50 ˚C for overnight to produce the PU-OH Res IVR segments.

After that, the PU-OH Res IVR were washed with plenty of water and dried under vacuum. In the second step, PU-OH Res IVR segments were immersed in a solution of n-hexane (50 mL) and triethylamine (4 mL). BIBB (2 mL in 5 mL n-hexane) was added into the reaction mixture, dropwise. The reaction mixture was gently stirred for 5 min at 0 ˚C and then 1 hr at room temperature. The PU-OH-BIBB Res IVR segments were washed with copious amounts of water/methanol (1:1 v/v) mixture and then dried under vacuum. (Scheme 3-3***Scheme 3-3***)

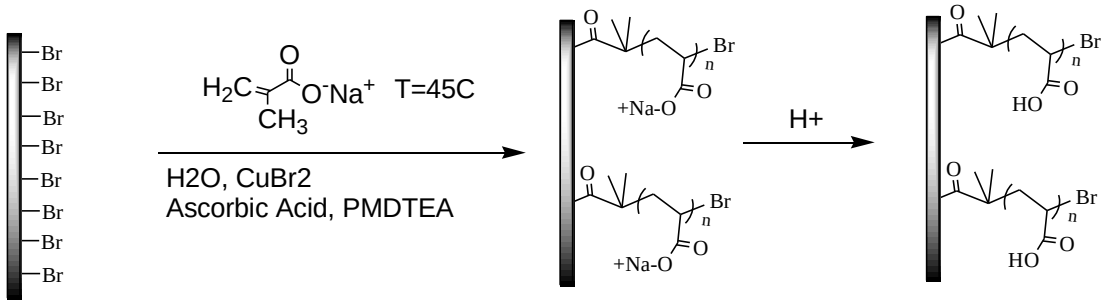


Scheme 3-3. Immobilization of ATRP initiator on the surface of PU 60D 35 Res IVR segments by formaldehyde method.

3.2.2 Surface-initiated ARGET-ATRP

3.2.2.1 |Surface-initiated ARGET-ATRP of poly(methacrylic acid) (PMAA) from the surface of BIBB-immobilized PU Res IVR segments:

PMAA brushes are grafted from the surface PU-OH-BIBB Res IVR segments. For this purpose, sodium methacrylate (NaMA) (1g, 9.25 mmol), $CuBr_2$ (7.75 mg, 0.034 mmol) and N, N, N, N, N-pentamethyldiethylenetriamine (PMDTEA) were dissolved in 4 mL of pure water and charged into a 25 mL RB flask containing PU-OH-BIBB Res IVR segments. Next, the reaction mixture was purged under N_2 atmosphere for 30 min. Afterwards, the flask was placed in water bath at 45 ˚C. The reaction was started by adding ascorbic acid (79.2 mg, 0.45 mmol) into the solution. The polymerization was stopped after 24 hr by exposing to the air. The samples were washed with lots of water, followed by immersing in 1 M HCl solution for 4 hr to exchange Na^+ for H^+. The prepared samples were dried under vacuum at 40 ˚C. (Scheme 3-4)



Scheme 3-4. SI-ARGET ATRP of poly (methacrylic acid) (PMAA) from the surface of PU-OH-BIBB Res IVR segments.

Experimental design based on the combination of SI-ARGET-ATRP and alkyne-azide click reaction:

3.2.3 Synthesis of Azide monomers:

3.2.3.1 Converting 2-bromobutyric acid monomer to 2-Azidobutyric acid monomer

For this reaction, 2-bromobutyric acid monomer (5 g, 30 mmol) was mixed with sodium azide (9.75 g, 150 mmol) in 20 mL water in a round bottom flask. Then the reaction mixture was refluxed overnight. After cooling down, the solution was acidified with 1 M HCl solution and extracted with ethyl acetate. The organic part was evaporated and 2-Azidobutyric acid was obtained.[103]

3.2.3.2 Converting 4-Chloromethylpyridine to 4-Azidomethylpyridine monomer

In a round-bottom flask, 2.5 g (15 mmol) of 4-Chloromethylpryidine dissolved in 20 ml of water and reacted with 4.57 g (150 mmol) of sodium azide. The reaction was continued at 50 ˚C for overnight, followed by quenching with sodium bicarbonate. An oily product was obtained after extraction with methylene chloride.[103]

3.2.3.3 Combination of SI-ARGT-ATRP and alkyne-azide click reaction

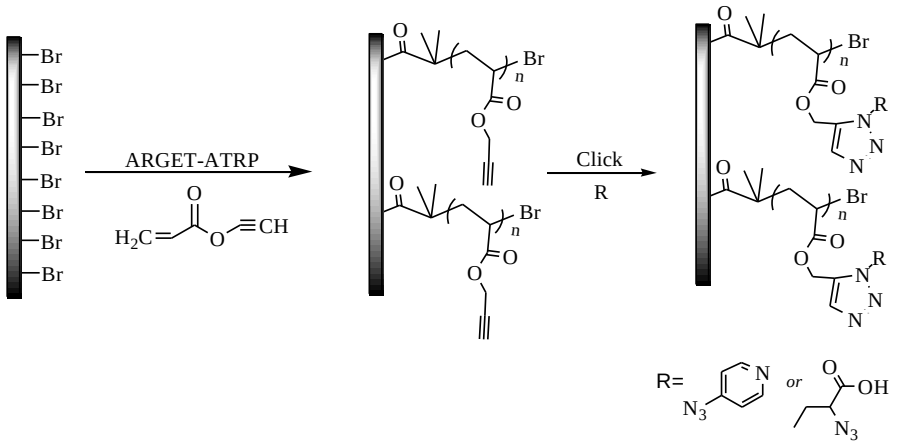
3.2.3.3.1 Surface-initiated ARGET-ATRP of poly(propargyl acrylate) (PPA) on the surface of PU-OH-BIBB Res IVR segments

Poly(propargyl acrylate) (PPA) brushes were grafted from the surface of PU-OH-BIBB Res IVR segments via SI-ARGET-ATRP. PPA (1g, 9.08 mmol), $CuBr_2$ (7.75 mg, 0.034 mmol) and N,N,N,N,N-pentamethyldiethylenetriamine (PMDTEA) (86.75 mg, 0.5 mmol) were dissolved in 4 ml of methanol and charged into a 25 mL RB flask containing PU-OH-BIBB Res IVR segments. The reaction mixture was purged with nitrogen for 30 min. After that, Ascorbic acid (79.2 mg, 0.45 mmol) was added to the reaction mixture and reaction mixture was purged with nitrogen for

extra 10 min. At the end, the flask containing the reaction mixture was placed in a 40 ˚C water bath and shaken for 36 hr. The polymerization was stopped by exposing the reaction mixture to air. The samples were washed with methanol and dried in a vacuum oven at 40 ˚C.

3.2.3.3.2 Alkyene-azide reaction of azide monomers on the surface of PU-PPA Res IVR segments.

In a 250 mL Erlenmeyer containing PU-PPA Res IVR segments, 10 mL water was mixed with 10 ml methanol. After that, the Azide monomers 1 mmol, 50 µl triton X were added into the solution. The reaction mixture was shaken for 30 min. Then, sodium ascorbate 1 mmol and copper sulfate 0.1 mmol were added to the solution and the mixture was shaken at room temperature overnight. Afterwards, samples were washed with methanol and acetone and dried at 40 ˚C under vacuum. PU-PPA Res IVR was grafted with butyric acid groups BAA and 4-vinyl pyridine 4VP were labeled as PU-PPA-BAA and PU-PPA-PY Res IVR respectively. Scheme 3-5 shows a combination of SI-ARGET-ATRP of PPA and alkyne-azide click reaction on the surface of PU 60D 35 Res IVR segments.



Scheme 3-5. Combination of SI-ARGET-ATRP and alkyne-azide click reaction of pH sensitive monomers from the surface of PU-OH-BIBB Res IVR segments.

3.2.4 Grafting density of acidic groups on the surface of PU-OH-PMAA and PU-PPA-BAA Res IVR

The grafting density of acidic groups on the surface of PU-OH-PMAA and PU-PPA-BAA were evaluated using the toluidine blue (TBO) dye method.[104] Res IVR segments were immersed in 10 mL of 0.5 mM TBO solution (pH 10) for 6 hr at room temperature while stirring. Samples were then rinsed using NaOH solution (pH 10) to remove unreacted TBO. The stained samples were immersed into 50% acetic acid which unbinds the TBO from the carboxylic acid groups of PMAA or PBAA.

The absorbance of the solution containing TBO was measured by UV-spectrophotometer (Ultrospec 4300 pro, Biochrom, England) at 630 nm. At pH 10, TBO forms a one-to-one complex with carboxylic acid groups on PMAA or PBAA. A calibration curve of TBO with different concentration in 50% acetic acid against corresponding absorbance was made. (Figure 3-1) The concentration of TBO in solution was calculated using this calibration curve. The PU-OH-BIBB and PU-PPA Res IVR were used as controls for samples grafted with PMAA and BAA respectively. The experiment was done in triplicate. (Figure 3-2)

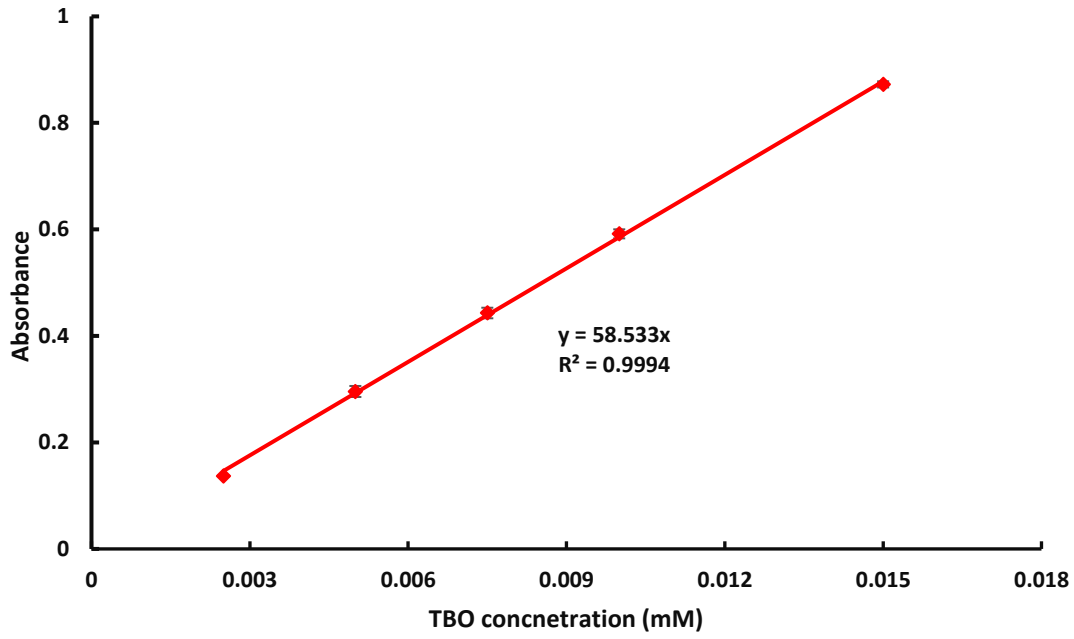


Figure 3-1. Absorbance of TBO against the concentration of TBO in % 50 acetic acid solution.

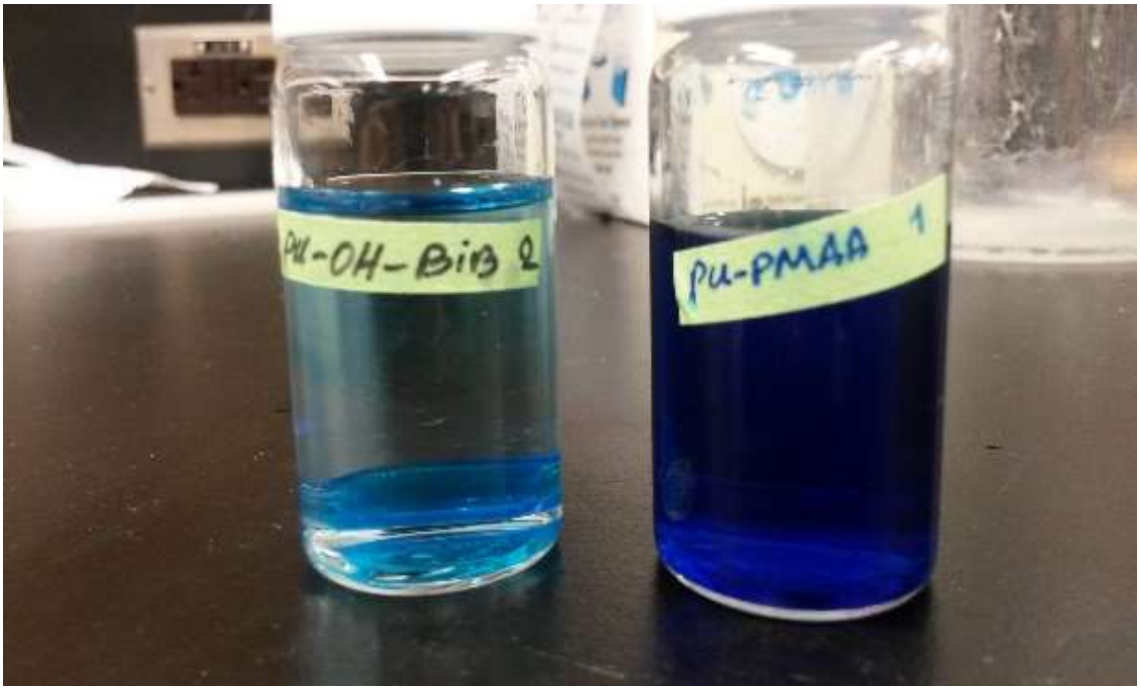


Figure 3-2. PU-OH-BIBB Res IVR (left) and PU-OH-PMAA Res IVR (Right) in 50% acid acetic solution

3.2.5 Grafting density of cationic groups on the surface of PU-PPA-PY IVR Res IVR

In order to determine the grafting density of pyridine groups on the surface of PU-PPA-PY IVR Res, the Acid Orange II (AO) method was applied. PU-PPA-PY Res IVR and PU-PPA Res IVR segments (as controls) were immersed into a 0.5 mM Acid Orange II (AO) solution in deionized water (D.I.) with pH adjusted to 3 with hydrochloric acid. At this pH, amino groups form complexes with AO.

After shaking overnight at 37 ˚C, samples were washed 3 times for at least 15 mins with D.I. water at pH 3. The AO concentration, which is proportional to the amine groups, was determined with a UV-spectrophotometer (Ultrospec 4300 pro, Biochrom, England) at 485 nm. A calibration curve of AO with different concentrations in water with pH=12 (which is adjusted with NaOH) verses their absorbance values was made. (Figure 3-3) The grafting density of PY was determined with reference to the calibration curve.[105,106]

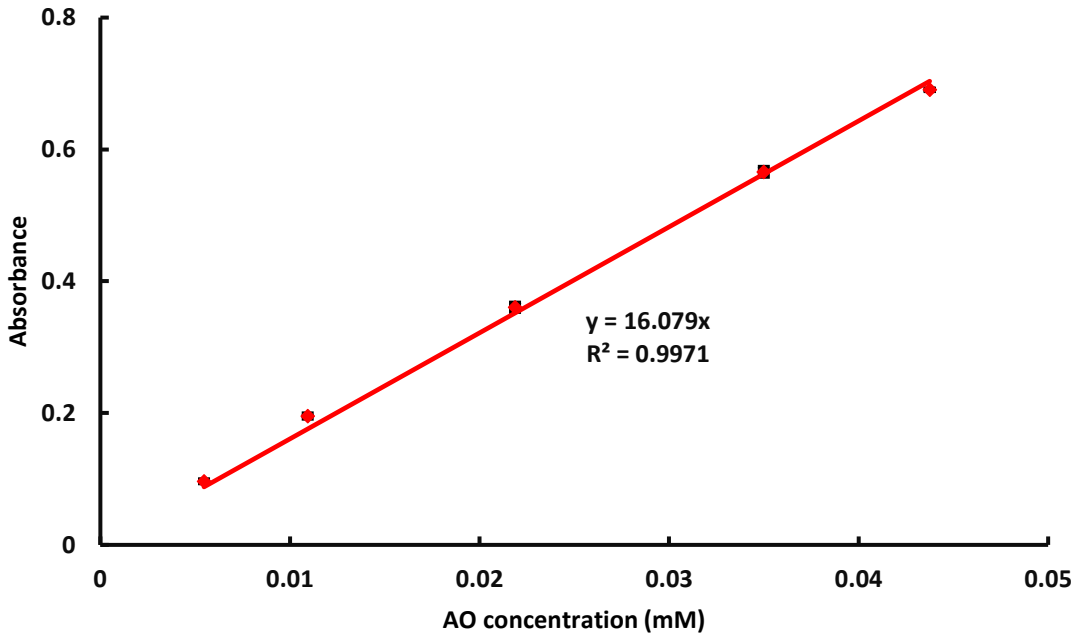


Figure 3-3. Absorbance of AO against the concentration of AO in water with pH=12

3.2.6 Release studies.

3.2.6.1 Semisolid HCQ-HPMC preparation

The preparation of a semi-solid containing HCQ-HPMC has been reported elsewhere, by Yannick et al. [8] In brief, HCQ-HPMC semi-solid was prepared by weighing 160 mg of HCQ and dissolving it in 1.28 ml of distilled water, followed by adding 160 mg of HMPC to the solution. The mixture was mixed with a spatula, then transferred into a 5 ml syringe connected to another syringe. The content of the syringe was passed in and out of the syringe, 80 times to make a homogenous mixture.

