Control of drug release rate by partition effect via drug immobilization

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2011

Shang, L. (2011). Control of drug release rate by partition effect via drug immobilization.
Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/43700>

<https://doi.org/10.32657/10356/43700>

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**CONTROL OF DRUG RELEASE RATE BY PARTITION
EFFECT VIA DRUG IMMOBILIZATION**

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**SCHOOL OF MECHANICAL AND AEROSPACE
ENGINEERING**

2011

CONTROL OF DRUG RELEASE RATE BY PARTITION EFFECT VIA DRUG IMMOBILIZATION

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A thesis submitted to the Nanyang Technological University

in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2011

Acknowledgement

I would like to express my sincere and deepest gratitude to my supervisor Prof. Sam Zhang Shanyong and A/P Du Hejun for their continual guidance and endless patience. I am grateful to them for their essential advice.

Deepest appreciation and gratitude also goes to Prof. Subbu S. Venkatraman from school of Materials Science and Engineering for the fruitful discussion and generous help.

Most sincere appreciation also goes to all the technical staff of Centre of Mechanics of Micro-systems, MicroMachines Centre, Materials Lab A in school of Mechanical and Aerospace Engineering and Biomaterials lab in school of Materials Science and Engineering for their technical support and logistic assistance.

Table of Content

Acknowledgement.....	I
Table of Content.....	II
List of Figures.....	VI
List of Tables	IX
List of symbols and abbreviations.....	X
Summary	XIV
Chapter 1 Introduction	1
1.1 Historical Perspective.....	1
1.1.1 Sustained and Targeted Drug Delivery.....	3
1.1.2 Pulsatile/Controlled Drug Delivery.....	4
1.2 Research trend in drug delivery	6
1.3 Objectives.....	8
1.4 Scope	8
1.5 Organization of the thesis.....	10
Chapter 2 Literature Review.....	12
2.1 Diffusion controlled drug delivery systems	12
2.1.1 Hydrogel and other polymeric drug delivery systems	12
2.1.1.1 Sustained hydrogel drug delivery systems.....	14
2.1.1.2 Pulsatile drug delivery systems	22
2.1.2 Hybrid systems for drug delivery.....	24

2.1.2.1	Hydrogel incorporated BioMEMS drug delivery systems	25
2.1.2.2	Surface Functionalization and coating for fabrication of hybrid systems	27
2.2	Drug release mechanisms and control principles	31
2.2.1	Mathematical description of release mechanism of diffusion controlled DDS	31
2.2.1.1	Release mechanism from a simple matrix based system	32
2.2.1.2	Release mechanism from a membrane based system	34
2.2.2	Control principles of diffusion controlled drug delivery systems	36
2.2.2.1	Diffusivity	37
2.2.2.2	Partition: Thermodynamics of mixture	44
2.3	Comment on the reviewed literatures	54
Chapter 3	Problem statement and proposed approach	55
Chapter 4	Experimental details	58
4.1	Drug Immobilization in hydrogel	58
4.1.1	Synthesis of hydrogel membrane	58
4.1.2	Drug immobilization in hydrogel	60
4.1.2.1	Drug functionalization	61
4.1.2.2	Simultaneous immobilization	62
4.1.2.3	Two-step immobilization	62
4.2	Drug immobilization in hydrogel incorporated BioMEMS membrane	63
4.2.1	Membrane microfabrication	64
4.2.2	Surface functionalization	65
4.2.3	Hydrogel Incorporation	65
4.2.4	Drug immobilization in the BioMEMS membrane	66
4.3	Embedding microsphere in drug immobilized poly (ethylene glycol) film	66
4.3.1	Synthesis of alginate microspheres	66
4.3.2	Drug loading into the microspheres	67
4.3.3	UV polymerization of microsphere embedded poly(ethylene glycol) film	67

4.4	Characterization	68
4.4.1	Swelling experiment.....	68
4.4.2	Particle size and Florescence microscopy	69
4.4.2.1	Florescence of drug immobilized hydrogel.....	69
4.4.2.2	Florescence of microsphere embedded PEG film	69
4.4.3	X-ray Photoelectron Spectroscopy (XPS).....	70
4.4.3.1	XPS of drug immobilized pH-sensitive hydrogel	70
4.4.3.2	XPS of functionalized silicon membrane.....	70
4.4.4	Scanning electron microscopy (SEM).....	70
4.4.5	Drug loading efficiency determination.....	71
4.4.6	Release experiment	71
4.4.6.1	Release experiment for membrane based systems	72
4.4.6.2	Release experiment for matrix based systems.....	73
4.4.7	Partition coefficient Measurement	74
4.4.8	Cell viability	75
Chapter 5	Results	77
5.1	Proof of concept: drug immobilized hydrogel	77
5.1.1	X-ray photoelectron spectroscopy (XPS) and Florescence.....	77
5.1.2	Swelling behavior.....	80
5.1.2.1	Static swelling result	81
5.1.2.2	Dynamic swelling results	84
5.1.3	Release rate	87
5.1.3.1	Flux of BSA	88
5.1.3.2	Diffusion coefficient.....	90
5.1.3.3	Partition coefficient	90
5.1.4	Cell viability	92
5.2	Application of partition control principle in hydrogel incorporated BioMEMS membrane	93
5.2.1	X-ray Photoelectron Spectroscopy.....	93
5.2.2	Scanning electron microscopy observation.....	94
5.2.3	Release rate	97
5.3	Application of partition control principle in microsphere embedded polymeric film	99

5.3.1	SEM and florescence observation	99
5.3.2	Loading efficiency.....	100
5.3.3	Release	101
Chapter 6	Analysis and discussion.....	104
6.1	Structure of the drug immobilized hydrogel	104
6.2	Control of drug release rate by partition effect via drug immobilization	106
6.2.1	Classical partition coefficient study	107
6.2.1.1	Steric effect.....	107
6.2.1.2	Chemical potential equilibrium.....	107
6.2.1.3	Summary of classical partition analysis	110
6.2.2	Thermodynamic modeling study.....	110
6.2.3	Molecular simulation study	115
6.3	Enhancement of switching effectiveness of pulsatile drug delivery systems by partition control principle	119
6.4	Alteration of release kinetics of sustained drug delivery system by partition control principle.....	121
Chapter 7	Conclusions	124
List of Publications		127
Appendix.....		I
Appendix A: Prediction of unloading time		I
Appendix B: Matlab code for partition coefficient calculated from hydrogel partition model.....		III
Appendix C: Matlab code for UNIQUAC three-component system.....		VI
Appendix D: Matlab code for UNIQUAC four-component system.....		VIII
References.....		I

List of Figures

Figure 1	Open- and close-loop control systems	5
Figure 2	Two types of drug delivery systems: matrix based and reservoir/membrane based	7
Figure 3	Release kinetics of Bovine Serum Albumin for different crosslinking density (crosslinking agent: Rose Bengal, in short RB) [19]	16
Figure 4	20% PHEMA with different amount of crosslinking concentration (left); 40% PHEMA with different amount of crosslinking (right, the intermediate curves between the two boundaries are 1 wt%, 2 wt% and 3 wt%) [21]	17
Figure 5	Proxiphylline release from PHEMA matrix with different concentration [21]	17
Figure 6	Microsphere morphology with and without pore introducing agent [23]	18
Figure 7	Schematic of fast and slow-release coating (left: slow release; right: fast release)	20
Figure 8	Microsphere embedded film	21
Figure 9	Cytarabine released from (a) chitosan microspheres and (b) the comatrix [34]	21
Figure 10	On-off release profile presented with therapeutic window	23
Figure 11	pH-dependent ionization of polyelectrolytes. Poly(acrylic acid) (positive pH-sensitive top) and poly(N,N-diethylaminoethyl methacrylate) (negative pH-sensitive)[43]	24
Figure 12	Hydrogel loaded silicon membrane [49]	26
Figure 13	Flow rate vs. time for thermo sensitive microvalve, with temperature alternated between 25 °C and 50 °C and pressure head of 74mmH ₂ O [49]	27
Figure 14	Reaction scheme of silane coupling agents [57]	30
Figure 15	Illustration of diffusion process through membrane based system	35
Figure 16	Drug partition into the membrane without immobilized drug (left) and with immobilized drug : free drug coming from donor : immobilized drug of the same type as free drug	56
Figure 17	Positive (right) and negative (left) hydrogel incorporated membrane	63
Figure 18	Silicon membrane fabrication flowchart in crosssectional view: (a) bare	

	silicon; (b) front side patterning and DRIE etching; (c) back side patterning and DRIE etching.....	64
Figure 19	Home made side-by-side diffusion cell.....	72
Figure 20	Florescence image of simultaneously drug immobilized hydrogel: no FITC-BSA immobilized (upper); with FITC-BSA immobilized (bottom) ...	78
Figure 21	XPS spectrum of C 1s narrow scan of two-step drug immobilized hydrogel (upper); C3 content as a function of immobilization level (bottom).....	80
Figure 22	Static swelling of simultaneously drug immobilized hydrogel.....	81
Figure 23	Static swelling of two-step drug immobilized hydrogel synthesized at different soaking concentration.....	83
Figure 24	Swelling ratio comparison of two-step drug immobilized hydrogel at different pH values	84
Figure 25	Dynamic swelling of simultaneously drug immobilized hydrogel at pH 2.4 and pH 7.4	85
Figure 26	Curve fitting of the dynamic swelling ratio	85
Figure 27	Comparison of order of release kinetics at pH 7.4 and pH 2.5	87
Figure 28	Flux of simultaneously drug immobilized hydrogel at pH 7.4	88
Figure 29	Flux of two-step drug immobilized hydrogel.....	89
Figure 30	Change in flux with respect to pH variation at different soaking concentrations (two-step immobilization method).....	89
Figure 31	Comparison of diffusion coefficient between pH 7.4 and pH 6.5 at different soaking concentrations (two-step immobilization method)	90
Figure 32	Comparison of partition coefficient between pH 7.4 and pH 6.5 at different soaking concentrations (two-step immobilization method)	91
Figure 33	Partition coefficient as a function as immobilization levels	92
Figure 34	Cell viability with different amount of immobilization	92
Figure 35	XPS spectra of silane functionalized silicon membrane (upper: surface scan; bottom: C 1s narrow scan)	94
Figure 36	SEM of fabricated silicon membrane.....	96
Figure 37	SEM of hydrogel incorporated silicon membrane	97
Figure 38	Flux of BSA from hydrogel incorporated membrane at different soaking levels: pH 7.4 for on state and pH 6.5 for off state	98

Figure 39	Illustration of expansion direction of hydrogel	98
Figure 40	Florescence and SEM images of microsphere embedded film, and the particle size distribution curve	100
Figure 41	Loading efficiency of BSA in alginate microspheres at different drug concentration	101
Figure 42	Accumulative release of FITC-BSA from microsphere embedded film within 15 days	102
Figure 43	Accumulative release of FITC-BSA from microsphere embedded film at different immobilization levels within 30 days	103
Figure 44	Osmotic pressure in the membrane as a function of BSA immobilization levels	109
Figure 45	BSA activity coefficient with different amount of immobilization in bulk solution; in membrane; in membrane with 200mg/mL added during polymerization in membrane	110
Figure 46	Partition-coefficient curve The black dots are the average of effective partition coefficients ($K_{eff} = K / K_{steric}$), calculated from the experimentally measured data (K) of three independent experiments, and K_{steric} is calculated from Appendix C. The error bar is the standard deviation of the measured data. For curve fitting, $\gamma_{M,free}$ is regarded as a constant in the interested concentration range. The curve fitting was performed by a freeware run under Mac OS 10.5: qtiplot.	115
Figure 47	Schematic of the simulation volume of the membrane	117
Figure 48	Loading capability of membranes with different amount of immobilization of PEGMA	118
Figure 49	Molecular simulation of partition coefficient as a function of different immobilization of PEGMA	119
Figure 50	Concentration profile of two different systems with the same total release time but different concentration profile	122

List of Tables

Table 1	BSA properties	60
Table 2	Surface charge of BSA at various ion concentration and pH values.....	60
Table 3	Reactant ratio between Bovine Serum Albumin and Acryloyl Chloride	62
Table 4	Multi step DRIE etching parameters for front side etching	65
Table 5	Composition of microsphere embedded film	68
Table 6	Order of swelling kinetics before and after drug immobilization	86
Table 7	Order of the release profile	103

List of symbols and abbreviations

Symbols

u_i^0	Reference chemical potential of substance i
η	Solvent viscosity
γ_i	Activity coefficient of i
v_i	Molar volume of i
V_f	Free volume
ϕ_p	Polymer volumetric fraction
χ	Flory thermodynamic interaction parameter
ξ	Mesh size
φ	Electric potential
π	Osmotic pressure (Equation 91)
M_∞	Amount of solvent absorbed at equilibrium
$\overline{M_c}$	Number average molecular weight
A	Area
a	Hydrodynamic radius
C	Concentration
D	Diffusion coefficient in a solvent
D_{eff}	Effective diffusion coefficient
D_0	Diffusion coefficient at infinite dilution
F	Fugacity
F	Faraday's constant
G	Gibbs free energy
GI	Gastrointestinal
g^E	Excess Gibb's free energy
J	Flux
k	Rate constant (section 2.1.2)
Kp	Partition coefficient
L	Thickness of membrane
M_t	Amount of solvent absorbed by hydrogel at time t
$n-1$	Order of release kinetics

P	Pressure
Q	Volume degree of swelling (Equation 58)
r	Pore radius
R	Ideal gas constant
S	Entropy
V	Volume

Abbreviations

AA	Acrylic acid
ADME	Absorption, distribution, metabolism and excretion
ATCC	American Type Culture Collection
ATPS	Aqueous two-phase system
BioMEMS	Bio-Mechanical Eelectro MicroSystems
BSA	Bovine serum albumin
CD	Cyclodextrin
CDDS	Controlled Drug Ddelivery Systems
CNS	Central nervous system
CS	Chondrotin sulfate sodium salt
DDS	Drug delivery systems
DEAEMA	2-(diethylamino) ethyl methacrylate
<i>DI</i>	Deionized
DMAEMA	<i>N,N'</i> –dimethylaminoethylmethacrylate
DMEM	Dulbecco's modified Eagle's Medium
DMPA	2,2-dimetoxy-2-phenyl acetophenone
DRIE	Deep reactive ion etching
<i>e.g.</i>	exempli gratia or for example
EC	Ethyl cellulose
EDTA	Ethylenediaminetetraacetic acid
<i>et al.</i>	et alii or the others
FAD	Flavin adenine dinucleotide
FBS	Fetal Bovine Serum
FITC-BSA	Fluorescein isothiocyanate labeled Bovine Serum Albumin
HF	hydrofluoric acid
HPMA	N-(2-hydroxypropyl) methacrylate
IDDM	Insulin-dependent diabetes mellitus
LCST	Low critical solution temperature
MAA	Methacrylic acid

MEMS	Micro electro mechanical systems
MTS	[3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
PA	Pharmacological availability
PEG	Poly (ethylene glycol)
PEGDMA	Poly (ethylene glycol) dimethacrylate
PHEMA	Poly 2-hydroxyethyl methacrylate
PK	Pharmacokinetics
PLGA	Poly (lactic-co-glycolic acid)
PLL	Poly-L-lysine hydrobromide
PMAA	Poly (methacrylic acid)
PNIPPA _m	Poly (<i>N</i> -isopropylacrylamide)
PVA	Poly (vinyl alcohol)
SEM	Scanning electron microscopy
TEGDMA	Tetraethylene glycol diacrylate
UNQUAC	Universal QUASI-Chemical theory
VA	Vinyl acetate
VEGF	vascular endothelial growth factor
XPS	X-ray photoelectron spectroscopy
μ-TAS	Micro-total analysis systems

Summary

Controlled Drug Delivery Systems (CDDS) are designed to deliver hormones and other protein drugs in an on-off manner, which better mimics real physiological release fashion. Although pulsatile release profile can be achieved by various materials and designs so far, the exact release rate is still difficult to control with reasonable flexibility. However, the exact release rates at both 'on' and 'off' states are critical to the success of CDDS. At 'on' state, high release rate will result in over-dose, which may cause severe side effects and low release rate may not be able to induce therapeutic effect, whereas at 'off' state, a non-zero release shortens the device lifetime and may eventually lead to over-dose if the off-state release (leakage) is considerably high.