3.2.6.2 pH responsive behavior at pH 4 or pH 7

Untreated PU 60D 35 IVR and modified PU 60D 35 Res IVR segments were filled with 80 mg of semisolid containing HCQ-HPMC. The end of each segment was capped with silicon caps and sealed with Loctite® 4013™ Prism® instant adhesive (Henkel Corporation). The "capped" segments were immersed in 5 ml Phosphate buffered saline PBS at pH 4 or pH 7, and placed in an incubator equipped with an orbital shaker set at 100 rpm and a temperature of 37 °C. Sink conditions were maintained by replacing the entire release medium after a desired time. The amount of released HCQ was determined using a UV spectrophotometer (Ultrospec 4300 pro, Biochrom, England) at 343 nm. (Figure 3-4)

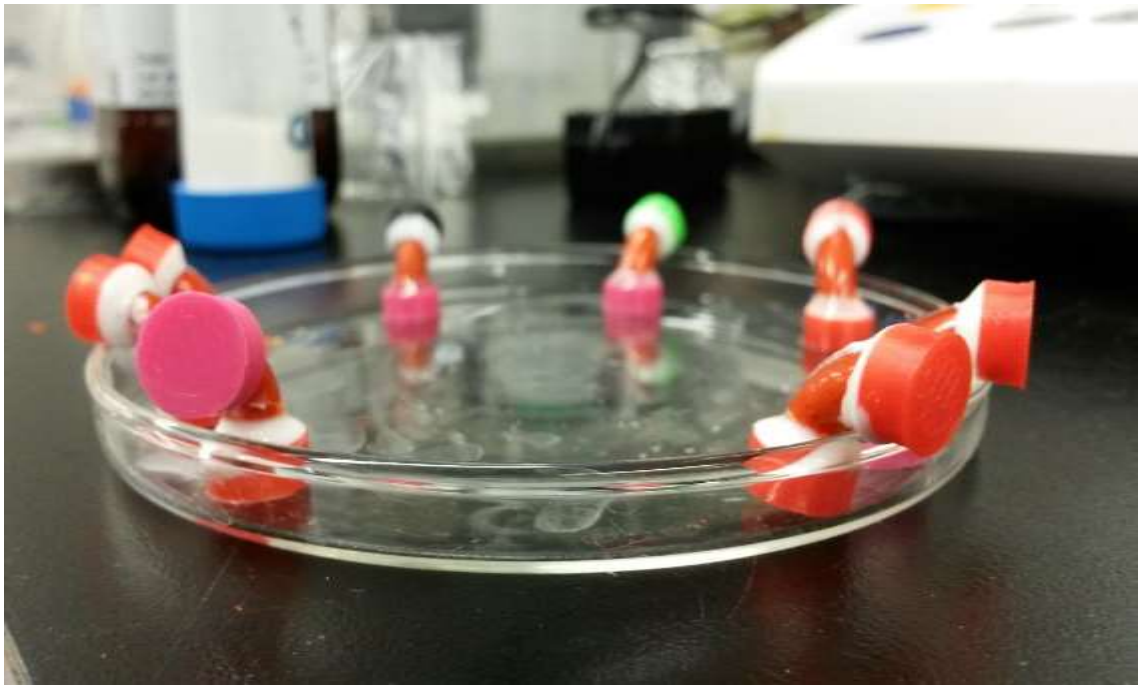


Figure 3-4. Silicon-capped PU 60D 35 and modified PU 60D 35 Res IVR segments.

3.2.6.3 Release study at pH 4 and pH 7 switch on/off cycle

For this set of experiments, the capped Res IVR segments filled with HCQ/HPMC semisolid were immersed in 5 ml of PBS solution (pH 4). After a desired time (4 hr), samples were taken out and immersed in pH=7 PBS solution. This cycle was repeated several times (minimum 5 times). All experiments were done in triplicate and the mean ±1 standard deviation is reported.

3.2.7 Surface characterization

The Attenuated Total Reflectance Fourier-transform infrared (ATR-FTIR) spectra were recorded with a Nicolet S10 spectrometer (ThermoScientific) with a resolution of 4 cm^{-1} for 16 scans. The ^{1}H NMR spectra of synthesized samples were recorded on a Bruker MSL 300 spectrometer (300 MHz). The surface chemical composition of the PU samples was analyzed by X-ray photoelectron

spectroscopy (XPS). XPS measurements were performed on a Kratos Axis Ultra spectrometer using a monochromatized Al K X-ray source (1486.7 eV photons).

The topography of the unmodified and modified PU Res IVR segments was studied by atomic force microscopy (AFM) using a Vecco D3100. In each case an area of 20×20 μm^2 was scanned using the tapping mode with a scan rate of 1 Hz. The arithmetic means of the surface roughness (Ra) was calculated from the roughness profile determined by AFM. NanoScope Software 6.13 was employed to analyze the data.

Scanning electron microscope (SEM) images of unmodified and modified PU 60D 35 Res IVR segments at different pH values were obtained on a FEI Quanta 650 FEG. Unmodified and modified PU 60D 35 Res IVR segments were immersed in PBS solution at pH 4 and pH 7 separately for 24 hr. After removing samples from PBS solutions and removing extra water solution from the surface, samples were frozen at -80 ˚C followed by freeze-drying. In order to study the cross-sectional view of samples, they were broken using a mortar and pestle, after immersing in liquid nitrogen. The samples were mounted on the sample studs and coated with a thin layer of gold. (

Figure ***3-5***)

Figure 3-5. Samples after coating with a gold layer.

Chapter 4
Results and discussion

Chapter 4. Results and Discussion

As mentioned previously in the first chapter, the position of polymer brushes relative to pores on the surface of the substrate determines the final response of the responsive substrate. For instance, if an anionic polymer brush such as PMAA is grafted only on the opening end of pores on a substrate, higher release rate would be observed at basic pH values compared to acidic pH values. At basic pH values, polymer chains are deprotonated and because of the repulsion forces between negatively charged polymer chains, they adopt a "brush-like" conformation. As a result, Pores are open and release rate increases.[64–66] In contrast, if polymer brushes are grafted inside the pores along the thickness of pores' walls, at higher pH values, the negatively charged polymer chains are extended along the thickness of walls in the pores. Therefore, they cover the pores and release rate decreases. While in acidic pH values, polymer brushes adopt a "mushroom" conformation and solutes can release easily from the substrate.[69,70,72]

Surface modification methods, i.e. the techniques used to immobilize initiators and the grafting techniques, can affect the position of polymer brushes relative to the pores on the surface of the substrate, pore size and their distribution which in turn leads to different responses of polymer brushes to external stimuli.[107]

Hence, in the first part of this project, I aimed to find a suitable surface priming and modification method for imparting PU Res IVR with the property of tuning the release of the encapsulated model drug HCQ in response to changes of environmental pH. In the second part, it was aimed to study and obtain a clear understanding of the correlation between specific pH responsive functional polymers and the pattern of pH-responsive drug release according to the

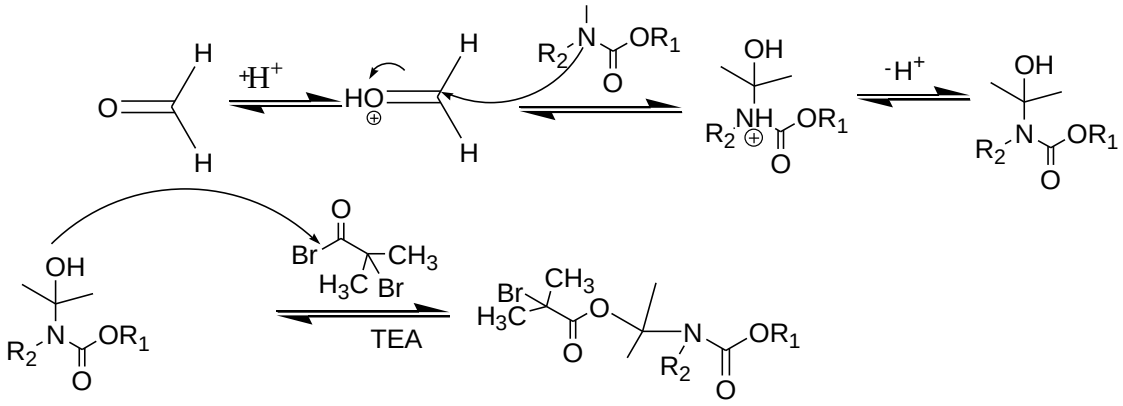
specific surface engineering technique. Moreover, it was aimed to use this correlation to achieve a heterosexual-triggered drug released from PU IVR Res.

4.1 Finding a feasible and suitable approach to achieve desirable pH sensitivity:

4.1.1 The choice of suitable surface priming and modification technique

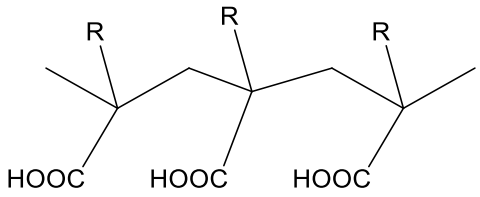
As it was mentioned, the most important step in the SI-polymerization technique is the immobilization of initiator on the surface of a substrate. We applied two methods to immobilize BIBB as an ATRP initiator on the surface of PU IVR Substrate. The first method is polydopamine (PDA) coating which is a universal method that can be applied to immobilize an ATRP initiator such as BIBB on the surface of a wide range of substrates.[108] In this method, the dopamine monomer reacts with BIBB under base catalysis before polymerization of PDA happens. This reaction is performed in a polar aprotic solvent such as DMF and in the absence of air to avoid premature polymerization. After that, polymerization happens in an aqueous solution in the presence of air.

Also an alternative method, activation with formaldehyde in an acidic condition, was applied for BIBB immobilization on the surface of PU substrate.[84] In this method, formaldehyde reacts with a nitrogen in urethane group in an acidic condition to form hydroxyl groups on the surface of the PU membrane. After that, theses hydroxyl groups react with BIBB to produce alkyl bromide-terminated PU membrane (Scheme 4-1).



Scheme 4-1. Schematic illustration of activation of the surface of PU IVR substrate and subsequently the reaction of hydroxyl groups with BIBB.

Also in our first attempt, PMAA (***Scheme 1-1***Scheme 4-2) with pKa 6.5 [109] was chosen to be grafted form the surface of PU Res IVR using SI-ARGET-ATRP.



Scheme 4-2. The chemical structure of PMAA

Controlled polymerization of acid monomers such as (acrylic acid) (AA) or (methacrylic acid) (MAA) using ATRP is very challenging because the acid monomer poisons the catalysts by converting the Cu(I) catalyst to Cu(I) carboxylate which retards the catalyst activity.[87] Also, ligands can be protonated, which interfere with the metal complexation ability.[87,110,111]

Alternatively, the PMAA brushes can be prepared through ATRP using precursor monomers of MAA such as (trimethylsilyl) methacrylate, tert-butyl methacrylate or p-nitrophenol methacrylate, followed by hydrolysis or pyrolysis of the protective groups. However, the de-protection of the initial monomer such as tert-butyl group not only introduces an additional synthetic step, but also, the prolonged hydrolysis times were found to cause partial cleavage of polymer chains from the surface.[111] Another possibility for grafting PMAA brushes from the surface is using sodium methacrylate (NaMA) instead of MAA. Billingham et al., for the first time reported the direct ATRP of sodium methacrylate in aqueous media.[112] Huang et al., grafted PMAA brushes from the surface of cross-linked chitosan microspheres using NaMA[87] to remove Cd(II) ions from aqueous solutions. In another work, PMAA brushes were grafted the surface of a quartz crystal microbalance (QCM) using NaMA to study the influence of thickness and density of polymer brushes on the pH responsiveness of PMAA brushes.[109] In this dissertation, poly(sodium methacrylate) PNaMA brushes were grafted from the surface of PU 60D 35 Res IVR via SI-ARGET-ATRP, followed by immersing in 1 M HCl solution to convert carboxylate groups to carboxylic acid groups.

4.1.2 In vitro HCQ release

Figure 4-1 shows a non-cumulative release of HCQ from PU 60D 35 Res IVR segments at pH 4 and pH 7. The PU used to fabricate IVR Res is an aliphatic hydrophilic polyether-based resin which can absorb water up to 35% of the weight of dry resin. As it can be seen in Figure 4-1 the release profile of HCQ was the same at pH 4 and pH 7 with the average release of 86.33±6.1 µg/mL after 24 hr. Also the pH sensitivity of PU 60D 35 Res IVR samples was studied at pH 4 and pH 7 cycles in a 4 hr time interval. First, the average release of HCQ was 1.16±0.88µg/mL at

pH 4 and then it increased to 5.33±1.77 µg/mL at pH 7, and it continued to increase to 8.6±0.5 µg/mL at pH 4 and finally it reached a constant release of 9.75±1.11 µg/mL every 4 hr. The reason might be related to the fact that, at first PU is in its dry state. However, after immersing in PBS solution PU starts to absorb water until it reaches its equilibrium point. These results suggest that, the release of HCQ from PU 60D 35 Res IVR is pH independent (Figure 4-2).

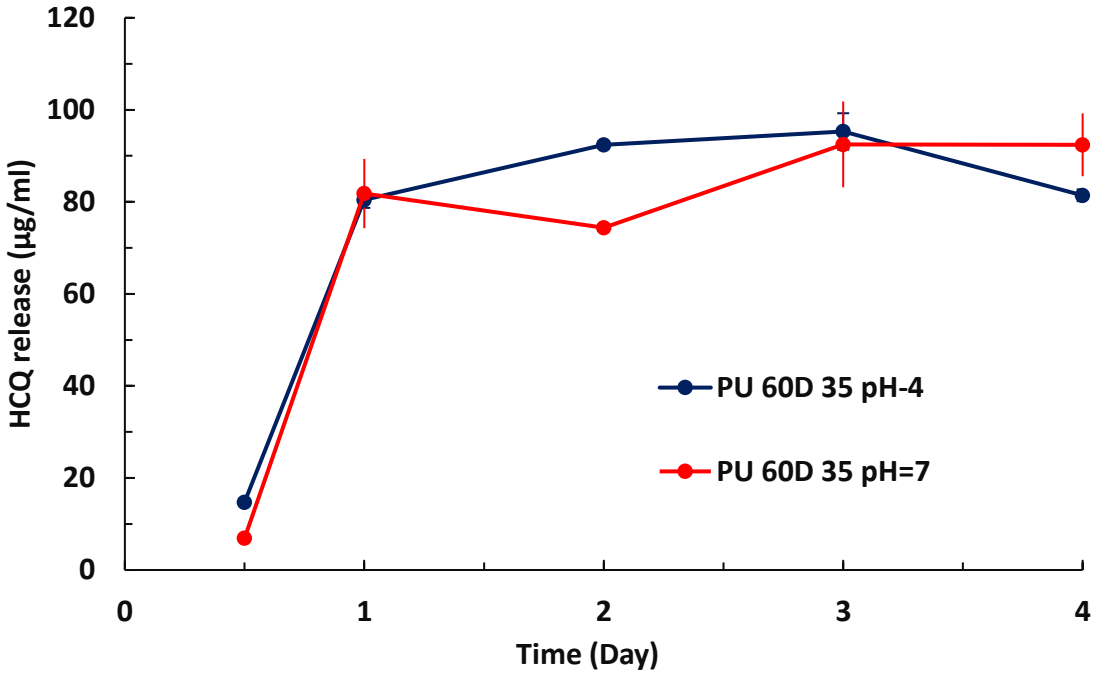


Figure 4-1. Release of HCQ from PU 60D 35 Res IVR at pH 4 (blue line) and pH 7 (red line) (n=3).

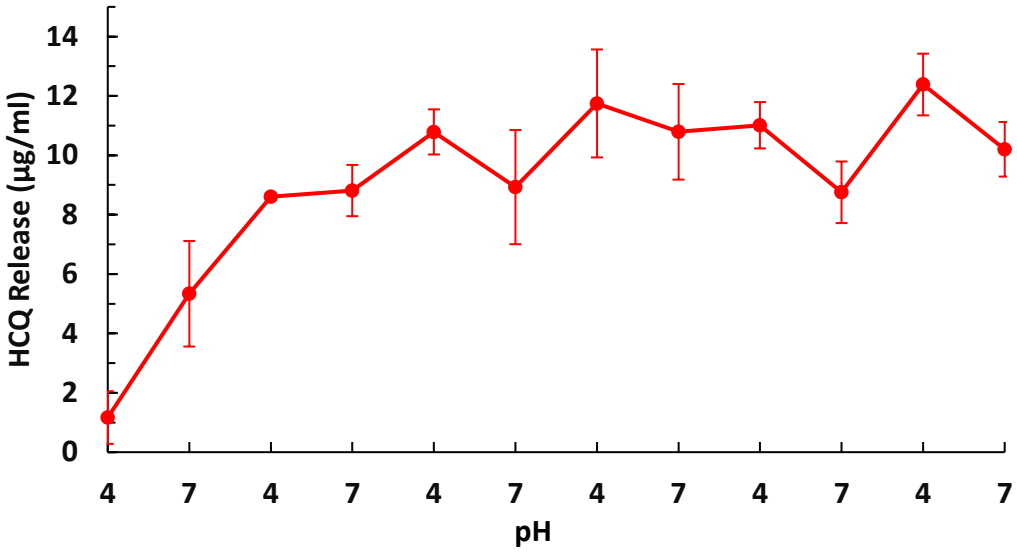
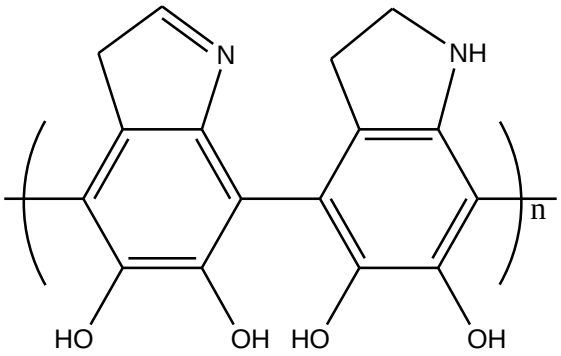


Figure 4-2. Release of HCQ from PU 60D 35 Res IVR at pH 4/pH 7 cycle at 4hr time interval n=3.