On the other hand, sustained drug delivery systems are designed for diseases, which require constant drug release for a prolonged period of time. Burst release (initial quick release) is usually observed in such systems, which shortens device lifetime and may cause severe side effects. It is apparent that precise or quantitative control over release rate is critical in both types of Drug Delivery Systems (DDS).

In membrane based DDS, drug partitions (or dissolves) into the membrane, and diffuses along a concentration gradient to be released. Therefore drug release rate can be controlled by two factors: partition and diffusion. Most research effort in release rate control has been devoted to the second step: diffusion step, however little attention is given to the partition step. Moreover partition coefficient is usually affected by the diffusion control method but the degree of influence is largely unknown. Therefore

quantitative control of drug release rate is difficult to achieve by the existing control method. Thus it is the objective of this project to develop a release rate control method, which separately controls the diffusion and the partition coefficients. The attempt to achieve this objective starts from partition coefficient analysis.

Thermodynamical analysis of the partition step suggests that drug partition from donor to membrane could be controlled (especially lowered) by altering the chemical potential of the drug in the membrane. If a certain amount of drug is immobilized in the membrane, the chemical potential of the drug in the membrane will be contributed by both the immobilized drug and the free drug coming from the donor; hence at chemical potential equilibrium between the total amount of drug in the membrane and the amount of drug in the donor, the effective amount of drug that could dissolve (or partition) into the membrane should be reduced. On the other hand, diffusion coefficient of the drug is not dependent on chemical potential of the drug in the membrane. Therefore in a membrane based DDS, it is expected that partition coefficient of free drug coming from the donor can be controlled by drug immobilization in the membrane, while diffusion coefficient is unaffected. The key requirement of the proposed partition control method is that the immobilized drug in the membrane has to be the same type as the free drug in the donor that is to be released.

It is found that the immobilization of the model drug (Bovine Serum Albumin, BSA) in the membrane results in a slower release rate for the drug (BSA) from the donor. The reduction in release rate can be attributed to partition coefficient reduction rather than diffusivity reduction. It is also found that partition coefficient and the amount of immobilization are inversely related, and the mathematical equation that depicts this

relation is derived. Moreover drug immobilization is not detrimental to the biocompatibility of the hydrogel. The partition control principle by drug immobilization has been applied to both pulsatile and sustained drug delivery systems.

In pulsatile drug delivery systems, the change in release rate upon switching (switching effectiveness) is enlarged after a suitable amount of immobilization because the concentration of immobilized drug in the membrane is different between ‘on’ and ‘off’ states (reduction effect is more prominent at ‘off’ state due to less solvent, hence higher concentration of the immobilized drug).

In sustained drug delivery systems, duration of release can be extended from a few hours to more than two weeks due to reduced release rate after drug immobilization. Burst release is not observed; rather a delayed release profile is achieved with a suitable amount of drug immobilization.

Chapter 1 Introduction

Drug is a substance that can induce a biological response in the human body. There are three phases that a drug undergoes after administration, namely pharmaceutical phase, pharmacokinetics phase and pharmacodynamic phase. Pharmaceutical phase is the initial phase of the drug in the body after administration, formulation breaks apart and active ingredient dissolves. The pharmacokinetics (PK) phase deals with absorption, distribution, metabolism and excretion of drugs in the body (ADME). Pharmacodynamic phase is the one, in which drug reaches the targeted site and takes action.

Drug delivery focuses on the second phase: pharmacokinetics. From the site of administration to the targeted site, various mechanisms, both biochemical and physical, which select or transform or even destroy the drug, take place in human body, either of which may severely affect drugs' bioavailability, hence limits its therapeutic effect. Therefore for a potential drug to be effective, not only is its pharmacodynamic property important, but also the successful delivery from its site of administration to the target site is crucial. The ultimate goal of drug delivery is to improve the drug therapeutic efficacy.

1.1 Historical Perspective

The concept of drug delivery is not new. Egyptian physicians employed pills, ointments, salves, and other forms of treatment over 4,000 years ago. The ancient Chinese herbs are still used today. Intravenous injections were first performed in humans in 1665, only a few years following Wren's infusion of opium into dogs and a

few decades after Havre's description of the circulatory system in 1616. Subcutaneous injections were introduced by Wood in 1853 and modern hypodermic syringe was developed by Luer in 1884 [1]. Many of these "traditional" drug delivery methods resulted from the fact that most drugs before 20th century lacked potency and specificity.

New technology and clearer biological insight have led to new classes of therapeutic agents; typical examples are antibodies, gene-based drugs, antisense oligonucleotides, virus-like particles, recombinant proteins and hormone. But the potency and activity of these biotechnological agents are often thwarted by the wrong or the improperly constructed delivery systems, due to the fact that these agents frequently have short half-lives, poor permeability in membranes, and serious toxicity when delivered systemically in large doses.

Beginning in the 1960s the modern view of drug delivery began to unfold: the delivery system could substantially contribute to the performance of drugs through proper spatial placement or temporal delivery. From this period onward began the serious effort on understanding the disposition of drugs and delivery systems in the various routes of administration and devising strategies for drug delivery to overcome biological barriers and to improve the physical-chemical properties of modern drugs [2].

Because a selection of a proper Drug Delivery System (DDS) can significantly enhance the therapeutic efficacy of drugs, drug delivery has become an integral part of drug development [3], and various methods were proposed to address different aspects of pharmacokinetics of various biotechnological drug products.

1.1.1 Sustained and Targeted Drug Delivery

One of the main objectives of drug delivery is to maintain the concentration of the drug sufficiently high at the site of action in order to exert the therapeutic effect; meanwhile the concentration should not be too high, since this may result in detrimental side effects. The upper and lower limit of drug concentration is also known as the therapeutic window. In order to maintain an essentially constant blood drug level within this therapeutic window over time, sustained drug delivery systems were designed and commercialized by Smith, Kline & French in the late 1940s [1]. Sustained drug delivery is ideal for drugs that are designed to be released slowly in the local tissue (potent drugs), or diseases, which require prolonged drug action time (immuno-suppression drugs). Therefore, toxicity and inconvenience of repeated dosage are greatly reduced. Depending on the nature of the disease and the medicine, the therapeutic window can be large or narrow. This imposes stringent requirement for drug delivery system to be able to control its release rate precisely, especially for diseases with narrow therapeutic windows.

The drug delivery system should preferably be designed such that a preferential accumulation of the drug is reached at the site of action, whereas the drug concentration elsewhere in the body should be as low as possible. The reason for this need of “targeting” is that a high concentration of the drug in tissues or cells other than those being targeted may cause problems related to side effects. A typical example of the latter is cancer therapy, where accumulation of chemotherapeutic agents in areas other than the tumor frequently causes severe side effects. Once the drug has performed its action, it should either be excreted or metabolized to harmless compounds [4].

1.1.2 Pulsatile/Controlled Drug Delivery

Most physiological systems work in a negative feedback manner, and only vary in a narrow range. Typical example is blood glucose concentration. Glucose is a major energy source for human beings. Glucose coming from food is converted into glycogen in liver and muscles, where glycogen can be converted back to glucose, as shown in Scheme 1

At equilibrium, k_1 is equal to k_2 , resulting in a constant blood glucose concentration of 70-100 mg/dL (3.9-6.1 mmol/L) for healthy people to guarantee that sufficient amount of energy is supplied to the system [2].

In a simplified picture, if the glucose concentration goes higher than the normal level (after food), the body will send signaling molecules (insulin) to the liver and muscle to speed up conversion of glucose to glycogen ($k_1 > k_2$), so as to drag down the glucose concentration; whereas if the blood glucose concentration is lower than the normal level (hungry), messengers will be absent, hence the reverse reaction is dominating ($k_1 < k_2$), resulting in an increase in the glucose concentration.