Figure 4-3 shows the release of HCQ from PU-PDA-BIBB Res IVR at pH 4 and pH 7. Since the first data point was collected in 12 h instead of 24 h, the average release of HCQ from PU-PDA-BIBB Res IVR at pH 4 and pH 7, was determined without considering the first data point. The average release of HCQ from PU-PDA-BIBB was determined to be 102.38±6.49 µg/ml at pH=4 and 53.92±3.42 µg/ml at pH=7 per day respectively ($p<0.05$ n=3). The higher release at pH=4 than pH=7 is because of decrease in interaction of PDA and HCQ because of protonation of amino and catechol groups of the PDA at low pH values. [113]



Scheme 4-3. Chemical structure of PDA.

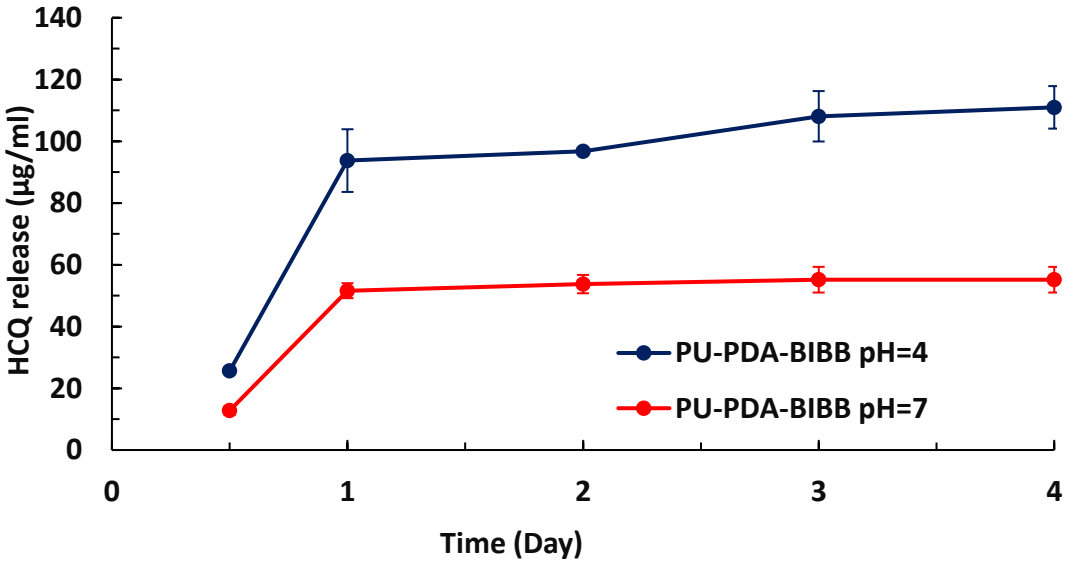


Figure 4-3. Release of HCQ from PU-PDA-BIBB Res IVR at pH 4 (blue line) and pH 7 (red line) (n=3).

Based on the release studies above, because PU-PDA-BIBB showed pH sensitivity at different pH values, this method cannot be considered as a good option to immobilize ATRP initiator on the surface of PU substrate.

In the next step the release profiles of HCQ from PU-OH-BIBB Res IVR segment at different pH values were studied and the results are shown in Figure 4-4. It can be seen that release curves at both acidic and basic pH are the same, indicating that release of HCQ from PU-OH-BIBB Res IVR is pH independent. The higher release of HCQ from PU-OH-BIBB Res IVR compared with PU Res IVR might be related to some degradation that can happen to the PU substrate during the immobilization of BIBB on the surface of the PU IVR Res. Also, the pH sensitivity studies of PU-OH-BIBB Res IVR were performed at pH 4/pH 7 cycles. Figure 4-5, also confirms that PU-OH-BIBB Res IVR does not show any pH sensitivities at different pH values.

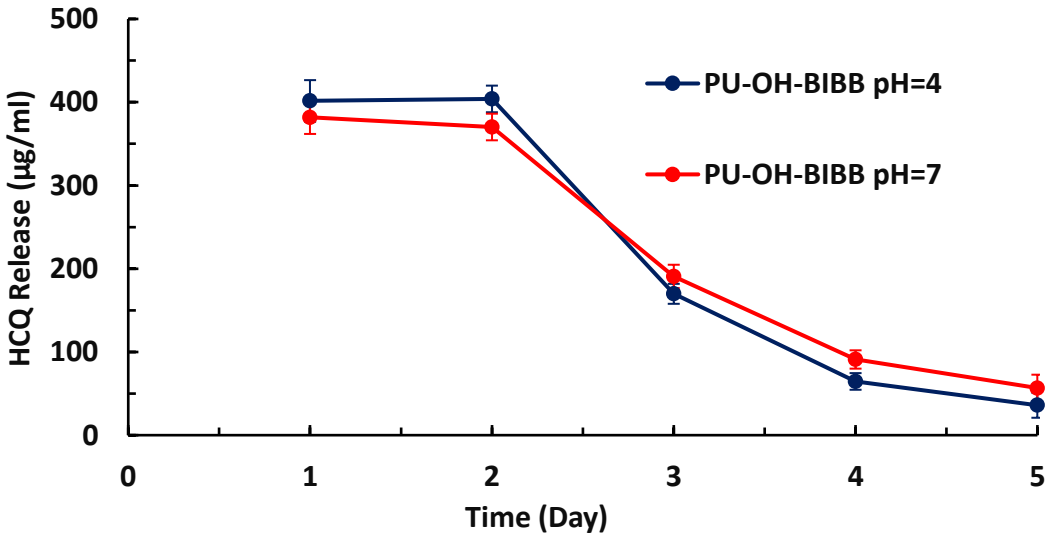


Figure 4-4. Release of HCQ from PU-OH-BIBB Res IVR at pH 4 (blue line) and pH 7 (red line) (n=3).

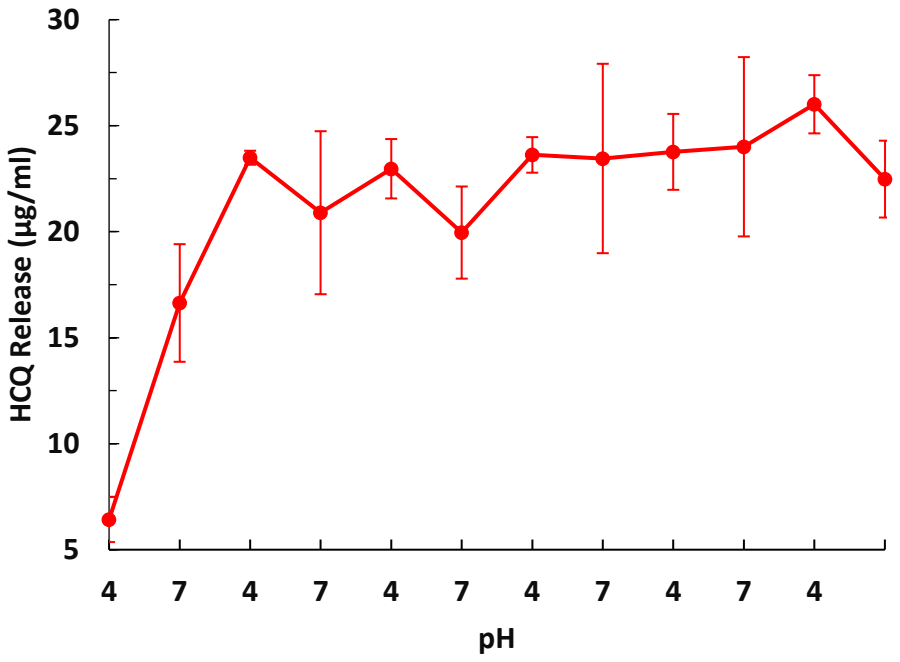


Figure 4-5. Release of HCQ from PU-OH-BIBB Res IVR at pH 4/pH 7 cycle at 4hr time intervals (n=3).

Since PU-OH-BIBB Res IVR does not show pH sensitivity upon pH changes, this method is applied to immobilize BIBB on the surface of PU Res IVR.

Figure 4-6 shows an XPS spectrum of (a) PU 60D 35 Res IVR and (b) PU-OH-BIBB Res IVR segments. Compared to PU 60D 35 IVR Res, a small peak appears in the spectrum of PU-OH-BIBB Res IVR at 70 eV. The presence of this peak corresponding to Br core level electron suggests the incorporation of 2-bromoisobutyrate groups onto the PU substrate.

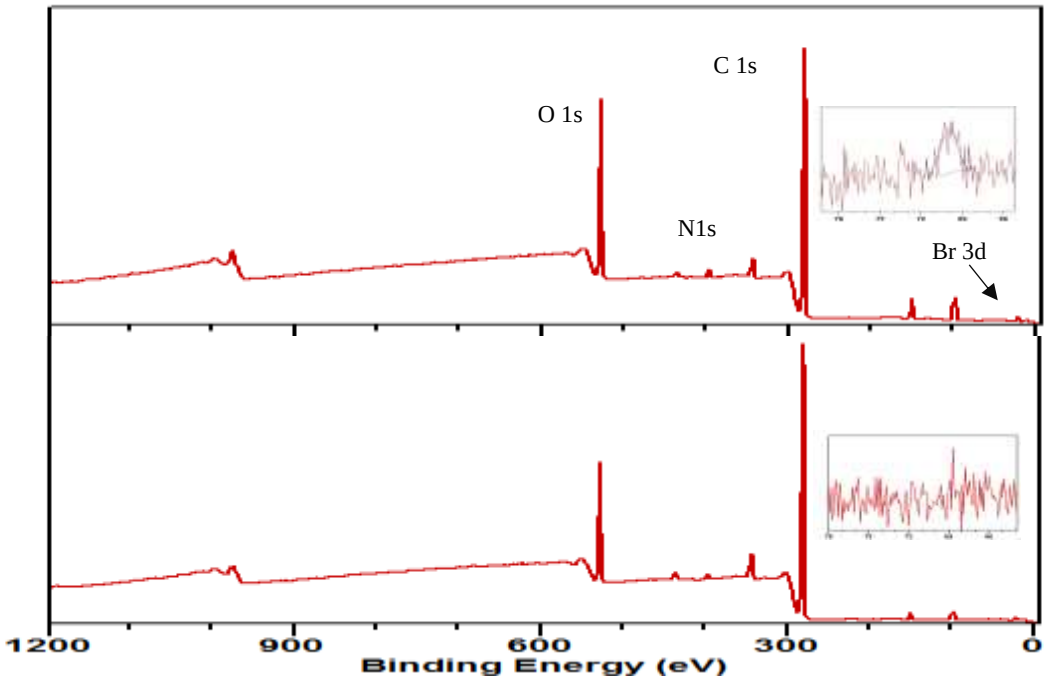


Figure 4-6. XPS spectra of (a) PU 60D 35 Res IVR segment and (b) PU-OH-BIBB Res IVR segment with inset showing Br core level scan.

The density of the BIBB (ρ^*, [Br]/nm^2) layer on the surface of PU-OH-BIBB Res IVR was obtained from the XPS results.[68,114,115] The thickness of the initiator layer h was determined to be 0.27 ρ^* based on Equation 4.1:

Equation 4.1 $$h = \rho^* \times {M_1}/{(N_A \times \rho_1)}$$

Where, M_1 and ρ_1 are molecular weight of BIBB (165.5 g/mol) and mass density of BIBB (1.0 g/cm^3, or 1.0 ×10^{-21} g/nm^3) respectively. NA is the Avogadro constant (6.022× 10^{23}).

The number (n) of the total C atoms per unit surface area (A=1 nm^2, with the XPS probing depth of 7.5 nm) is expressed in Equation 4.2:

Equation 4.2 $$n = 4\rho^* + 3((7.5 - h) \times N_A \times {}^{\rho_2}/_{M_2})$$

Where 4 and 3 refer to the number of C atoms in BIBB group ($C_4H_6O_2Br$) and the repeating unit of polyurethane ($C_3H_8N_2O$) respectively. ρ_2 is the mass density of the polyurethane (1.2 g/cm^3 or 1.2×10^{-21} g/nm^3), and M_2 is the molecular weight of the polyurethane repeating unit (88 g/mol).

We obtained n=184.725±2.65ρ^* after substituting h, N_A, ρ_2 and M_2 with data. Based on the [Br]/[C] ratio of 1.6×10^{-3} (determined from the sensitivity factor-corrected area of Br 3d and C 1s peaks in the high-resolution spectrum of PU-OH-BIBB Res IVR segment (**Error! Reference s ource not found.**Table 4-2), the initiator density ρ^* is estimated to be 0.29 [Br]/nm^2 from the following equation: $r = \frac{\rho^*}{n}$. This density is higher than the reported value of 0.21 [Br]/nm^2 which was shown to be high enough to initiate surface graft polymerization. [115]

In the next step, PMAA brushes were grafted from the surface of PU-OH-BIBB Res IVR segments. The grafting density of PMAA brushes is determined to be 2.43±0.055 mg/cm^2 using the TBO dye method. In one study the permeability of riboflavin was investigated from poly(acrylic acid) PAA grafted poly(vinylidene fluoride) PVDF membrane. It was observed that the permeability of the model drug depends on the graft density of PAA chains on the surface of the membrane. It was observed that when the grafting density of PAA chains is between 0.35 and 2.17 μmol/cm^2, the permeability of riboflavin decreased dramatically at pH 4-5 which is close to the pKa of PAA.[116] Therefore, the grafting density of PMAA grafted from the surface of PU-OH-BIBB is high enough to show pH responsiveness.

The response of PU-OH-PMAA Res IVR after application of the new immobilization method of ATRP initiator at different pH values is illustrated in Figure 4-7. Interestingly, it is observed that the release profiles of HCQ from PU-OH-PMAA Res IVR segments, at two pH values of 4 and 7 are the same ($p>0.05$ $n=3$). This suggests that although the presence of PMAA brushes decreases the rate of drug release compared to PU-OH-BIBB IVR Res, the presence of PMAA brushes does not affect the pH sensitivity of Res IVR segments. This could be related to the nature of the PMAA polymer chains. Poly(methacrylic acid) chains can be hydrated over the entire pH range. It has been shown that a layer by layer (LBL) hydrogel, which contains PMAA chains remains hydrated over the entire pH range with the lowest static water contact angle and the largest swelling ratio among the family of poly(2-alkylacrylic acid) (PaAAs).[117,118] Hence, changes in the conformation of PMAA chains upon different pH values do not have a dramatic influence on profile release of HCQ from PU-OH-PMAA IVR Res.

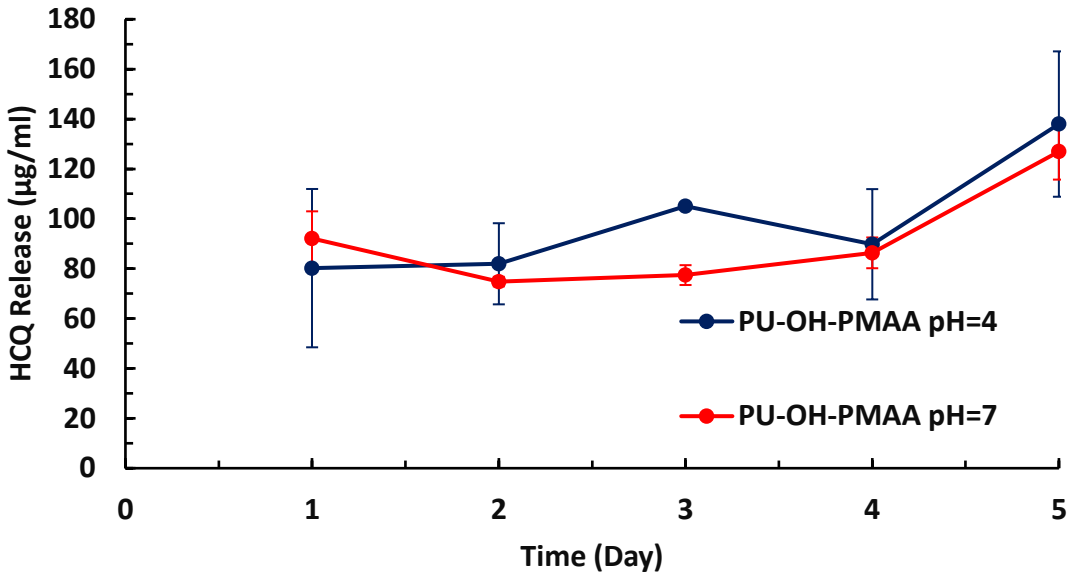


Figure 4-7. Release of HCQ from PU-OH-PMAA Res IVR at pH 4 (blue line) and pH 7 (red line) (n=3).

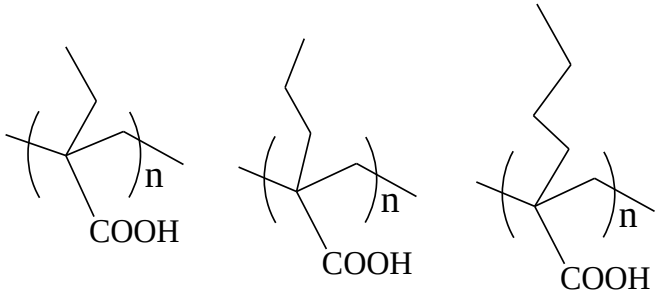
4.2 Alternative polymer brushes in order to achieve desirable pH responsiveness:

4.2.1 The choice of anionic polymer brushes

According to the pervious results, it seems that PMAA is not a good candidate for pH responsiveness, therefore a monomer which presents more changes in hydrophobicity and hydrophilicity in response to changes of pH is needed. Unlike PMAA and PAA, other PaAAs including poly(2-ethylacrylic acid) (PEAA) (pKa=5.2-6.2), poly(2-n-propylacrylic acid) (PPAA) (pKa=5.5-6.5) and poly(2-n-butylacrylic acid) (PBAA) (pKa=6.5-7.2) (Scheme 4-4) that become more hydrophobic in acidic pH values and show prominent changes upon pH changes were investigated.[117–119] We aimed to graft PaAAs with more hydrophobicity/hydrophilicity from PU Res IVR segments to achieve desirable pH responsiveness.

The main challenge in the first place was synthesis of these monomers. Huntington et al, reported a three-step method to synthesize 2-Alkylacrylic acid monomers. In this method, first dimethyl-2alkylmalonates were prepared by using dimethyl malonate and corresponding sodium alkyl ethoxide. Then dimethyl-2alkylmalonate was alkylated with the corresponding alkyl bromide and hydrolysis of the ester groups to give white solids of 2-alkylmalonic acids. Finally, acidic monomer can be synthesized from treatment of 2-alkylmalonic acid with formaldehyde or paraformaldehyde.[120,121] Lu et al., used free a radical polymerization method to synthesize PaAAs with average molecular weights of 100,000 g/mol and polydispersity (PDI) of 2.2.[118]

We tried to synthesize alkyl acylic acid monomers in our lab. However, not only the procedure to synthesize the monomers was too long, but also the purification steps results in a low yield of final products (less than 20%). Therefore, we tried to find an alternative method which can overcome these obstacles.



Scheme 4-4. Chemical structures of poly(2-alkylacrylic acids) (PaAAs). The abbreviations PEAA, PPAA, PBAA stands for poly(2-ethylacrylic acid), poly(2-n-propylacrylic acid) and poly(2-n-butyric acid), respectively.

4.2.2 Choice of cationic polymer brushes

In our attempt to understand the correlation between the position of polymer brushes on the surface, and the mechanism of release from modified PU 60D 35 Res IVR segments, a cationic polymer such as Poly(4-vinylpyridine) P4VP pKa 5-6 [122] was considered to be grafted from the surface of PU 60D 35 Res IVR as well.

Poly(4-vinylpyridine) (P4VP) is a pH-sensitive polymer which has attracted a great interest over past decades, because of its applications as a water-soluble polymer and coordination reagent for transition metals. Also this polymer can be used as a conductive polymer and a compatiblizer in block copolymers.[123–125]

Homopolymers and copolymers of P4VP and polymer brushes of P4VP have been produced by ATRP[123–125] and SI-ATRP.[57,126,127] However, the ATRP of 4VP is very challenging, because of the complex behaviour of the pyridine rings of 4VP and P4VP with the copper catalysts. Both 4VP and P4VP can compete for the binding of the metal catalysts in ATRP systems due to the fact that the monomer and polymer are strong coordinating ligands. Furthermore, because of the excess amount of monomer in the solution compared to the ligand used, there is a possibility of the formation of a tspyridine-coordinated metal complex in the polymerization solution. These metal complexes are not effective catalysts for ATRP and in many cases, this leads to partial or complete loss of catalyst activity.[123,125] The second challenge in ATRP of 4VP is the necessity of chloride-based initiators and catalysts for a successful polymerization. The chloride-based initiator or catalysts avoid side reactions that supress the main reaction. For instance, it was observed that a polymer with a polymodal molecular weight distribution was obtained when a bromide-based initiator or catalyst was used. The bromine-terminated P4VP can react with either the monomer or

the polymer, yielding pyridinium salts and hence producing a branched polymer.[128] This reaction can be supressed when a chloride based initiator was used in the reaction.

Because of these challenges related to the synthesis of PaAAs and P4VP, finding a versatile approach that can overcome these obstacles is needed. In our effort to solve the mentioned problem, we used a novel method to graft acidic and pyridine groups the surface of PU 60D 35 IVR Res. This novel method is a combination of the SI-ARGET-ATRP and the alkyne-azide click reactions. In this approach, first poly(propargyl acrylate) (PPA) brushes were grafted from the surface of PU-OH-BIBB using SI-ARGET-ATRP. PU-PPA Res IVR can be used as a versatile platform where monomers with different functionalities can be easily immobilized on the surface of this platform with an alkyne-aizde reaction for desirable applications.

4.2.3 Synthesis of Azido compounds:

In order to impart pH sensitive groups onto the surface of PU-PPA Res IVR segments, first pH-sensitive monomers were synthesized in their azide format. 2-Azidobutyric acid was synthesized from 2-bromobutyric acid.

The formation of an azide compound was detected by ATR-FTIR and ^{1}H-NMR. Figure 4-8 demonstrates ATR-FTIR spectra of 2-bromobutyric acid and 2-Azidobutyric acid. A strong $N\equiv\equiv N$ asymmetric stretching absorption appears at 2100 cm^{-1} which is related to the azido group.

^{1}H-NMR spectra of 2-bromobutyric acid and 2-azidobutyric acid are demonstrated in Figure 4-9 and Figure 4-10 respectively. It can be observed that the methine group shifted from 4.2 ppm to 3.5 ppm after replacing the bromide group with an azide group in 2-azidobutryic acid. (Figure 4-10)

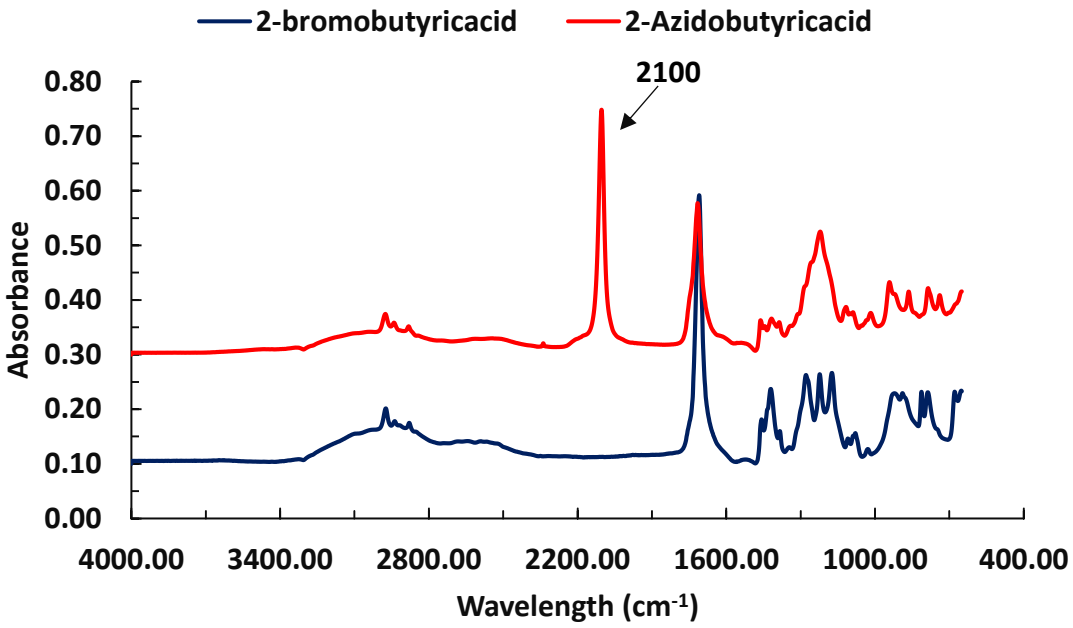


Figure 4-8. ATR-FTIR spectra of 2-bromobutyric acid (blue line) and 2-Azidobutyric acid (red line).

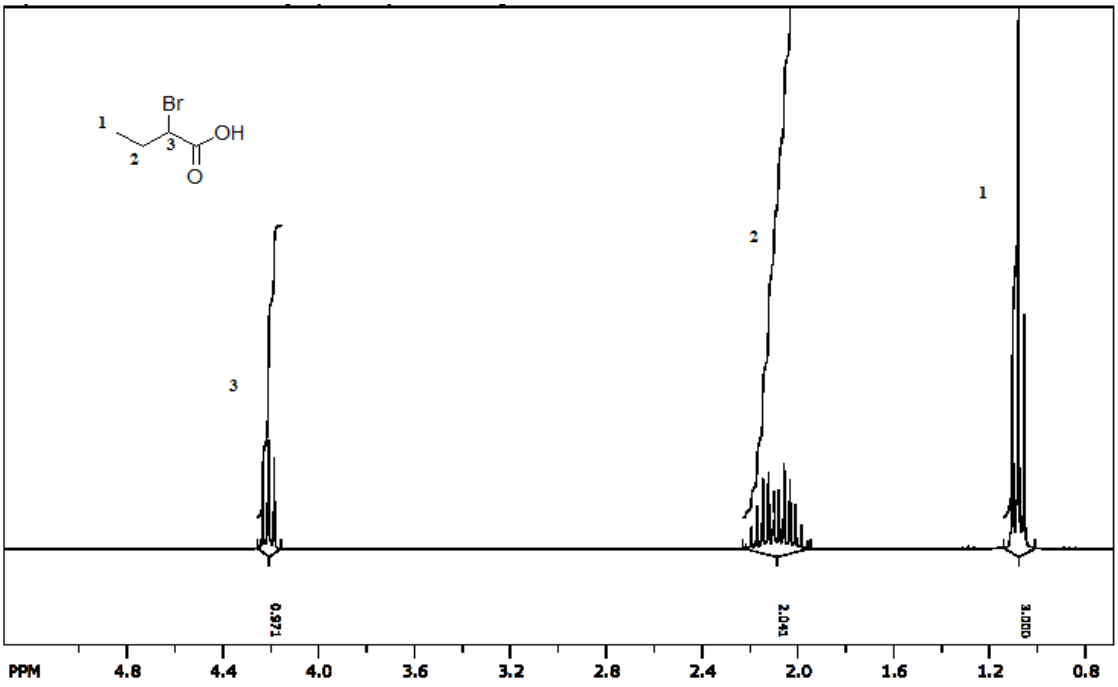


Figure 4-9. [1]HNMR peaks for 2-bromobutyric acid.

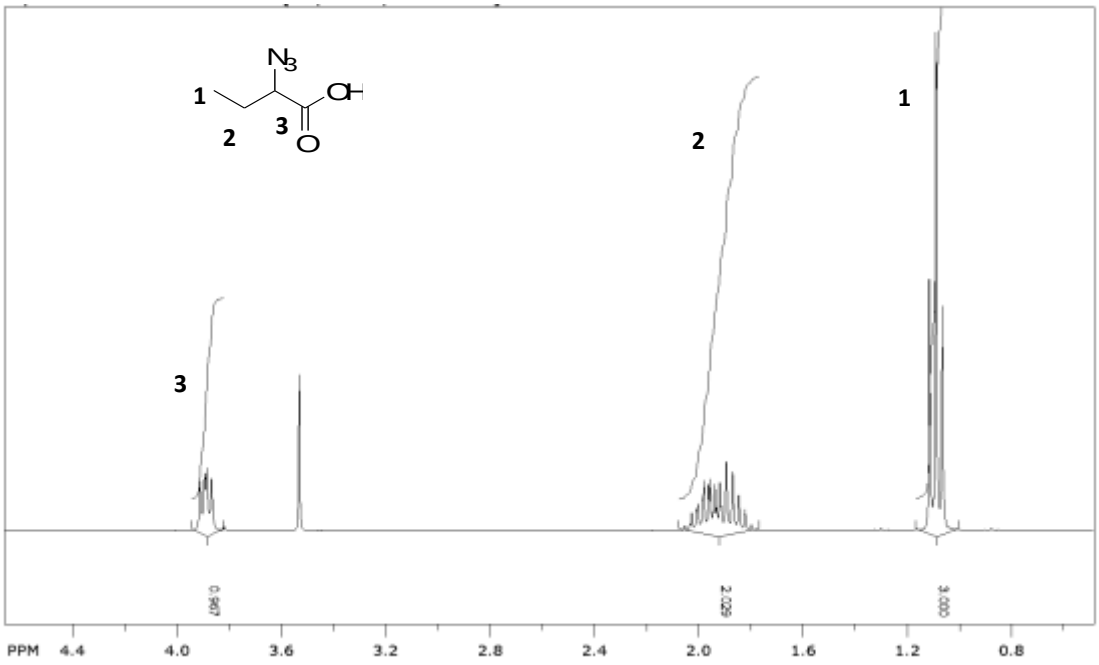


Figure 4-10. [1]HNMR peaks for 2-azidobutyric acid.

Moreover, 4-azidomethylpyridine was synthesized from 4-chloromethylpyridine as mentioned in Chapter3. ATR-FTIR analysis of 4-chloromethylpyridine and 4-azidomethylpyridine are illustrated in Figure 4-11. The strong peak at 2100 cm^{-1} is related to the azide group in the synthesized compound. [1]H-NMR results of 4-chloromethyl pyridine and 4-Azidomethyl pyridine are illustrated in Figure 4-12 and Figure 4-13. After replacing the chlorine group with an azide group, there is a shift from 4.8 to 4.6 of the C-H bond connected to the azide group. (Figure 4-13)

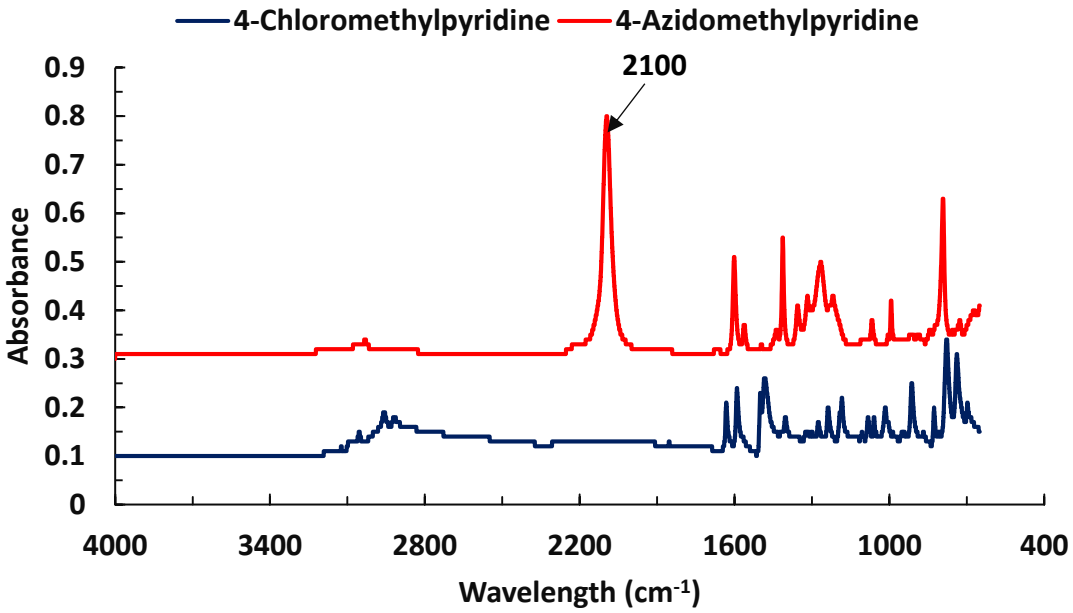


Figure 4-11. ATR-FTIR spectra of (a)4-chloromethylpyridine (red) (b) 4-azidomethylpyridine (blue).

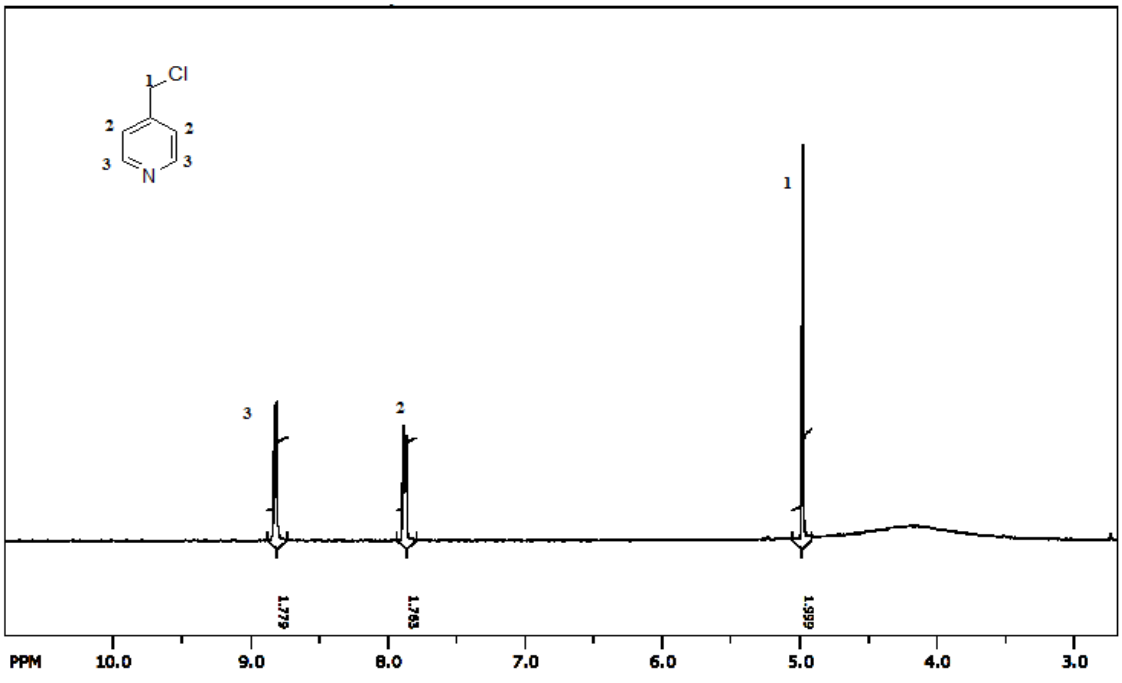


Figure 4-12. ^{1}H-NMR peaks for 4-chloromethylpyridine.