Physiological peptides and proteins, which act as messengers to maintain equilibrium of the physiological system, are collectively known as hormones. They are released in the body in a pulsatile fashion on a need basis as opposed to continuous release. Diseases caused by functionality or secretion deficiency of this kind of proteins are often treated by administration of the same kind of proteins obtained from other sources (animal or human recombinant proteins). A significant challenge in hormone delivery is to prepare delivery systems that release hormones in a non-zero-order

fashion (section 2.2.1.1) to better mimic physiological hormone release. Pulsatile/controlled delivery systems working in an on-off manner to delivery protein or peptide drugs in response to internal or external stimuli are an ideal option. Externally regulated pulsatile delivery systems may be considered open-loop systems (Figure 1a) and the internal or self-regulated systems may be considered closed-loop systems (Figure 1b). It is obvious that close-loop system is a more suitable choice due to its ability to be self-controlled. Overdose of hormone drugs such as insulin is highly undesirable because it leads to coma and death [5]. A good control over the release rate, especially at off-state, is thus the key issue in pulsatile/controlled delivery systems to minimize any leakage.

(a)

(b)

Figure 1 Open- and close-loop control systems

1.2 Research trend in drug delivery

Research on drug delivery systems in *vitro* is focused on material selection and release profile optimization. Polymer remains one of the most popular candidates under investigation. Among different types of polymers, natural constituents of physiological systems, i.e. alginate and chitosan, and synthetic hydrophilic polymers such as Poly(ethylene glycol) (PEG) and Poly(vinyl alcohol) (PVA) are widely used in DDS design [1]. Those polymers fall into a general category of polymer, known as hydrogels. Different design approaches have been utilized in hydrogel based DDS to realize distinct release profiles with controllable drug release rate. In order to control drug release rate, it is the driving force of release that should be controlled. Among different driving forces, which can be used for drug delivery, diffusion is by far the most commonly used driving force [6]. Hence in most systems, it is the diffusion coefficient that should be controlled to realize a desired release profile. At device level, there are basically two types of diffusion controlled drug delivery systems, matrix based and membrane based (Figure 2). The difference between these two is that matrix based DDS (e.g. tablets) carries the drug inside the material and allows it to diffuse out either in a sustained or controlled manner. The membrane based DDS, on the other hand, carries the drug in a separate reservoir and the drug is released by diffusion through the membrane (e.g. capsules).

Figure 2 Two types of drug delivery systems: matrix based and reservoir/membrane based

However, even for a simple reservoir/membrane based system, factors other than diffusion coefficient dramatically affect the overall release rate, such as drug redistribution coefficient, also known as partition coefficient, which is a measurement of the amount of drug dissolved in the membrane [1]. Since drug molecules have to go into the membrane first before they diffuse out of the system, both partition coefficient and diffusion coefficient are important to determine the overall drug release rate. In most cases, partition coefficient is also important in matrix-based systems because coating of the matrix with another material is a common practice to control release rate (another form of the reservoir/membrane based system: the matrix serves as a drug reservoir, and the coating serves the same function as membrane). To be able to control the release rate more precisely, it would be great if diffusion coefficient and partition coefficient can be controlled separately. It has been demonstrated that partition coefficient can be controlled by using materials with different drug solubility and binding properties [7 -9]. Drug solubility in a polymeric matrix is a function of compatibility (hydrophilic drug-hydrophilic matrix) and specific binding, which can be altered by introducing binding sites or hydrophilic/hydrophobic segments to the matrix. However it is expected that diffusivity and partition coefficient are both changed at the same time by those two methods.

Although hydrogel is an ideal choice for DDS, it often suffers from poor mechanical property. On the other hand, silicon based Micro Electro Mechanical systems (MEMS) have much better mechanical integrity. It is advantageous to make use of the good mechanical integrity and sophisticated sensing-actuating ability of MEMS devices in the drug delivery field. However, integration of sensing, controlling and actuating components in a single device is still a challenging job for MEMS design and fabrication. To compensate the weaknesses from both sides, hybrid systems, which are composed of basic structure made from MEMS process and functional polymers, have been proposed by various research groups.

1.3 Objectives

The objective of this project is to establish a method to control partition coefficient of protein drugs quantitatively with their diffusion coefficient largely intact, by examining the chemical potential equilibrium of the drug at the donor-membrane interface. It is hypothesized that partition coefficient of the free drug in the donor could be controlled by immobilizing a certain amount of the same type of drug in the membrane because the immobilized drug would contribute to the total chemical potential of the drug in membrane so as to lower the partition coefficient of the free drug. In this way, drug release rate from membrane based systems (including matrix with coating) will be controlled more precisely since the immobilization level can be precisely controlled.

1.4 Scope

To achieve the objectives, this project covers the following work:

(a) Drug immobilization in the hydrogel membrane

A pH-sensitive hydrogel film will be chosen as the basis of this project. Two different drug immobilization methods will be used to easily manipulate the immobilization levels: simultaneous immobilization and two-step immobilization. In the simultaneous immobilization method, C=C attached model drug will be mixed with hydrogel precursor and the mixture will be polymerized so that immobilization and membrane synthesis will be done simultaneously. In the two-step method, drug-free membrane will be first synthesized and then soaked in C=C attached model drug solution of various concentrations to load the drug. Finally a second polymerization will be induced to immobilize the loaded drug. Swelling, release rate analysis and biocompatibility assay will be conducted to the prepared samples.

(b) Thermodynamic modeling of the partition control principle

Thermodynamic analysis will be carried out to establish the relation between effective partition coefficient and the amount of immobilization. Data fitting will be used to verify the developed model. Molecular simulation will be carried out to understand molecular events of the partition behavior in the presence of the immobilized drug.

(c) Application of the partition control principle in pulsatile drug delivery systems

A pH-sensitive hydrogel incorporated silicon membrane will be fabricated to provide better mechanical integrity to the polymer. Silicon membrane with 10 μm holes will be first fabricated and hydrogel will be incorporated in the silicon membrane via

polymerization. Prior to hydrogel incorporation, silane functionalization of the silicon membrane will be carried out. Drug will be immobilized in the hydrogel by the two-step immobilization method. Electron microscopy and release studies will be carried out to study the controllability of the partition control principle.

(d) Application of the partition control principle in sustained drug delivery systems

Because there has to be a partition step to apply the partition control principle, a microsphere embedded film will be used in sustained drug delivery study so that drug inside the microsphere has to dissolve in the film before being released. Alginate microsphere will be synthesized by double emulsion method and drug will be loaded by soaking. Drug will also be immobilized in the hydrogel film to provide chemical potential and to affect the release rate of the drug inside the microspheres. Florescence and electron microscopy will be used to observe the structure of the fabricated device and release study will be carried out.

1.5 Organization of the thesis

This thesis is divided into seven chapters.

Chapter 1: A brief introduction of the background of the proposed research area is presented. Objectives, scope and the organization of the thesis is defined in this chapter.

Chapter 2: Literature review on hydrogel based drug delivery systems, drug release mechanism from hydrogel based delivery systems, release rate control principles

(diffusion and partition) and current state of art control methods are provided in this chapter.

Chapter 3: The defined problem that this project is meant to solve and the proposed solution are presented in this chapter. Novelty and scientific contribution of this project is also highlighted in this chapter.

Chapter 4: Procedures and method to conduct various experiments involved in this project are detailed in this section.

Chapter 5: Experimental results are summarized and described in this chapter.

Chapter 6: Experimental results are analyzed in this chapter to understand how the partition control principle works. Thermodynamic modeling and molecular simulation are used to depict the control mechanism. Various application prospects are discussed in this chapter as well.

Chapter 7: Major conclusion and future research directions are given.

Chapter 2 Literature Review

In this chapter, a detailed literature review on current matrix and membrane based diffusion controlled drug delivery systems, the related control principles and their mathematical models are presented.