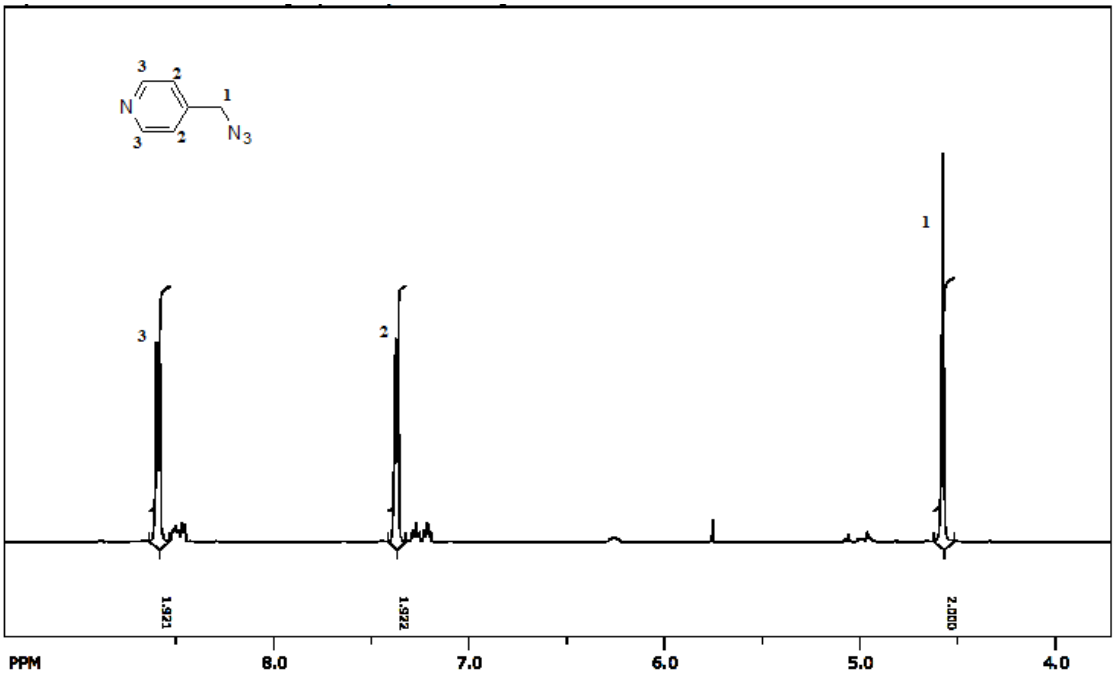


Figure 4-13. ^{1}H-NMR peaks for 4-azidomethyl pyridine.

4.2.4 Surface characterization:

ATR-FTIR and XPS were conducted to analyze the surface of modified samples. The ATR-FTIR spectra of PU-PPA Res IVR before and after alkyne-azide reaction with 2-azidobutyric acid and 4-azidomethylpyridine are demonstrated in Figure 4-14 and Figure 4-15 respectively**Figure 4-13**. A broad N=N stretch can be observed around 1610 cm^{-1} which confirms the formation of a triazole ring from the click reaction.

Grafting density of acidic groups on the surface of PU-PPA Res IVR segments was determined to be 3.05±0.021 μmol/cm^2 using the TBO dye method. As for the PU-PPA-PY, the grafting density of cationic groups on the surface of PU-PPA-PY Res IVR was determined to be

2.02±0.044 μmol/cm^2 using the AO dye method. As it was mentioned in the previous section this amount is high enough for pH responsiveness.[87,116] Table 4-1 summarizes the grafting density of all modified PU 60D 35 Res IVR segments with anionic and cationic groups used in this project.

Table 4-1. Grafting density of anionic and cationic groups on the surface of PU Res IVR segments.

Sample	Grafting density (μmol/cm^2)
PU-OH-PMAA	2.43±0.055
PU-PPA-BAA	3.05±0.021
PU-PPA-P4VP	2.02±0.044

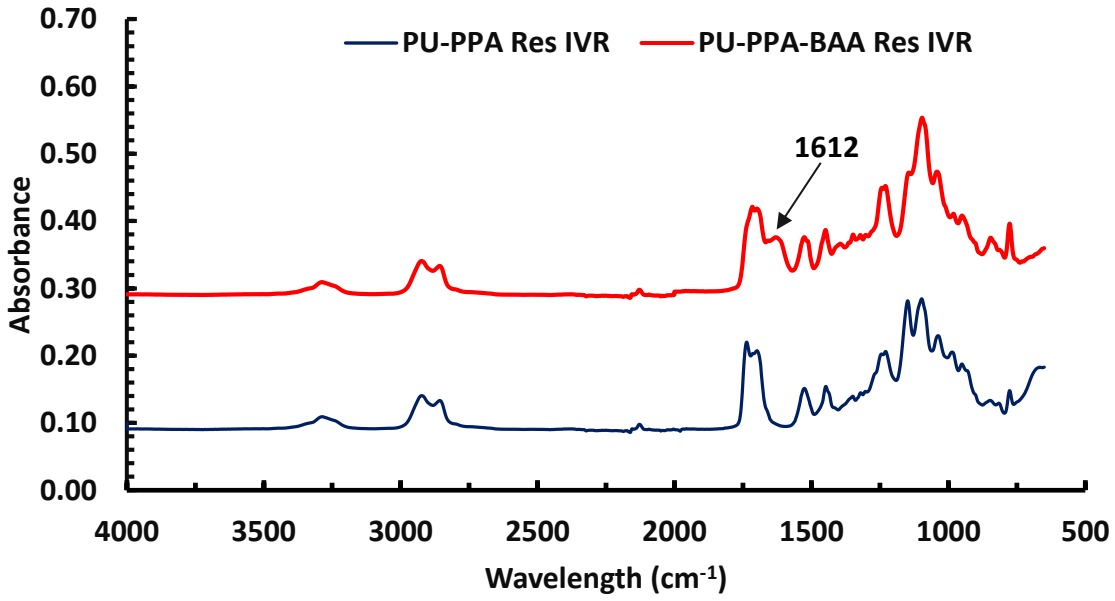


Figure 4-14. ATR-FTIR spectra of (a) PU-PPA Res IVR (blue) and (b) PU-PPA-BAA Res IVR (red) segment.

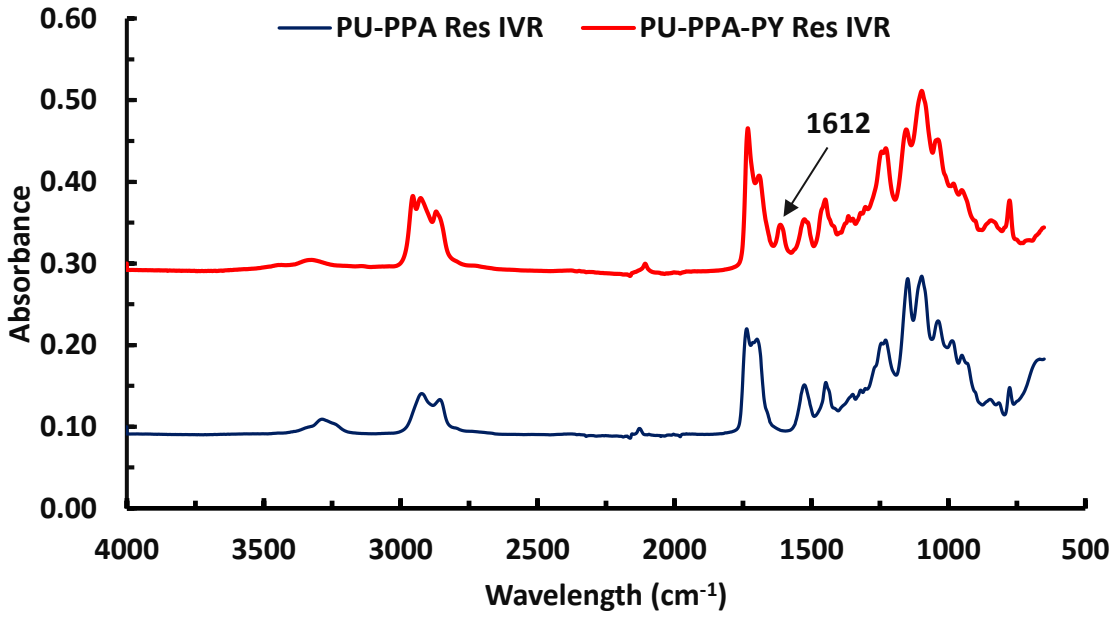


Figure 4-15. ATR-FTIR spectra of (a) PU-PPA Res IVR (red) and (b) PU-PPA-PY (blue) Res IVR segment.

Figure 4-16 shows the C 1s high-resolution spectrum of untreated PU 60D 35 Res IVR and PU Res IVR with pH sensitive functional grroups after the combination of SI-ARGET-ATRP and a click reaction. Three peaks are observed in each spectrum. For untreated PU (

Figure ***4-16***,a), peaks are (A) aliphatic or aromatic hydrocarbon (C-C/C-H) at a binding energy of 285 eV, (B) the carbon linked to an ether group (COC), at the binding energy of 286.5 eV and (C) carbon related to a carbamate group (OCON) at the binding energy of 289 eV. However,

in the spectrum of PU-PPA-BAA and PU-PPA-PY Res IVR, the peak related to the carbon attached to the nitrogen atoms in the triazole ring is observed at 287 eV (Figure 4-16, b, c).

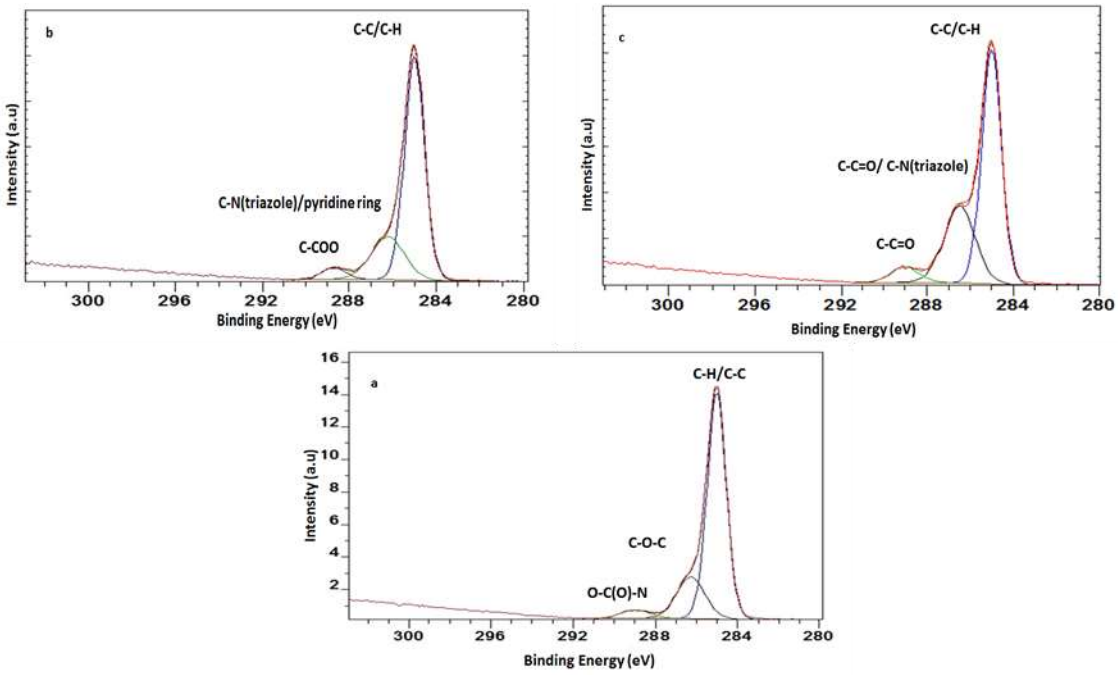


Figure 4-16. XPS C 1s high-resolution spectrum of (a) PU 60D 35 (b) PU-PPA-BAA and (c) PU-PPA-PY Res IVR segments.

Also N1s high-resolusion spectra of ummodified and modified segments are shown in Figure 4-17. It is observed that a new peak at 401 related to N1s of triazole ring appears after click reaction for PU-PPA-PY (b) and PU-PPA-BAA (c).

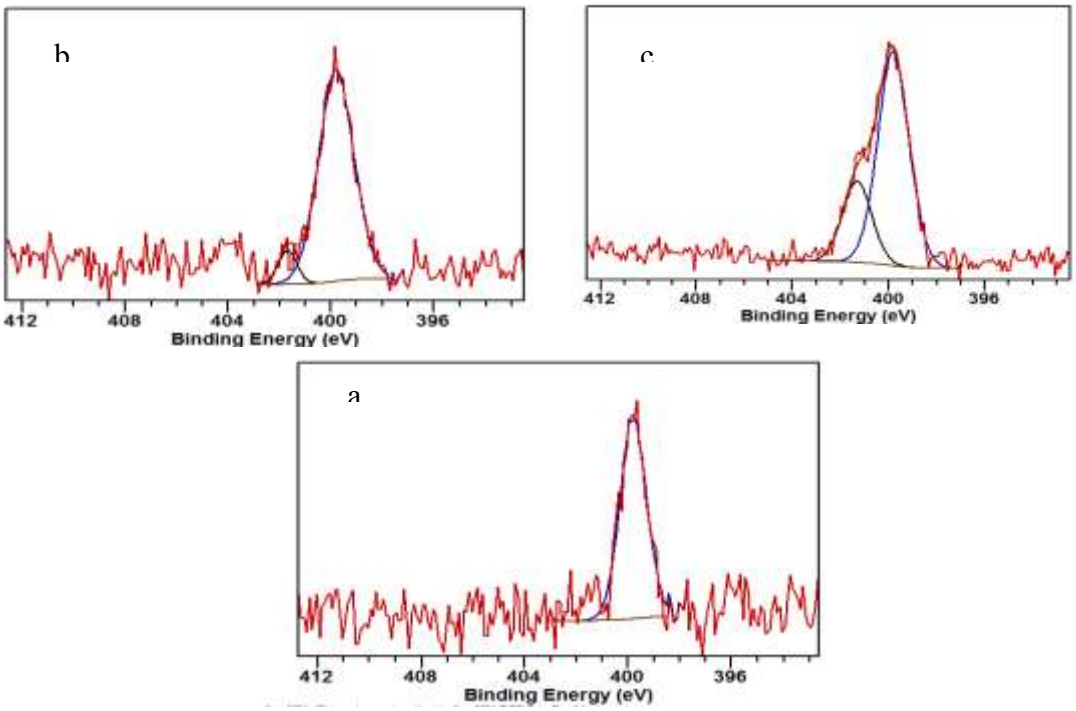


Figure 4-17. XPS N 1s high-resolution spectra of PU 60D 35 (a), PU-PPA-PY (b) and PU-PPA-BAA(c) Res IVR segments.

Surface elemental concentrations of unmodified and modified PU 60D 35 Res IVR segments obtained from the peak-area ratios, are summarized in Table 4-2. The amount of N on the surface of PU Res IVR decreases after PPA grafting due to the fact that PPA does not contain nitrogen. The N seen in PU-PPA is related to the N existing in PU Res IVR. After alkyne-azide click reaction, because of trizole groups, the amount of N increases from 1.27 %w for PU-OH-BIBB IVR Res to 3.14 % w and 2.49 %w for PU-PPA-BAA Res IVR and PU-PPA-PY Res IVR

respectively. These results confirm that click reaction was performed sucessfully on the surface of PU Res IVR.

Table 4-2. XPS-derived surface elemental compositions of untreated PU and modified PU's.

Sample	Atomic concentration (%)			
	C 1s	N 1s	O 1s	Br 3d
PU 60D 35	82.27	1.11	16.62	0
PU-OH-BIBB	81.24	1.27	17.36	0.13
PU-PPA	83.6	0.7	15.7	0
PU-PPA-PBAA	78.06	3.14	18.8	0
PU-PPA-P4VP	81.3	2.49	16.21	0

The topography of the unmodified and modified PU Res IVRsegments using AFM; are illustrated in Figure 4-18. An area of 20×20 μm^2 was scanned using the tapping mode. As for the untreated PU 60D 35 Res IVR, the surface was rather smooth with a root-mean-square surface roughness value (Ra) of about 15.1 nm (Figure 4-18a). However, after immobilization with BIBB and grafing of PPA onto? the surface of the PU Res IVR, the roughness of the surface increased to 30.1 nm for PU-OH-BIBB (Figure 4-18b) and 46.5 nm for PU-PPA (Figure 4-18c). Interestingly, after alkyne-azide click reaction the roughness increased to 341 nm for PU-PPA-PY (Figure 4-18d) and 427 nm for PU-PPA-BAA (Figure 4-18e). This dramatic increase in roughness after click reaction can be explained on the basis that carboxylic acid or pyridine moities can generate significant steric hindrance between polymer chains and prevent the polymer chains from folding.

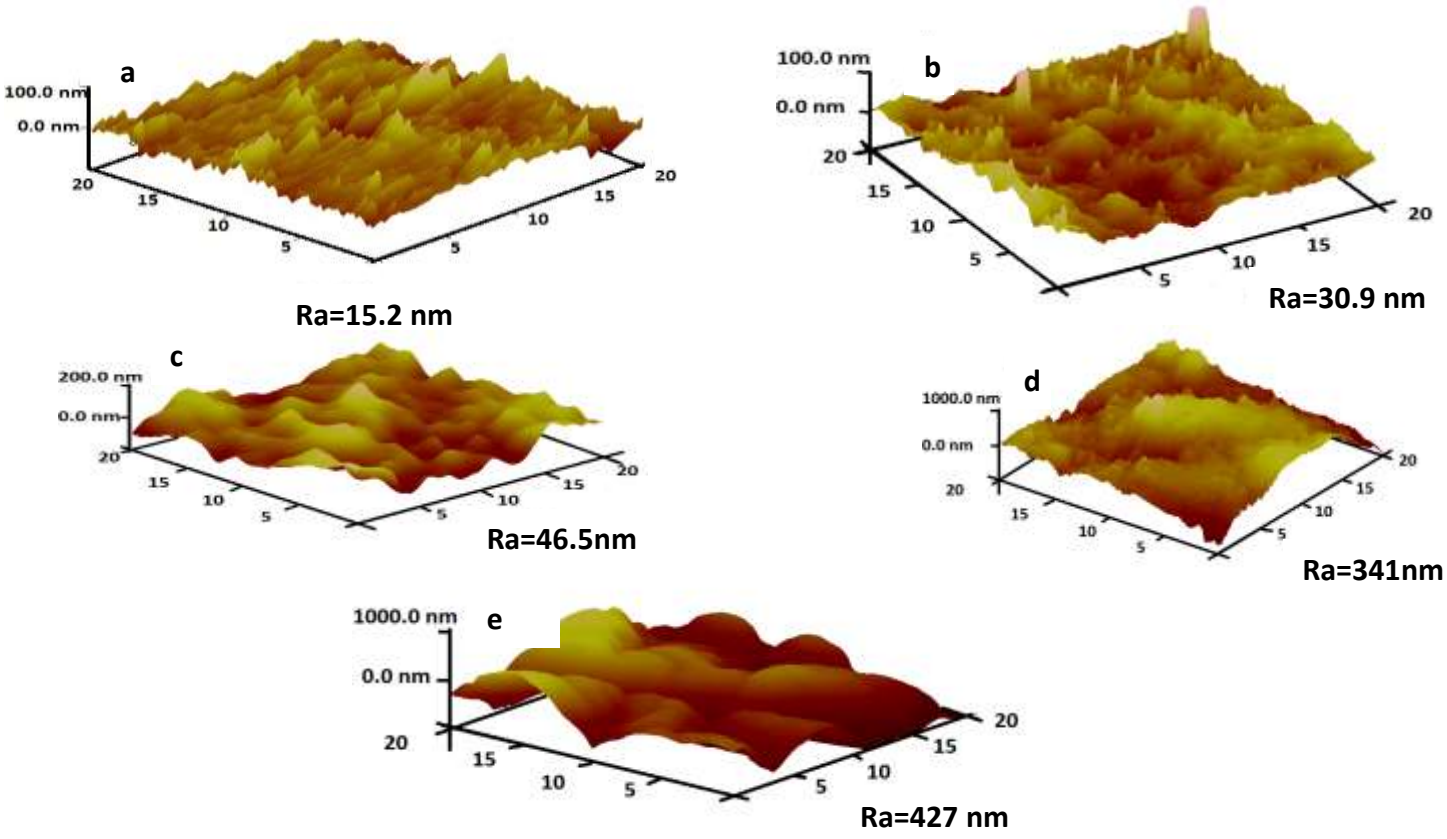


Figure 4-18. AFM images of (a) PU 60D 35, (b) PU-OH-BIBB, (c) PU-PPA, (d) PU-PPA-P4VP and (e) PU-PPA-PBAA IVR Res.

4.2.5 pH responsiveness of modified PU 60D 35 Res IVR with the new method:

Figure 4-19 shows the pH responsiveness of PU-PPA Res IVR segments at pH 4/pH 7 cycle. The average amount of released HCQ from PU-PPA Res IVR segment at pH 4 and pH 7 is 0.90±0.37 and 0.86±0.3µg/ml in 4 hr time intervals at pH 4 and pH 7 ($p>0.05$, $n=3$). According to these results, PU-PPA IVR Res, does not show any pH sensitivities in drug release.

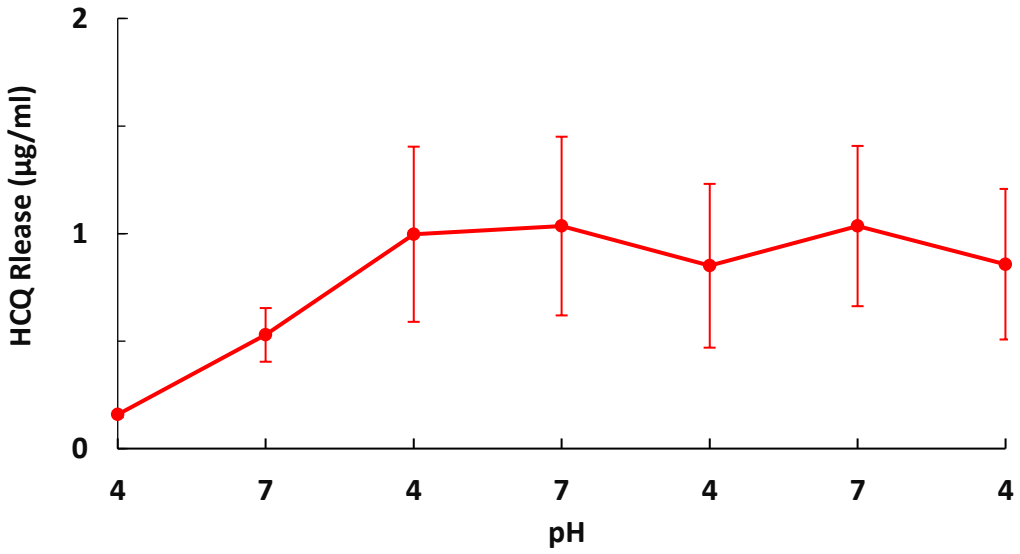


Figure 4-19. Release of HCQ from PU-PPA Res IVR at pH 4/pH 7 cycle at 4 hr time intervals (n=3).

The release profiles of HCQ from PU-PPA-BAA Res IVR and PU-PPA-PY Res IVR in pH 4/7 cycles for 4hr time intervals are shown in Figure 4-20 and Figure 4-21 respectively. As for PU-PPA-BAA Res IVR, the amount of released HCQ at pH 4 is 65.67 ±3.83 µg/ml and it decreases to 40.86±1.31 µg/ml at pH 7. These results reveal that the rate of release was higher at pH 4 than pH 7 when butyric acid groups were clicked onto the surface of PU-PPA IVR Res. In contrast when pyridine groups were clicked onto the surface of PU-PPA Res IVR segments as a cationic

polymer, the rate of release increased from 3.87±1.29 µg/ml at pH 4 to 11.90±0.72 µg/ml at pH 7 (Figure 4-21).

This behavior can be explained by the "through pore" mechanism. According to this mechanism, when, butyric acid groups are grafted inside the pores along the thickness of the pore walls, with an increase in the pH values, most carboxylic acid groups are converted to carboxylate ions. A possible explanation is as follows: Electrostatic repulsion among the carboxylate groups along with their interaction with the aqueous solution, forces the polymer brushes to adopt an extended or "brush-like" conformation. The extended polymer chains in the pores could reduce the effective dimension of the pores. As a result, the release of HCQ thorough PU Res IVR would decline. On the other hand, PPA-BAA chains adopt a "mushroom" conformation under low-pH conditions. As a result, steric obstruction of the pores decreases and the drug release rate increases.[107]

In contrast, when the pyridine groups of the grafted PPA-PY chains are protonated in an acidic solution, the electrostatic repulsion among the positively charged pyridinium nitrogen atoms overcomes the hydrophobic interactions among the alkyl segments of the chains. The extended polymer chains would reduce the effective pore size and thus the release of HCQ from PU-PPA-PY IVR Res. On the other hand, with an increase in pH values, PPA-PY brushes are expected to be in their mushroom conformation. Therefore, release of HCQ would be expected to increase because of an increase in the pores' dimensions.

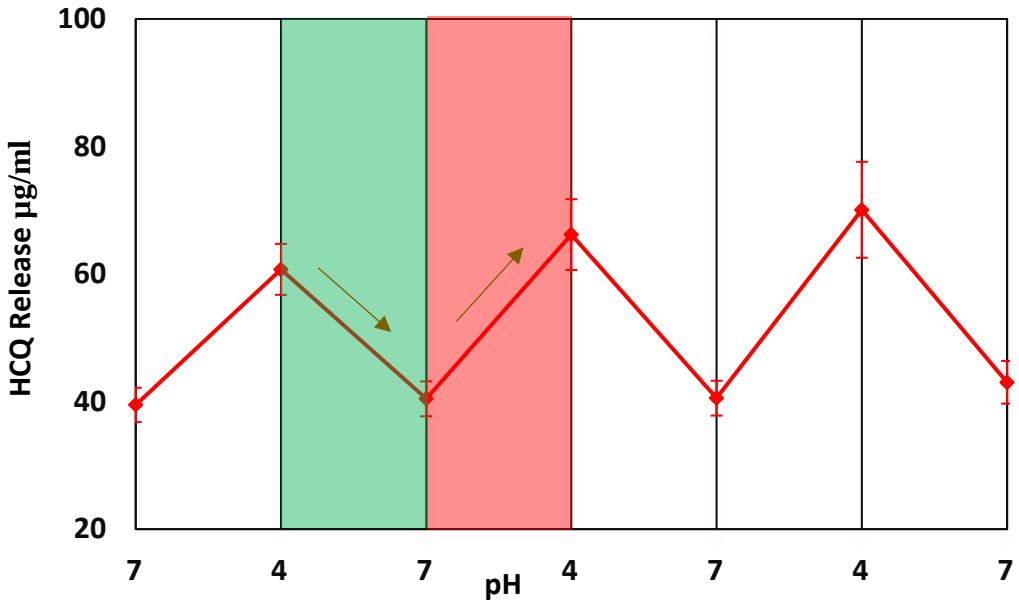


Figure 4-20. Release of HCQ from PU-PPA-BAA Res IVR at pH 4/pH 7 cycle at 4h time intervals (n=3).

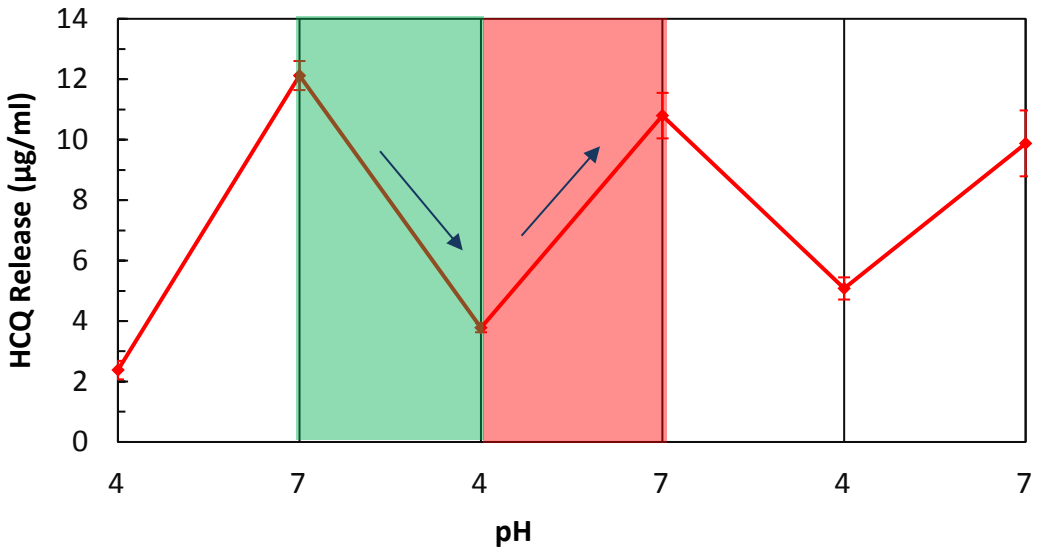


Figure 4-21. Release of HCQ from PU-PPA-PY Res IVR at pH 4/pH 7 cycle at 4hr time intervals (n=3).

4.2.6 Surface morphology of the untreated PU 60D 35 Res IVR and modified PU IVR Res

SEM analysis was performed to study the surface morphology of untreated PU 60D 35 Res IVR and modified PU Res IVR segments. Figure 4-22 shows cross-section views of PU 60D 35 Res IVR and PU-PPA Res IVR swollen at pH 4 and pH 7. The SEM images indicate, crevices on the surface of PU 60D 35 Res IVR with an average length of 207.27±2.47 nm and width of 31.82±0.68 nm among 17 crevices at pH 4 (Figure 4-22, a). The size of crevices did not change significantly at pH 7, with the length of 197.11±2.64 nm and width of 25.10±0.64 nm (Figure 4-22,b) ($p>0.05$ $n=17$). These results demonstrate that PU 60D 35 Res IVR structure is pH-independent. SEM images reveal that when PPA brushes are grafted from the surface of PU Res IVR segments, there is no significant change in surface morphology in comparison to the untreated material (Figure 4-22 c, d). Also changes in pH values do not affect the crevice size on the surface of PU-PPA IVR Res. At pH 4, crevices on the surface of PU-PPA Res IVR have an average length of 209.09±2.10 nm and width of 28.57±0.51 nm. At pH 7, the size of the crevices on the surface of PU-PPA Res IVR remain constant with the length of 194.23±1.94 nm and width of 28.82±0.49 nm ($p>0.05$ $n=17$).

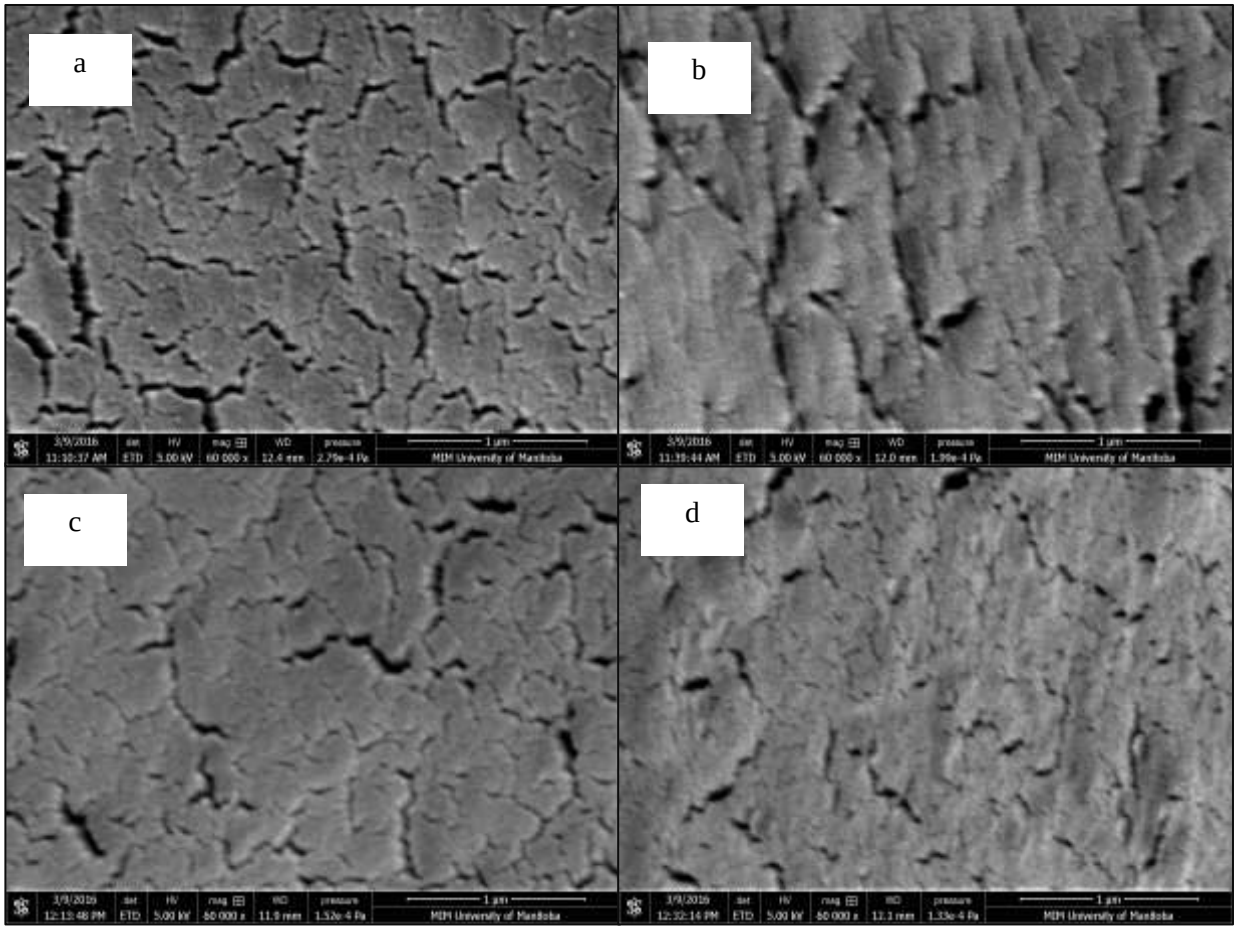


Figure 4-22. SEM of a cross-sectional view of PU Res IVR segments at (a) pH 4, (b) pH 7 and PU-PPA Res IVR segments at (c) pH 4 and (d) pH 7.