2.1 Diffusion controlled drug delivery systems

The ultimate task a drug delivery system performs is to release the drug; therefore drug delivery systems can be classified according to the driving forces for drug release. Diffusion, osmotic pressure and erosion of matrix are common driving forces for drug release, among which, diffusion controlled drug delivery systems are more popular due to its simple realization. Yet its release rate cannot be precisely controlled to date. The purpose of this subsection is to review drug delivery systems that are mainly based on diffusion, and the different release behavior associated with each of them.

2.1.1 Hydrogel and other polymeric drug delivery systems

Gels are three dimensional polymer networks in which chains are crosslinked [7]. The gel formation (crosslinking) is caused by formation of intermolecular bonds as a result of physical (reversible) or chemical (irreversible) interconnection between macromolecular chains. In a proper solvent, which should be able to dissolve the polymer, gels will only absorb the solvent to a certain extent without dissolving, due to crosslinking between the chains. The phenomenon is known as swelling. The degree of swelling depends on the elasticity of the polymer networks and compatibility between

the polymer network and solvent [10]. If the gel is made of water-soluble polymers, the gels are known as hydrogels [6]. Hydrogels are three-dimensional networks of hydrophilic polymers that can absorb a large amount of water (more than 200% of the total weight) but remain insoluble [11].

The key feature of hydrogel is the ability to absorb water, and the ability to absorb water is correlated to osmotic pressure of ions in water and compatibility of the polymer and water, whereas it is inversely related to crosslinking density [10]. The higher the ion concentration is in water, the lower the degree of swelling will be. This can be understood by a high reverse osmotic pressure generated by high ion concentration in the water. Other conditions being the same, the more water-soluble the polymer is, the higher the degree of swelling will be [12]. In contrary, higher crosslinking decreases elasticity of the polymer matrix, hence reduces the degree of swelling. In addition to its ability to swell, hydrogel shows good biocompatibility [13]. Diffusion rates at swollen and dry states are usually different, which enables hydrogel to be used in controlled drug delivery systems. On the other hand, cells and proteins generally display a low tendency for adhesion to hydrogel surfaces because of the low interfacial free energy of the hydrogels when in contact with body fluid [14]. Good biocompatibility and ability to swell make hydrogels good candidates for drug delivery systems.

The main interest in current hydrogel based drug delivery system is to control the degree of swelling at different conditions so as to achieve desired release profile. For the purpose of sustained drug delivery, release rate can be controlled by degree of swelling under physiological conditions (ionic strength, pH, temperature), whereas for controlled drug delivery, distinct swelling behavior is desired upon presenting a stimuli.

The focus of this thesis is on hydrogel based drug delivery systems, however for the purpose of this project, namely to develop a method to control the drug release rate more precisely, drug delivery systems reviewed in this session are not limited by the type of materials used in various studies, rather this session is organized according to the different control methods used in the cited examples. Therefore this section covers more than just hydrogel based systems. The other reason to cover polymeric drug delivery systems in general is because hydrogel is essentially a type of polymer; drug release control methods used in other types of polymeric drug delivery systems are normally applicable to hydrogel systems as well. Hence in order to develop a novel control method, the review of drug release rate control methods will cover not only hydrogel based, but also other relevant polymeric systems. Although the control method will be tested mainly on hydrogel, it is desirable to design the control method with other systems in mind so that the control methods proposed in this project will have a wider applicability.

2.1.1.1 Sustained hydrogel drug delivery systems

Sustained drug release profile refers to the one that the amount of the released drug is linearly proportional to the duration of the release [15] (also known as zero-order release profile, the mathematical definition of it will be covered in section 2.2.1.1), hence a constant release rate over time. It is highly desirable because a constant release rate makes it easier to maintain the plasma concentration within the therapeutic window for a longer period of time, therefore to extend the duration of action of a given dosage, and because of which, the dosing frequency and total dose will be effectively reduced. Both matrix and membrane configurations for sustained delivery can be found in the literature.

2.1.1.1.1 Matrix based/monolith systems

In a simple configuration, drug can be dispersed in a polymeric matrix (monolith system). Rate of sustained drug release is eventually determined by rigidity of the matrix and diffusivity of the drug. A proper selection of the degree of crosslinking helps to achieve a specific release rate as well as mechanical integrity of a drug carrier [16, 17]. Therefore, when a limited solute diffusion is required as in the case of guar galactomannan for oral colon specific drug delivery [18], a high crosslinking density is needed. However high degree of crosslinking makes drug loading very restrictive in applying absorption loading method (synthesized hydrogels are left inside a drug solution so that drug can diffuse into the hydrogel), or if simultaneous loading method is applied (drug molecules are added into the gel precursor before polymerization), high crosslinking density may cause the chemical crosslinker to react with the drug, creating toxic species.

In a collagen matrix drug delivery system, the effect of crosslinking density on protein release kinetics was studied [19, 20]. A strong dependence of release rate on crosslinking density was observed: a slower release rate at higher crosslinking density (Figure 3).

Figure 3 Release kinetics of Bovine Serum Albumin for different crosslinking density (crosslinking agent: Rose Bengal, in short RB) [19]

In most cases, crosslinking is done throughout the polymer matrix; but local alteration of crosslinking density (such as surface crosslinking) can be used to modify overall release rate. Brazel *et al.* reported surface crosslinked poly 2-hydroxyethyl methacrylate (PHEMA) discs, where crosslinking of the PHEMA is only induced at the surface by controlling the contacting time between crosslinking agent and the matrix [21]. It can be shown that a much longer sustained release profile can be achieved at a suitable crosslinking density (Figure 4 left). It is worth nothing that a similar effect can be achieved by changing the PHEMA concentration during preparation (Figure 5). But most interestingly, a delayed release profile was found for a high PHEMA concentration hydrogel with a high surface crosslinking density (Figure

4 right): drug is only released after an initial silent period. A delayed formulation is beneficial for oral delivery because it takes hours for the delivery system to reach small intestine and colon. Moreover it solved burst release problem. The surface crosslinking approach has been applied to PVA hydrogels for delayed and sustained release as well [22].

Figure 4 20% PHEMA with different amount of crosslinking concentration (left); 40% PHEMA with different amount of crosslinking (right, the intermediate curves between the two boundaries are 1 wt%, 2 wt% and 3 wt%) [21]

Figure 5 Proxiphylline release from PHEMA matrix with different concentration [21]

Besides crosslinking density, release behavior can be tailored by changing the porosity of the matrix as well. Ahmed and coworkers used NaCl and Glycerol monooleate as pore forming agent (Figure 6) [23]. Their results showed that Poly(lactic-co-glycolic acid) (PLGA) microspheres usually demonstrated a biphasic release profile: a burst release in the initial stage and a slower release profile at a later stage. However a direct comparison between the release rate between the one without introducing pores and those with pores is not available.

Figure 6 Microsphere morphology with and without pore introducing agent [23]

A common practice to induce pores into coating is to use pore-forming agents. Pore-forming agents are usually hydrophilic polymers, which dissolve in body fluid. When the pore-forming agents are blended with hydrophobic polymers to form a coating, hydrophilic polymers will dissolve and leach out from the coating, hence making the coating more porous. Cellulose derivatives are often used to alter porosity of the hydrophobic coatings [24].

Drug-matrix interactions can affect the drug release rate dramatically. Cyclodextrin (CD) has been incorporated into a hydrophilic hydrogel matrix so that hydrophobic drug can form non-covalent binding with the hydrophilic matrix which increases the drug solubility in the matrix and drug can be released over a longer period of time [25-27]. Tada *et al.* synthesized hydrogel using albumin as the crosslinker [28, 29]. Their results showed that drug molecules with albumin affinity had higher drug loading

contents in the matrix and demonstrated slower release profiles. This is because binding of the drug molecules increases the drug solubility in the matrix so that drug molecules prefer to stay in the matrix rather than to be released.