SEM images of cross-sectionals view of the PU-PPA-BAA Res IVR and PU-PPA-PY Res IVR segments at different pH values are illustrated in Figure 4-23 and Figure 4-24 respectively. It is observed that the surface morphology of Res IVR segments is significantly altered when BAA and PY groups are added to the PPA chains after click reaction. In the case of PU-PPA-BAA Res IVR segments, pores are covered at pH 7 (Figure 4-23a) due to the fact that negatively charged polymer chains of BAA are extended and cover the pores on the surface of PU IVR Res. However,

with decreasing pH to 4, the size of pores increases to the average size of 576 ± 87 nm of length and 124±51 nm of width (Figure 4-23b) (n=10).

Interestingly, as for PU-PPA-PY Res IVR segments, the size of the pores increases with increasing pH values to pH 7 with an average size of 459 ±24 nm (Figure 4-24a) compared to pH 4 with the average size of 149 ±26 nm (Figure 4-24b) ($p<0.05$, n=10). These results are in agreement with results obtained from release studies and confirm that the dominant mechanism that controls release of drug from polymer brushes grafted from PU Res IVR segments is the "through pore" mechanism.

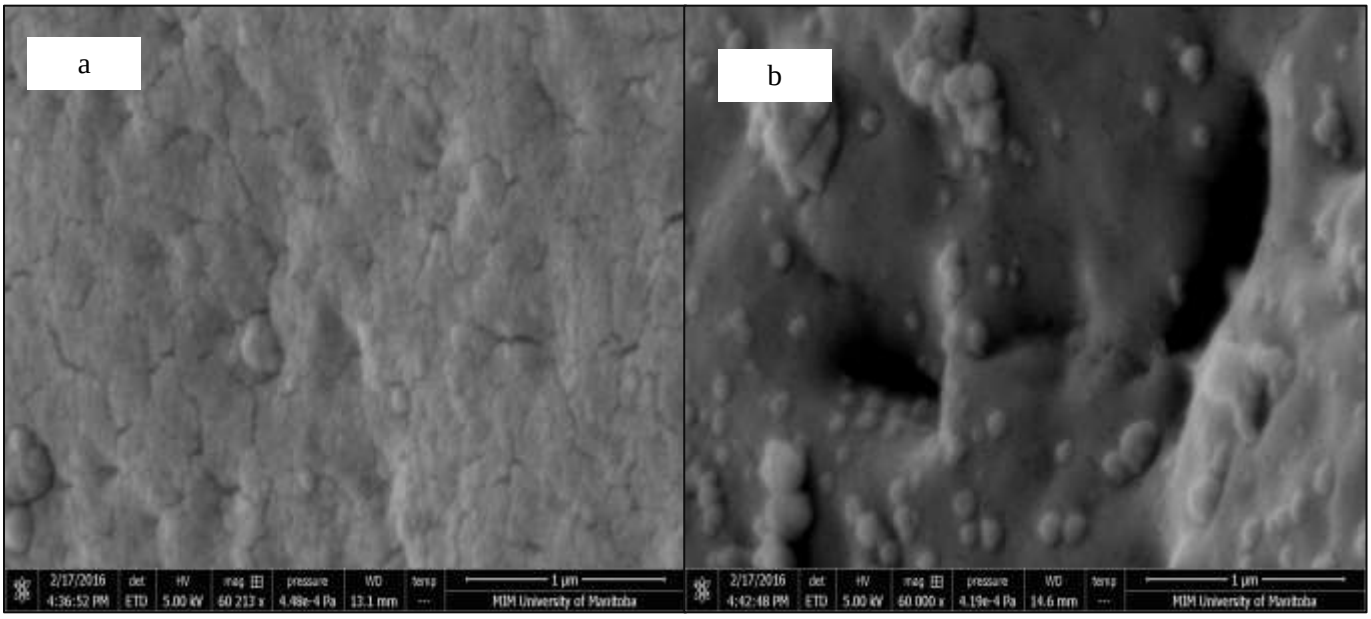


Figure 4-23. SEM of cross-sectional view of the PU-PPA-BAA Res IVR segments at pH 7 (a) and pH 4 (b).

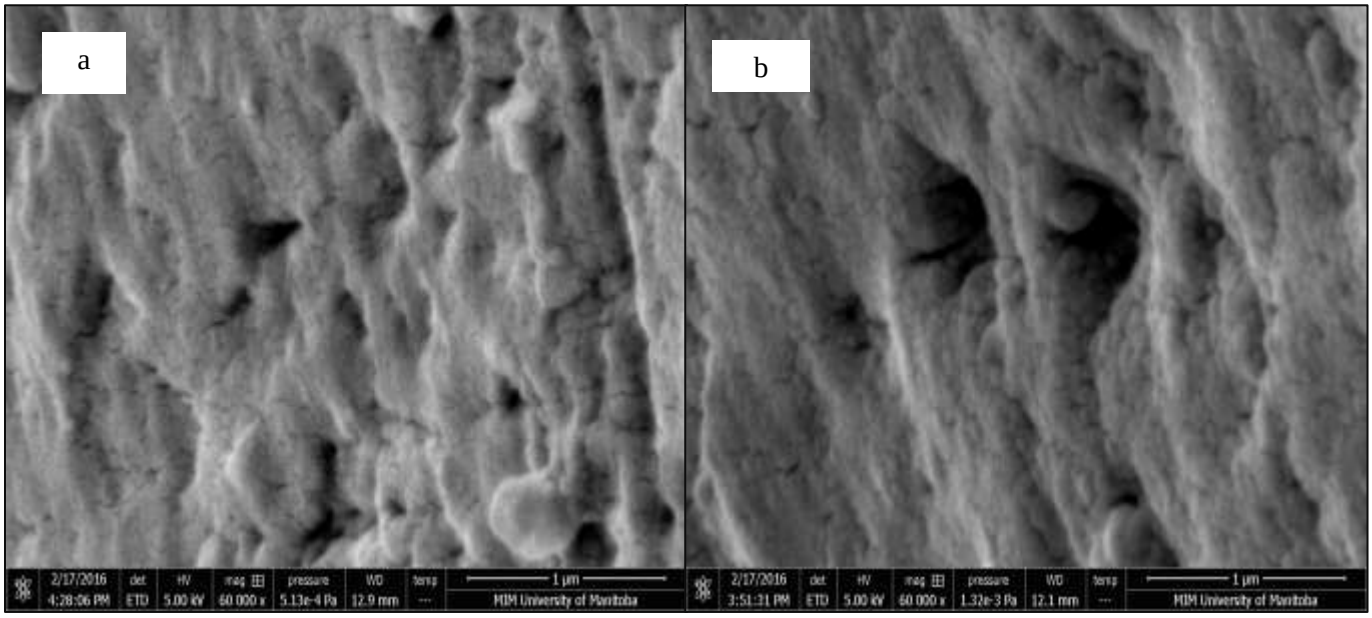


Figure 4-24. SEM of cross-sectional view of the PU-PPA-PY Res IVR segments at pH 4 (a) and pH 7 (b).

4.2.7 Influence of reaction time of PPA on pH responsiveness of PU-PPA-PY Res IVR segments

In the next step, it was aimed to study the effect of polymerization time of PPA on pH responsiveness of PPA-PY polymer brushes grafted from the surface of PU Res IVR segments. Figure 4-25 shows the release of HCQ from PU-PPA-PY Res IVR segments at different reaction times. It is observed that the amount of release at 12 h is lower that ($p<0.05$, $n=3$) than the release at 36h. However, increasing the reaction time from 36 hr to 60 hr did not have a significant effect on pH responsiveness of PPA-PY brushes ($p>0.05$, $n=3$).

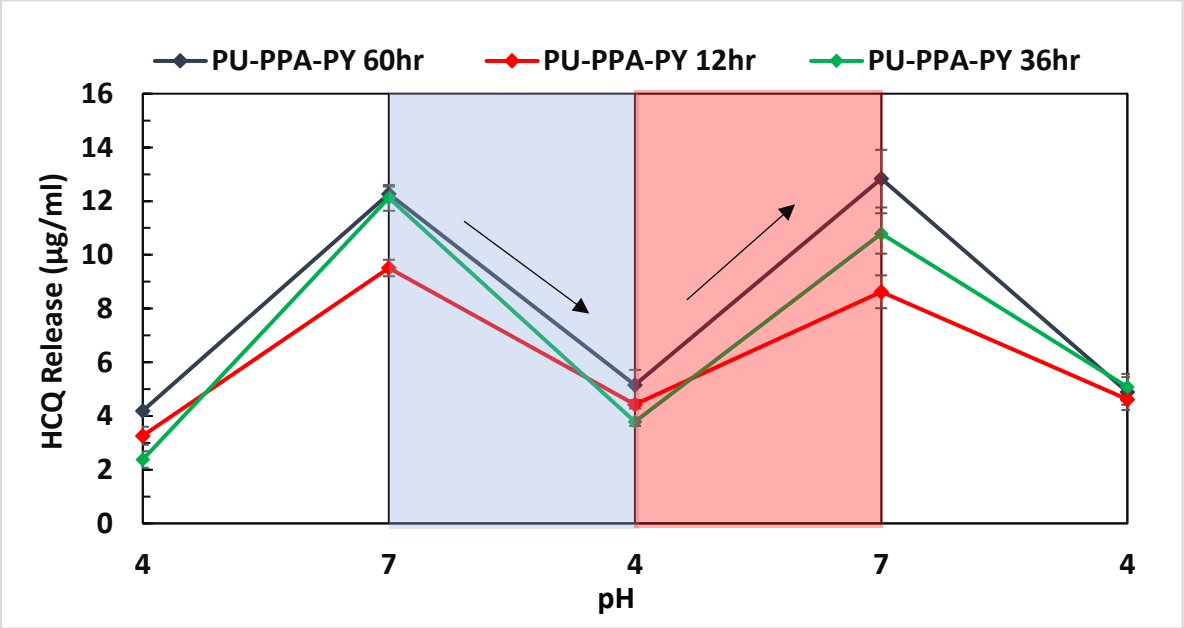


Figure 4-25. HCQ Release from PU-PPA-PY Res IVR segments in pH 4/pH 7 cycles at different reaction times of PPA (red) 12 hr, (dark blue) 36 hr, (green) 60 hr (n=3).

.

Chapter 5
Summary and Conclusion

Chapter 5. Summary and Conclusions

Stimuli-responsive polymer drug delivery systems have drawn much interest in the field of drug delivery systems, since they can reduce the side effects of drugs on tissue toxicity or the emergence of drug resistance in viruses and bacteria in the long term. Grafting of stimuli- responsive polymer brushes from the surfaces of drug delivery systems can be an effective method to functionalize the system with desirable properties for controlled drug release without changing the bulk properties.

Depend on where pH responsive polymer brushes are grafted the mechanism of release can be different. For instance, in case of an anionic polymer such as PMAA, it was observed that the release of dyes from PMAA-g-hollow silica is very low at pH=2.0, but increased dramatically when pH increased to pH=7.4.[64] On the other hand it was observed that the water permission from PAA grafted PP declined when pH increased from 3 to 6 because of changes in conformation of PAA chains.[75] Therefore, the position of polymer brushes can dictate different mechanism of releases. In this project we studied the correlation of pH responsive polymer brushes and the pattern of pH responsive drug release. The main purpose of the present work was to graft pH-sensitive polymer brushes from the surfaces of intravaginal rings (IVR Res) made of polyurethanes (PUs) to achieve a switchable rapid release of the immunomodulatory drug hydroxychloroquine (HCQ), as a potential drug for prevention of HIV at basic pH (pH 7) and zero or close to zero drug release at acidic pH (pH 4).

Release studies showed that PU 60D 35 Res IVR is not pH responsive upon pH changes. 2-bromoisobutyrylbromide (BIBB) was immobilized onto the surface of PU 60D 35 Res IVR through two methods: PDA coating and the formaldehyde activation method. Release studies indicated that, PU-PDA-BIBB Res IVR shows pH responsiveness upon pH change, while PU-OH-

BIBB Res IVR is pH-independent. Therefore, the formaldehyde activation method was applied to immobilize BIBB onto the surface of PU 60D 35 Res IVR segments.

In our attempt, in order to find the suitable polymerization technique with suitable polymer brushes, PMAA (pK_a 6.5) was chosen to be grafted from the surface of PU Res IVR using SI-ARGET-ATRP. Release studies showed that PU-PMAA Res IVR does not show pH responsiveness at different pH values. This can be explained by the fact that the PMAA brushes can be hydrated at all different pH values.

In the next step, a combination of SI-ARGET-ATRP with alkyne-azide click reaction as a versatile technique was applied to graft PPA-BAA and PPA-PY brushes from the surface of PU-OH-BIBB Res IVR segments. First an alkyne polymer such as poly(propargyl acrylate) (PPA) was grafted from the surface of PU-OH-BIBB Res IVR segments. In the next step, pH responsive monomer in the form of an azide compound was reacted with the alkyne polymer brushes to form PU-PDA-BAA and PU-PDA-PY Res IVR segments.

Release studies showed that, when BAA groups are clicked from the surface of PU-PPA Res IVR, more release of HCQ was observed at pH 4 compared to pH 7. On the contrary, when PY groups are clicked onto the surface of PU Res IVR, the opposite behavior is observed. These results suggest that the mechanism that controls drug release from PU Res IVR segments is the “through pore” mechanism. According to this mechanism, when an anionic polymer such as PPA-BAA is grafted from the surface of PU Res IVR, with an increase in the pH, carboxylic acid groups are converted to carboxylate ions. Electro-statistic repulsion among the carboxylate groups along with their interaction with the aqueous solution, forces the polymer brushes to adopt an extended or “brush-like” conformation. The extended polymer chains reduce the effective dimensions of the pores. As a result, the release of HCQ thorough PU-PDA-BAA Res IVR declines. On the other

hand, under the low-pH conditions, PPA-BAA brushes adopt a "mushroom-like" conformation. As a result, steric obstruction to the pores of the PU Res IVR is reduced and release increases. The release of HCQ from PU-PPA-PY at different pH values can also be explained using this mechanism. Therefore, according to the results obtained from the release studies at different pH values and SEM images, PPA-PY as a cationic polymer is the suitable polymer in order to have a switchable rapid release of HCQ at pH 7 and zero or less release at pH 4.

In summary, a combination of SI-ARGET-ATRP with alkyne-azide click reaction was performed to graft pH responsive polymer brushes onto the surfaces of PU Res IVR segments to achieve a rapid switchable release at a specific pH value. Indeed, this method is a versatile approach in which different polymers with different functionalities can be easily grafted onto? the surface of drug delivery systems and used for different applications.

A suggestion for extending this research is to try other surface priming methods in order to increase the grafting density of polymer brushes to achieve the therapeutic concentration of HCQ. Also it is recommended to graft pH-sensitive polymer brushes onto the surface of a PU matrix IVR and study the release of an anti-HIV drug from a pH sensitive matrix. Furthermore, the release behavior of an anti-HIV drug such as Tenofovir can be studied from the developed Res IVR systems.

Bibliography

(1) Rohan, L. C.; Sassi, A. B. *AAPS J.* **2009**, *11* (1), 78–87.

(2) Thurman, A. R.; Clark, M. R.; Hurlburt, J. A.; Doncel, G. F. *Int. J. Womens. Health* **2013**, *5*, 695–708.

(3) Sheet, F. *UNAIDS 2014 Factsheet*; 2015.

(4) Yang, S.; Chen, Y.; Kaien, G.; Dash, A.; Sayre, C. L.; Davies, N. M.; Ho, E. a. *Int. J. Nanomedicine* **2013**, *8*, 2847–2858.

(5) Ho, E. A. *J. Mol. Pharm. Org. Process Res.* **2013**, *1* (2), 1–2.

(6) Lack of Effectiveness of Cellulose Sulfate Gel for the Prevention of Vaginal HIV Transmission — NEJM http://www.nejm.org/doi/full/10.1056/NEJMoa0707957 (accessed Feb 27, 2015).

(7) Chen, Y.; Traore, Y. L.; Li, A.; Fowke, K. R.; Ho, E. A. *Drug Des. Dev. Ther.* **2014**, *8*, 1801–1815.

(8) Traore, Y. L.; Chen, Y.; Bernier, A.-M.; Ho, E. A. *Antimicrob. Agents Chemother.* **2015**, *59* (12), AAC.01819–15.

(9) UNAIDS. *Women living with HIV speak out against violence*; 2014.

(10) Hussain, A.; Ahsan, F. *J. Control. Release* **2005**, *103* (2), 301–313.

(11) Haugen, T. B.; Grotmol, T. *Int. J. Androl.* **1998**, *21* (2), 105–108.

(12) Stein, Z. a. *Am. J. Public Health* **1990**, *80* (4), 460–462.

(13) Ndesendo, V. M. K.; Pillay, V.; Choonara, Y. E.; Buchmann, E.; Bayever, D. N.; Meyer, L. C. R. *AAPS PharmSciTech* **2008**, *9* (2), 505–520.

(14) De Clercq, E. *Expert Opin. Emerg. Drugs* **2005**, *10* (2), 241–274.

(15) Canada, P. H. A. of. *Hiv Transmission Risk : a Summary of the Evidence*; 2012.

(16) Gupta, S. K. *Indian J. Med. Res.* **2011**, *134* (6), 939–949.

(17) Klasse, P. J.; Shattock, R.; Moore, J. P. *Annu. Rev. Med.* **2008**, *59*, 455–471.

(18) Hladik, F.; Doncel, G. F. *Antiviral Res.* **2010**, *88*, 3–9.

(19) Mehellou, Y.; De Clercq, E. *J. Med. Chem.* **2010**, *53* (2), 521–538.

(20) Debyser, Z.; Pauwels, R.; Andries, K.; Desmyter, J.; Kukla, M.; Janssen, P. A.; De Clercq, E. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88* (February), 1451–1455.

(21) Basavapathruni, A.; Anderson, K. S. *FASEB J.* **2007**, *21* (14), 3795–3808.