2.1.1.1.2 Reservoir/membrane systems

Drug can be stored inside a matrix, and it can also be stored in a separate reservoir. If one or more surfaces of the reservoir are permeable to drug, the system is referred to as reservoir/membrane system. Partition behavior or drug solubility in the membrane is found to be an important factor to alter the release rate of reservoir/membrane systems. It is well known that release rate of hydrophilic drug from hydrophilic matrix is rather rapid, hence to lower down its release rate. Coating the hydrophilic matrix with a hydrophobic material is a feasible approach to have slower release formation. Shin *et al.* studied the effect of composition of ethylene-vinyl acetate membrane on the release rate of a hydrophobic drug [7]. It was found that with increasing vinyl acetate (VA) content, the drug permeability of the membrane was increased. The authors argued that since permeability was governed by both partition coefficient and diffusivity, the increase in VA content might increase the partition coefficient of the drug, but no experimental evidence was reported.

Another type of reservoir system is multilayer structure. When a drug containing film is coated on the surface of biomedical devices (i.e. drug eluting stent), it also suffers from burst release, hence is lack of long-term effect. A practical approach to enhance the long-term effectiveness of a drug-containing film/matrix is to capsule it with another layer, which is essentially a reservoir/membrane system as well [30]. S. Venkatraman and F. Boey reviewed several this types of designs in the application of cardiology stents [31]. In one of the applications where drug is supposed to be released

over a longer period of time, a drug free coating is applied on the surface of the drug-containing layer (Figure 7). Since drug has to pass through an extra layer, its release rate is supposed to be reduced. And in contrast, a faster release design is the one without the drug free layer (Figure 7).

Figure 7 Schematic of fast and slow-release coating (left: slow release; right: fast release)

One form of reservoir/membrane design is to embed drug loaded microspheres in another polymer matrix [32]; therefore drug has to partition into the matrix before it can be released. Hence a slower release rate is expected (Figure 8). This is especially usefully for hydrophilic drugs because hydrophilic drugs usually diffuse out from hydrophilic matrix quickly, but they diffuse slowly in hydrophobic matrix; however they barely incorporate into hydrophobic polymer matrix. To improve the compatibility between drugs and their carrier, meanwhile to retard its release rate, hydrophilic drugs are loaded into hydrophilic matrix (microsphere/nanospheres), and then embed the drug-loaded spheres into a hydrophobic polymer to control its release rate. Another reason for this type of system is to ease the chemical stress encountered by the drug molecule during preparation.

Figure 8 Microsphere embedded film

C Varelas *et al.* [33] proposed a mathematical model to describe the release mechanism of the microsphere embedded polymeric film, and highlighted that partition coefficient is an important factor in governing both the release rate and release profile (zero-order or non zero-order).

Teijon *et al.* [34] reported a chitosan microspheres embedded poly (lactide-co-glycolide) film, and a slightly slower release rate was observed for the composite/comatrix (Figure 9).

Figure 9 Cytarabine released from (a) chitosan microspheres and (b) the comatrix [34]

The same group of researcher also embedded albumin microspheres in a poly(lactide-co-glycolide) film to study its release behavior [35]. It was found that a cancer therapy drug: cytarabine was quickly released from the albumin microsphere in the presence of a protease. However when the drug containing microsphere was embedded inside a poly (lactide-co-glycolide) matrix, the release of the drug was slowed down in the

enzymatic environment because the matrix protected the microsphere from being degraded so that it was only through diffusion that the drug could be released. It is worth noting that in a non-enzymatic environment, the release behavior of the comatrix is almost the same as the microsphere alone, suggesting that diffusion in the microsphere is the rate-limiting step, and the retardation is because of the protection against enzymatic degradation. Prolonged release profile was also observed by Zhang *et al.* when insulin loaded PLGA nanoparticles were embedded in PVA gel [36]. Li *et al.* [37] studied the water uptake property of a ethyl cellulose (EC) microspheres embedded chitosan film and found that the existence of EC microspheres increased the water uptake capability of chitosan films due to the more amorphous structure of the EC microspheres containing chitosan films.

2.1.1.2 Pulsatile drug delivery systems

For the purpose of controlled drug delivery, hydrogel can be made to swell under certain condition whereas de-swell if the condition changes. The rate of release is controlled by the swelling ratio of the hydrogel because diffusivity is generally higher at high swelling ratio. Typical external stimuli to control the degree of swelling include: temperature [38], electric field, magnetic field [39], pH [40], light, and ultrasonic. Nowadays, it is not a major issue to demonstrate on-off release profile for responsive hydrogel delivery systems, but more stringent requirement is imposed when they are used for controlled delivery; the release rate during on state shall be dramatically different from the release rate during off state, so that the plasma drug concentration in the off state can be maintained at a much lower level compared to the lower limit of the therapeutic window while the on state plasma drug concentration falls within the therapeutic window (Figure 10). Switching effectiveness is defined as the percentage

change of release rate during on/off switching, which can be used to measure the degree of difference in release rate during switching.

Figure 10 On-off release profile presented with therapeutic window

As a typical example among different stimuli, pH sensitive hydrogels are reviewed in this thesis. The release behavior of different types of pH sensitive hydrogel is presented as well.

pH varies a lot in some parts of the body, noticeably in the Gastro-Intestinal Tract (GI tract), hence pH controlled drug delivery system is suitable to target diseases in a particular site. pH sensitive hydrogel either swells or de-swells upon pH increase. This is especially beneficial to oral protein/peptide delivery. Conventionally, less than 0.1% of orally dosed insulin reaches the blood stream intact [41] due to strong acidic environment, enzymatic degradation in stomach and low epithelial permeability [42]. pH sensitive hydrogel can be used to block direct contact between protein and the harsh environment, so that protein drugs can be safely transported through stomach and reach the small intestine, where most of absorption occurs.

pH responsiveness is because of basic or acidic groups in the hydrogel, which are capable of accepting or donating protons as shown in Figure 11 [43]. Swelling is due to

electro-static repulsion between ionized chains.

Figure 11 pH-dependent ionization of polyelectrolytes. Poly(acrylic acid) (positive pH-sensitive top) and poly(N,N-diethylaminoethyl methacrylate) (negative pH-sensitive)[43]

Since swelling is mainly due to electro-static repulsion, any factors, such as pH, and ionic strength that may affect electro-static repulsion will influence swelling behavior [44]. Besides external conditions, intrinsic properties of hydrogel, such as molecular weight and elasticity of the monomer, crosslinking density and type of solvent used are all determinants of the swelling behavior of a hydrogel.

2.1.2 Hybrid systems for drug delivery

Micro Electro Mechanical Systems (MEMS) devices are manufactured by standard microfabrication process that are used in Integrated Circuit fabrication. The device is small (μm scale), yet serves the same function as their larger counterparts. Integration of mechanical and electrical components in a single chip is still challenging for most MEMS design. Moreover, most MEMS drug delivery systems are of non-self controlled. An external controller is needed to trigger the device. This is because of fabrication difficulty to integrate sensing, signal processing, actuating, and drug reservoir in a single chip.

Polymers such as hydrogel are able to respond to external stimuli. It is equivalent to say the material itself is able to sense, process and actuate based on the given signal. But mechanical property of hydrogel is generally poor. More advanced design integrates hydrogel and other devices (mostly MEMS) to compensate the weaknesses of each individual candidate.

2.1.2.1 Hydrogel incorporated BioMEMS drug delivery systems

Crosslinked Poly (*N*-isopropylacrylamide) (PNIPAAm), a temperature sensitive hydrogel, is brittle in the dry state and becomes very soft in the swollen state [45]. To improve its mechanical property, co-polymerization with different monomer is the most common approach [45,46], which affects drug release rate as well. Another approach is to combine thermo-responsive hydrogel with another device, which has better mechanical properties. If a membrane made from other material were loaded with thermo-responsive hydrogel, a stimuli-responsive membrane would be achieved. Polymer [47], glass filters [48] and silicon membranes [49] were used to incorporate with environmental-sensitive hydrogel for better mechanical properties and controllability. Although different membrane offers different pore size and mechanical property, the release mechanism is the same. At temperature below Lower Critical Solution Temperature (LCST), hydrogel is swollen and hence blocks the pores of the membrane; this state is known as off state. As temperature increases to LCST, hydrogel shrinks and leaves the pores open; hence it is referred to 'on' state. In one such design [47], cellulose nitrate membrane (0.2 μm) is immersed into a monomer solution, and vacuum dried. They reported a low penetration amount at off state and a

high penetration amount at on state. Although effect of different parameters on drug diffusion has been studied, a dramatic leakage at off state is always observed; again it is because of diffusion through swollen hydrogel at off state.