(22) Deeks, S. G.; Smith, M.; Holodniy, M.; Kahn, J. O. *Jama* **1997**, *277* (2), 145–153.

(23) De Clercq, E. *Int. J. Antimicrob. Agents* **2009**, *33* (4), 307–320.

(24) Briz, V. *J. Antimicrob. Chemother.* **2006**, *57* (4), 619–627.

(25) Reshef, R.; Luger, S. M.; Hexner, E. O.; Loren, A. W.; Frey, N. V; Nasta, S. D.; Goldstein, S. C.; Stadtmauer, E. A.; Smith, J.; Bailey, S.; Mick, R.; Heitjan, D. F.; Emerson, S. G.; Hoxie, J. A.; Vonderheide, R. H.; Porter, D. L. *N. Engl. J. Med.* **2012**, *367* (2), 135–145.

(26) Warhurst, D. C.; Steele, J. C. P.; Adagu, I. S.; Craig, J. C.; Cullander, C. *J. Antimicrob. Chemother.* **2003**, *52* (July), 188–193.

(27) Brouwers, J.; Vermeire, K.; Schols, D.; Augustijns, P. *Virology* **2008**, *378* (2), 306–310.

(28) Chiang, G.; Sassaroli, M.; Louie, M.; Chen, H.; Stecher, V. J.; Sperber, K. *Clin. Ther.* **1996**, *18* (6), 1080–1092.

(29) Browning, D. J. **2014**.

(30) Graham, G. G. *Antirheum. Ther. Actions Outcomes* **2005**, 13–36.

(31) Lewis, L. D. *Br. J. Clin. Pharmacol.* **2004**, *58* (3), 336–336.

(32) Coelho, J. F.; Ferreira, P. C.; Alves, P.; Cordeiro, R.; Fonseca, A. C.; Góis, J. R.; Gil, M. H. *EPMA J.* **2010**, *1* (1), 164–209.

(33) Hafner, A.; Lovrić, J. **2014**, 1005–1023.

(34) Promadej-Lanier, N.; Smith, J. M.; Srinivasan, P.; McCoy, C. F.; Butera, S.; Woolfson, a. D.; Malcolm, R. K.; Otten, R. a. *J. Med. Primatol.* **2009**, *38*, 263–271.

(35) Dobaria, N. *East Cent. African J. Pharm. Sci.* **2007**, *10*, 3–13.

(36) Acartürk, F. *Recent Pat. Drug Deliv. Formul.* **2009**, *3* (NOVEMBER 2009), 193–205.

(37) Woolfson, a. D.; Malcolm, R. K.; Morrow, R. J.; Toner, C. F.; McCullagh, S. D. *Int. J.*

Pharm. **2006**, *325*, 82–89.

(38) Dhondt, M. M. M.; Adriaens, E.; Remon, J.-P. *Sex. Transm. Dis.* **2004**, *31* (4), 229–235.

(39) Baloglu, E. etl al. **2009**, *12* (3), 312–336.

(40) El-Kamel, A.; Sokar, M.; Naggar, V.; Al Gamal, S. *AAPS PharmSci* **2002**, *4* (4), E44.

(41) Malcolm, R. K.; Woolfson, A. D.; Toner, C. F.; Morrow, R. J.; McCullagh, S. D. *J. Antimicrob. Chemother.* **2005**, *56* (5), 954–956.

(42) Malcolm, R. K.; Fetherston, S. M.; McCoy, C. F.; Boyd, P.; Major, I. *Int. J. Womens. Health* **2012**, *4*, 595–605.

(43) Kiser, P. F.; Johnson, T. J.; Clark, J. T. *AIDS Rev.* **2012**, *14*, 62–77.

(44) Baum, M. M.; Butkyavichene, I.; Gilman, J.; Kennedy, S.; Kopin, E.; Malone, A. M.; Nguyen, C.; Smith, T. J.; Friend, D. R.; Clark, M. R.; Moss, J. A. *J. Pharm. Sci.* **2012**, *101* (8), 2833–2843.

(45) Taylor, M. J.; Tanna, S.; Sahota, T. *J. Pharm. Sci.* **2010**, *99* (10), 4215–4227.

(46) Gupta, K. M.; Pearce, S. M.; Poursaid, A. E.; Aliyar, H. A.; Tresco, P. A.; Mitchnik, M. A.; Kiser, P. F. *J. Pharm. Sci.* **2008**, *97* (10), 4228–4239.

(47) Thurman, A. R.; Clark, M. R.; Hurlburt, J. A.; Doncel, G. F. *Int. J. Womens. Health* **2013**, *5* (1), 695–708.

(48) Malcolm, R. K.; Edwards, K. L.; Kiser, P.; Romano, J.; Smith, T. J. *Antiviral Res.* **2010**, *88* (SUPPL.), 30–39.

(49) Taylor, M. J.; Tanna, S.; Sahota, T. *J. Pharm. Sci.* **2010**, *99* (10), 4215–4227.

(50) Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. *Chem. Rev.* **1999**, *99* (11), 3181–3198.

(51) Lee, K. Y.; Peters, M. C.; Mooney, D. J. *Adv. Mater.* **2001**, *13* (11), 837–839.

(52) Schmaljohann, D. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1655–1670.

(53) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29* (12), 1173–1222.

(54) Wandera, D.; Wickramasinghe, S. R.; Husson, S. M. *J. Memb. Sci.* **2010**, *357* (1-2), 6–35.

(55) Zhao, B.; Brittain, W. J. **2000**, *25* (February), 677–710.

(56) Currie, E. P. K.; Sieval, a. B.; Fleer, G. J.; Stuart, M. a C. *Langmuir* **2000**, *16*, 8324–8333.

(57) Qiu, J.-H.; Zhang, Y.-W.; Zhang, Y.-T.; Zhang, H.-Q.; Liu, J.-D. *J. Colloid Interface Sci.* **2011**, *354* (1), 152–159.

(58) Adiga, S. P.; Brenner, D. W. *J. Funct. Biomater.* **2012**, *3* (2), 239–256.

(59) Laurent, P.; Souharce, G.; Duchet-Rumeau, J.; Portinha, D.; Charlot, A. *Soft Matter* **2012**, *8* (3), 715–725.

(60) Qiu, Y.; Park, K. *Adv. Drug Deliv. Rev.* **2012**, *64*, 49–60.

(61) Shen, Y.; Li, G.; Ma, Y.; Yu, D.; Sun, J.; Li, Z. *Soft Matter* **2015**, *11* (38), 7502–7506.

(62) Kumar, S.; Dory, Y. L.; Lepage, M.; Zhao, Y. **2011**, 7385–7393.

(63) Bajpai, a. K.; Shukla, S. K.; Bhanu, S.; Kankane, S. *Prog. Polym. Sci.* **2008**, *33* (11), 1088–1118.

(64) Lay, C. L.; Tan, H. R.; Lu, X.; Liu, Y. *Chem. - A Eur. J.* **2011**, *17*, 2504–2509.

(65) Gao, Q.; Xu, Y.; Wu, D.; Sun, Y.; Li, X. *J. Phys. Chem. C* **2009**, *113* (29), 12753–12758.

(66) Hong, C.-Y.; Li, X.; Pan, C.-Y. *J. Mater. Chem.* **2009**, *19*, 5155.

(67) Chiu, H. C.; Lin, Y. W.; Huang, Y. F.; Chuang, C. K.; Chern, C. S. *Angew. Chemie - Int. Ed.* **2008**, *47*, 1875–1878.

(68) Pour, S. N.; Ghugare, S. V.; Wiens, R.; Gough, K.; Liu, S. *Appl. Surf. Sci.* **2015**, *349*, 695–704.

(69) Ito, Y.; Park, Y. S.; Imanishi, Y. *Langmuir* **2000**, *16* (12), 5376–5381.

(70) Ito, Y.; Ochiai, Y.; Park, Y. S.; Imanishi, Y. *J. Am. Chem. Soc.* **1997**, *119* (21), 1619–1623.

(71) Ito, Y.; Park, Y. S.; Imanishi, Y. *J. Am. Chem. Soc.* **1997**, *119* (96), 2739–2740.

(72) Israeis, R.; Gersappe, D.; Fasolka, M.; Roberts, V. a; Balazs, A. C. *Macromolecules* **1994**, *27*, 6679–6682.

(73) Ferro, L.; Scialdone, O.; Galia, A. *J. Supercrit. Fluids* **2012**, *66*, 241–250.

(74) Park, Y. S.; Ito, Y.; Imanishi, Y. *Chem. Mater.* **1997**, *9* (14), 2755.

(75) Wang, Y.; Liu, Z.; Han, B.; Dong, Z.; Wang, J.; Sun, D.; Huang, Y.; Chen, G. *Polymer (Guildf).* **2004**, *45* (3), 855–860.

(76) Hansson, S.; Trouillet, V.; Tischer, T.; Goldmann, A. S.; Carlmark, A.; Barner-Kowollik, C.; Malmström, E. *Biomacromolecules* **2013**, *14*, 64–74.

(77) Minko, S. *Polym. Rev.* **2006**, *46* (May), 397–420.

(78) Weir, M. P.; Parnell, A. J. *Polymers (Basel).* **2011**, *3*, 2107–2132.

(79) Ballauff, M.; Borisov, O. *Curr. Opin. Colloid Interface Sci.* **2006**, *11* (6), 316–323.

(80) Barbey, R.; Lavanant, L.; Paripovic, D.; Schüwer, N.; Sugnaux, C.; Tugulu, S.; Klok, H.-A. *Chem. Rev.* **2009**, *109* (11), 5437–5527.

(81) Edmondson, S.; Osborne, V. L.; Huck, W. T. S. *Chem. Soc. Rev.* **2004**, *33* (1), 14–22.

(82) Lee, H.; Pietrasik, J.; Sheiko, S. S.; Matyjaszewski, K. **2010**, *35*, 24–44.

(83) Chan, N.; Cunningham, M. F.; Hutchinson, R. a. *Macromol. Chem. Phys.* **2008**, *209* (1), 1797–1805.

(84) Xu, F. J.; Zhao, J. P.; Kang, E. T.; Neoh, K. G.; Li, J. *Langmuir* **2007**, *23* (16), 8585–8592.

(85) Zheng, Y. Q.; Deng, S.; Niu, L.; Xu, F. J.; Chai, M. Y.; Yu, G. *J. Hazard. Mater.* **2011**, *192* (3), 1401–1408.

(86) Cui, L.; Meng, Q.; Zheng, J.; Wei, X.; Ye, Z. *Vacuum* **2013**, *89* (1), 1–6.

(87) Huang, L.; Yuan, S.; Lv, L.; Tan, G.; Liang, B.; Pehkonen, S. O. *J. Colloid Interface Sci.* **2013**, *405*, 171–182.

(88) Matyjaszewski, K.; Dong, H.; Jakubowski, W.; Pietrasik, J.; Kusumo, A. *Langmuir* **2007**, *23* (8), 4528–4531.

(89) Simakova, A.; Averick, S. E.; Konkolewicz, D.; Matyjaszewski, K. *Macromolecules* **2012**, *45* (16), 6371–6379.

(90) Zhu, B.; Edmondson, S. *Polymer (Guildf).* **2011**, *52* (10), 2141–2149.

(91) Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2007**, *28* (1), 15–54.

(92) Kali, G.; Georgiou, T. K.; Iván, B.; Patrickios, C. S. *J. Polym. Sci. Part a-Polymer Chem.*

2009, *47*, 4289–4301.

(93) Golas, P. L.; Matyjaszewski, K. *QSAR Comb. Sci.* **2007**, *26* (11), 1116–1134.

(94) Vogt, A. P.; Sumerlin, B. S. *Macromolecules* **2006**, *39* (16), 5286–5292.

(95) Tsarevsky, N. V.; Bernaerts, K. V.; Dufour, B.; Du Prez, F. E.; Matyjaszewski, K. *Macromolecules* **2004**, *37* (25), 9308–9313.

(96) Gao, H.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2007**, *129* (20), 6633–6639.

(97) Zhu, Y.; Shi, J.; Shen, W.; Dong, X.; Feng, J.; Ruan, M.; Li, Y. *Angew. Chemie - Int. Ed.* **2005**, *44* (32), 5083–5087.

(98) Cherng, J. Y.; Hou, T. Y.; Shih, M. F.; Talsma, H.; Hennink, W. E. *Int. J. Pharm.* **2013**, *450* (1-2), 145–162.

(99) McBane, J. E.; Sharifpoor, S.; Cai, K.; Labow, R. S.; Santerre, J. P. *Biomaterials* **2011**, *32* (26), 6034–6044.

(100) M, R. E.; Zhao, Q.; Anderson, J. M.; Hiltner, A. *Polymer (Guildf).* **1987**, *28*, 2032–2039.

(101) Huynh, T. T. N.; Padois, K.; Sonvico, F.; Rossi, A.; Zani, F.; Pirot, F.; Doury, J.; Falson, F. *Eur. J. Pharm. Biopharm.* **2010**, *74* (2), 255–264.

(102) Kohjiya, S.; Ikeda, Y.; Takesako, S.; Yamashita, S. *React. Polym.* **1991**, *15* (C), 165–175.

(103) Dutta, S.; Li, Y. L.; Rock, W.; Houtman, J. C. D.; Kohen, A.; Cheatum, C. M. *J. Phys. Chem. B* **2012**, *116* (1), 542–548.

(104) Kaur, S.; Ma, Z.; Gopal, R.; Singh, G. *Langmuir* **2007**, No. 12, 13085–13092.

(105) Hamerli, P.; Weigel, T.; Groth, T.; Paul, D. *Biomaterials* **2003**, *24* (22), 3989–3999.

(106) Yang, Z.; Wang, J.; Luo, R.; Maitz, M. F.; Jing, F.; Sun, H.; Huang, N. *Biomaterials* **2010**, *31* (8), 2072–2083.

(107) Wang, W. C.; Vora, R. H.; Kang, E. T.; Neoh, K. G.; Liaw, D. J. *Ind. Eng. Chem. Res.* **2003**, *42* (4), 784–794.

(108) Zhu, B.; Edmondson, S. *Polymer (Guildf).* **2011**, *52* (10), 2141–2149.

(109) Schüwer, N.; Klok, H. A. *Langmuir* **2011**, *27*, 4789–4796.

(110) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921–2990.

(111) Tugulu, S.; Barbey, R.; Harms, M.; Fricke, M.; Volkmer, D.; Rossi, A.; Klok, H. A. *Macromolecules* **2007**, *40*, 168–177.

(112) Ashford, E. J.; Naldi, V.; O'Dell, R.; Billingham, N. C.; Armes, S. P. *Chem. Commun.* **1999**, No. 14, 1285–1286.

(113) Lynge, M. E.; van der Westen, R.; Postma, A.; Städler, B. *Nanoscale* **2011**, *3* (12), 4916.

(114) Jiang, H.; Wang, X. B.; Li, C. Y.; Li, J. S.; Xu, F. J.; Mao, C.; Yang, W. T.; Shen, J. *Langmuir* **2011**, *27* (18), 11575–11581.

(115) Xu, F. J.; Yang, X. C.; Li, C. Y.; Yang, W. T. *Macromolecules* **2011**, *44* (7), 2371–2377.

(116) Lee, Y. M. O. O.; Shim, J. I. N. K. I. E. *J. Appl. Polym. Sci.* **1996**, *61*, 1245–1250.

(117) Lu, Y.; Sarshar, M. A.; Du, K.; Chou, T.; Choi, C. H.; Sukhishvili, S. a. *ACS Appl. Mater. Interfaces* **2013**, *5* (23), 12617–12623.

(118) Lu, Y.; Wu, Y.; Liang, J.; Libera, M. R.; Sukhishvili, S. a. *Biomaterials* **2015**, *45*, 64–71.

(119) Peljhan, S.; Zagar, E.; Cerkovnik, J.; Kogej, K. *J. Phys. Chem. B* **2009**, *113* (8), 2300–2309.

(120) Pichota, A.; Duraiswamy, J.; Yin, Z.; Keller, T. H.; Alam, J.; Liung, S.; Lee, G.; Ding, M.; Wang, G.; Chan, W. L.; Schreiber, M.; Ma, I.; Beer, D.; Ngew, X.; Mukherjee, K.; Nanjundappa, M.; Teo, J. W. P.; Thayalan, P.; Yap, A.; Dick, T.; Meng, W.; Xu, M.; Koehn, J.; Pan, S.-H.; Clark, K.; Xie, X.; Shoen, C.; Cynamon, M. *Bioorg. Med. Chem. Lett.* **2008**, *18* (24), 6568–6572.

(121) Huntington, K. M.; Yi, T.; Wei, Y.; Pei, D. *Biochemistry* **2000**, *39* (15), 4543–4551.

(122) Yameen, B.; Ali, M.; Neumann, R.; Ensinger, W.; Knoll, W.; Azzaroni, O. *Nano Lett.* **2009**, *9* (7), 2788–2793.

(123) Xia, J.; Zhang, X.; Matyjaszewski, K. *Macromolecules* **1999**, *32*, 3531–3533.

(124) Davis, K. a.; Matyjaszewski, K. *Macromolecules* **2001**, *34* (7), 2101–2107.

(125) Vidts, K. R. M.; Du Prez, F. E. *Eur. Polym. J.* **2006**, *42*, 43–50.

(126) Yameen, B.; Ali, M.; Neumann, R.; Ensinger, W.; Knoll, W.; Azzaroni, O. *Nano Lett.* **2009**, *9* (7), 2788–2793.

(127) Jiang, J.; Zhang, Y.; Cao, D.; Jiang, P. *Chem. Eng. J.* **2013**, *215-216*, 222–226.

(128) Tsarevsky, N. V; Matyjaszewski, K. *Chem. Rev.* **2007**, *107* (6), 2270–2299.