Besides polymer membrane, glass filter (pore diameter 161-250 μm) was used to provide mechanical support and thermo-sensitive membrane was prepared in a similar way as polymeric membranes [48].

To achieve a uniform pore size distribution, Baldi *et al.* [49] fabricated a silicon membrane of 100 μm thick (Figure 12). The pores were composed of an inner orifice (40 μm in diameter) and an outer orifice (140 μm in diameter).

Figure 12 Hydrogel loaded silicon membrane [49]

Thermo-sensitive or pH/glucose-sensitive hydrogel was loaded inside the pores at

swollen state. The control mechanism is exactly the same as previous designs. The result was promising especially with thermo-responsive hydrogel; almost no off-state leakage was observed (Figure 13). However, caution has to be taken when the design is to be applied in drug delivery applications. This is because the result reported was flow rate upon pressure gradient rather than cumulative amount of permeating drugs. Effect due to diffusion is not included in this result; however diffusion at off state, corresponding to swollen state of the hydrogel, should result in a dramatic off-state leakage based on the diffusion result presented in previous sections.

Figure 13 Flow rate vs. time for thermo sensitive microvalve, with temperature alternated between 25 °C and 50 °C and pressure head of 74mmH₂O [49]

Hydrogel controlled micro-valve for insulin release is also found in the literature [50]. The reservoir could be placed outside the body and the delivery of insulin is through a catheter. A glucose-sensitive micro-valve is connected to one end of the catheter and inserted into body to sense the glucose level and permit delivery once glucose level goes up.

2.1.2.2 Surface Functionalization and coating for fabrication of hybrid systems

Strong adhesion between hydrogel and silicon (Si) is critical in constructing hybrid drug delivery systems to preserve the functionality of the device and to prevent any

damage to the body due to the polymer wear particles. Preparation and patterning polymer (including proteins) on various surfaces (i.e. silicon surface) with direct chemical bonds between polymer and the surface has been extensively studied in recent years, largely because of the interest in bio-sensors [51].

The first step to establish the chemical bond between polymer and the surface is to introduce functional groups on the surface that are able to react with the polymer, which is known as surface functionalization.

Most efforts to make high quality functionalized silicon surface begin with the atomically flat hydrogen terminated Si (H/Si) [52]. Hydrogen termination could be obtained by first cleaning the silicon using $\text{H}_2\text{O}:\text{H}_2\text{O}_2:\text{NH}_4\text{OH}$ and $\text{H}_2\text{O}:\text{H}_2\text{O}_2:\text{HCl}$ both in (4:1:1) proportions at 80°C for 10 min followed by rinsing in deionized water (DI) [53] to oxidize the surface (-OH terminated, and Si-OH is known as silanol group). To form H/Si, 49% hydrofluoric acid (HF) can be used to etch the cleaned Si for (100) orientation, whereas ammonium fluoride (NH_4F) can be used for (111) orientation, followed by DI rinse. A wide variety of reactions have been used on H/Si surface, resulting in different modified surfaces. One of the most popular reactions used in functionalizing H/Si surface is to open up an alkene ($\text{C}=\text{C}$) bond, which reacts with the H/Si surface. The popularity of using modified alkenes is because various types of functionalized alkene molecules are readily available and could be covalently linked to the H/Si by activating the alkenes group using heat or ultra-violet light [54]. However, activating $\text{C}=\text{C}$ bond requires high energy source ($>3.5\text{eV}$, or equivalently $<350\text{nm}$) and may cause polymerization reaction between themselves [54], hence reduce the quality of functionalized surface. Halogen atoms are more reactive, and can be used to modify the H/Si surface for better reactivity. Halogen terminated Si surface with

subsequent reaction with alkyl lithium reagents [55] or R-OH [56] provides an alternative approach to functionalize the surface with low energy requirement (60-80 °C).

Besides direct covering the surface with desired terminal groups, di-functional molecules can be patterned on the H/Si or halogen terminated surface using one of their functional groups, leaving the other functional group on the surface for further modification. In the case of polymer coating on silicon surface, the most apparent way is to terminate the surface by C=C bonds, which will be polymerized with the coating material to establish covalent linkage between silicon surface and the coating. Alkyl-substituted silanes are widely used to react with the -OH terminated surface through condensation reaction between the silanol groups on the silane and the surface, and leaving C=C bond to react with the coating material. This type of silane is known as silane coupling agents. In general, the structure of silane coupling agents can be written as $\text{Si}(\text{OR}')_x\text{R}_{4-x}$, the R' position is usually occupied by hydrocarbons to prevent homo-condensation of the coupling agents during storage. The R position can be alkenes or acrylates to provide C=C for binding with the coating material. The simplified reaction scheme (Figure 14) of methoxysilane ($\text{Si}(\text{OCH}_3)_3\text{R}$) was given by W. Posthumus *et al.* [57]:

Figure 14 **Reaction scheme of silane coupling agents [57]**

Scheme A is the hydrolysis of Si-OCH₃, scheme B shows the condensation reaction with the OH- terminated surface, whereas scheme C is the homo-condensation. Scheme B and C are competing with each other, for the purpose of surface functionalization, scheme B should be dominating.

Solvent used to dissolve the silane coupling agent plays an important role in adhesion properties, study has shown that both aqueous and dry toluene are good solvent to produce high adhesion strength linkages [58]. If water is used as solvent, it was reported that at pH 4, homo-condensation was minimized [59], hence scheme B is dominating. To preserve the siloxane bond (Si-O-Si), basic environment shall be avoided (pH<9) [58].

2.2 Drug release mechanisms and control principles

Various designs of different drug delivery systems with distinct release behavior have been presented in the previous section. Theoretical foundations of those designs, contributing to different release characteristics, are the topic of this section.

2.2.1 Mathematical description of release mechanism of diffusion controlled DDS

Diffusion is one of the most commonly used driving forces for drug release. Hence this project will focus on diffusion controlled hydrogel drug delivery systems. In order to control the release rate and to understand the control principles used in the reviewed diffusion controlled systems, a comprehensive study of release mechanism from hydrogel DDS is provided in this part. The mathematical equations related to diffusion are cited from [60] unless otherwise stated.

Diffusion is a mass transport phenomenon from high concentration region to low concentration region and it is one of the most commonly utilized driving forces for drug release from reservoir or drug-loaded matrix. Diffusion is a result of random motion of particles due to thermal energy. This random motion is often modeled by ‘random walk’ theory [61], by which the famous Fick’s first law can be derived [1].

$$J = -D \frac{\partial C}{\partial x} \quad \text{Equation 1}$$

J is the flux (g/m²s), D is the diffusion coefficient (m²/s) in pure solvent and C is the concentration (g/m³). By coupling it with mass conservation, and assuming no bulk flow or chemical reaction, Fick’s second law can be written as:

$$D\nabla^2 C = \frac{\partial C}{\partial t}$$

where ∇^2 is the laplacian operator

Equation 2

$$\nabla^2 = \frac{\partial^2}{\partial^2 x} + \frac{\partial^2}{\partial^2 y} + \frac{\partial^2}{\partial^2 z}$$

2.2.1.1 Release mechanism from a simple matrix based system

Drug release from a matrix-based system is basically a diffusive transport process of drug molecule from a carrier to its exterior environment. The nature of this transport is the same as water transport during swelling/de-swelling. Therefore it is mathematically equivalent for both water and drug transport in a given DDS. The water transport will be discussed in this section, and the understanding of which can be extended to drug release.

In the hydrogel drug delivery systems, the first event happened to the hydrogel is absorption of water upon contacting with body fluid. The degree of swelling depends on the intrinsic property of the hydrogel and external stimuli. If the external environment does not change, hydrogel will absorb water and reach equilibrium.

The dynamic swelling behavior of hydrogel is often fitted to an empirical relation:

$$\frac{M_t}{M_\infty} = kt^n$$

where M_t is the amount of solvent absorbed at time t

Equation 3

M_∞ is the amount of solvent absorbed at equilibrium

Water absorption of the hydrogel can be described by three steps. 1, diffusion of solvent molecules into polymer networks; 2, relaxation of polymer chains from a glassy stage to a rubbery state by solvation; 3, polymer networks diffuse into the solvent.

One simple assumption to derive a theoretical model is that swelling is diffusion limited. Hence the water absorption can be modeled by solving Fick's second law. For a flat film with thickness L, the weight of the disc at swollen state is related to its initial weight by the following equation:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} (2n+1)^{-2} \exp[-D(2n+1)^2 \pi^2 t L^{-2}] \quad \text{Equation 4}$$

When $0 \leq \frac{M_t}{M_\infty} \leq 0.6$

$$\frac{M_t}{M_\infty} = 4 \left(\frac{Dt}{\pi L^2} \right)^{1/2} \quad \text{Equation 5}$$

The amount of solvent absorbed by hydrogel of various shapes can be expressed by [10]

$$\frac{M_t}{M_\infty} = 1 - \left(1 - \frac{k_0 t}{aC} \right)^N$$

where N=1 for flat film, N=2 for rod, and N=3 for sphere

k_0 is the relaxation constant

C is the solvent concentration

Equation 6

in the swollen region

a is the radius of the rod or sphere

or half the thickness of the flat film.

$n=0.5$ in the empirical equation corresponds to Fickian behavior, meaning that diffusion is the limiting factor for swelling. A value of $n=1$ indicates that diffusion is more rapid compared to rate of relaxation (case-II transport); therefore water absorption is limited by polymer chain relaxation. A value between 0.5 and 1 suggests that rate of diffusion is comparable to rate of relaxation. The order of the release profile is defined by the value of $n-1$.

Diffusion coefficient can be found by plotting M_t/M_∞ versus $\sqrt{t}$, and absorption type (n) can be found by taking the natural logarithm of Equation 6.

Equation 5 is valid for $0 \leq M_t / M_\infty \leq 0.6$, which is referred as early period of swelling.

As the degree of swelling increases, the rate of absorption decreases, which can be expressed by

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \exp\left(-\frac{\pi^2 D t}{h^2}\right)$$

Equation 7

$$(0.4 \leq \frac{M_t}{M_\infty} \leq 1)$$

If the hydrogel is loaded with drug, the drug release depends on both the rate of swelling and the rate of diffusion. If water absorption is more rapid than drug diffusion, the drug release can be described by the equations as we derived for water absorption ($n=0.5$). If the drug diffusion rate is more rapid than the rate of water absorption, the drug release depends on the rate of water absorption, which is case-II transport as discussed before ($n=1$). When the rate of water absorption is comparable to drug diffusion rate, the rate of drug release depends on both factors.

2.2.1.2 Release mechanism from a membrane based system

It can be seen from the review of different drug delivery systems, although it is more popular to use matrix based system for drug delivery, a lot of them require modifications to alter their release profile, and one of the most commonly used alteration method is to coat the surface of the original matrix with another material. Such systems are more similar to membrane-based systems when their release mechanism is concerned: the core serves the same function as the reservoir. Drug molecules have to dissolve into the coating/membrane first (partition step), and then diffuse along a concentration gradient in the coating to be released from the system. The release mechanism of such a system is more complex compared to a simple matrix

based system for two reasons: 1, drug diffuses in two different regions with different diffusivities; 2, besides diffusion there is an extra step governing the overall release rate, which is partition. The complication due to partition step is because only drug molecules inside the membrane/coating could be released, but drug concentration in the membrane might not be equal to the drug concentration in the donor/matrix. This scenario is illustrated in Figure 15 where C_D and C_R are the donor and receptor drug concentration respectively, C_A and C_B are the drug concentration in the membrane at two interfaces, L is the membrane thickness. Under perfect sink condition ($C_R(t) = 0$), it is reasonable to assume that $C_B = C_R$, but C_D is usually different from C_A , and the ratio between those two is called partition coefficient ($K_p = \frac{C_A}{C_D}$).

Figure 15 Illustration of diffusion process through membrane based system

Assuming the donor chamber to be an infinite source for diffusion, and a linear concentration distribution inside the membrane, a relation between concentration at both sides of the membrane can be established [62]:

$$\ln\left(\frac{C_A - 2C_B}{C_A}\right) = -2 \frac{AD}{V_B L} t \quad \text{Equation 8}$$

If we extend the domain of interest slightly to include a layer of water in both sides of the membrane then the above equation becomes:

$$\ln\left(\frac{C_D - 2C_B}{C_D}\right) = -2 \frac{AD_{eff}}{V_B L} t \quad \text{Equation 9}$$

where D_{eff} is the effective diffusion coefficient, taking into account of the transport from donor to the membrane. It is equal to

$$D_{eff} = K_p D \quad \text{Equation 10}$$

In practice, a finite period of time is needed to establish the equilibrium in the membrane. By non-steady state analysis, under perfect sink and infinite source condition, the flux from the donor to the receptor is

$$J(t) = -D \left(\frac{\partial C}{\partial x} \right)_{x=l} = 2C_A \sum_1^{\infty} \left(\frac{D}{\pi t} \right)^{\frac{1}{2}} \exp \left\{ - (2m+1)^2 l^2 / (4Dt) \right\} \quad \text{Equation 11}$$

The exponential term converges to unity as time goes by. By taking only the leading term, the flux at donor side of the membrane is simplified to [63]

$$\ln(t^{0.5} J_t) = \ln \left\{ 2C_A \left(\frac{D}{\pi} \right)^{0.5} \right\} - \frac{L^2}{4Dt} \quad \text{Equation 12}$$

According to this equation, partition coefficient and diffusivity can be separately calculated.

2.2.2 Control principles of diffusion controlled drug delivery systems

It is clear that diffusion and partition are two steps affecting the overall release rate for a lot of diffusion controlled DDS. Mathematical relationships among release rate,

diffusivity and partition coefficient has been reviewed in the previous subsection so that diffusivity and partition coefficient can be extracted from experimental data. It is the purpose of this section to review factors that may affect diffusivity and partition coefficient respectively, and to correlate those factors with synthesis or fabrication parameters.

2.2.2.1 Diffusivity

Diffusion coefficient is extremely important in diffusion because it measures the number of solute molecules being transported through a unit cross sectional area in a unit time, so it tells how fast a solute molecule could travel in a given system. Because diffusion is a type of motion, any factors, i.e. interaction between solute molecules, interaction between solute and solvent, barriers to move, that influence the mobility of a solute would affect the solute diffusion coefficient. In drug delivery devices, where drug (solute) is often released (through diffusion) from membrane or matrix, the release rate could be affected by all the factors that affect its diffusion coefficient. Control of drug release rate is one of the essential issues in drug delivery; therefore, it is the control of the diffusion coefficient critical in the design of drug delivery devices. In this section, limiting factors of diffusion coefficient and their mathematical models will be discussed.

The simplest case of diffusion in solution is a binary system where solutes are non-electrolytes, and the interaction between solutes is ignored (dilute solution). By considering thermal energy as a force exerted onto the solute, and assuming that solute molecules are sphere in shape (with radius a) and are much greater in diameter than solvent molecules, the well known Stokes-Einstein equation would be derived [64]:

$$D = \frac{k_B T}{6\pi\eta a} \quad \text{Equation 13}$$

Where k_B is Boltzmann's constant, $1.38054 \times 10^{-23} \text{ J / K}$, T is the absolute temperature and η is solvent viscosity. If the solute is not a sphere, yet its diffusion coefficient is known, the resulting radius (a) is called hydrodynamic radius. Sometimes, the denominator of the above equation is also known as the frictional drag coefficient, denoted by f .

The diffusion system described by Stokes-Einstein equation considers only hydrodynamic interactions between solute and solvent. In drug delivery systems, the medium that drugs diffuse through is not only composed of solvent, but also other components (i.e. polymer chains), which contribute to dragging forces in diffusion. The effect of the membrane or the matrix on diffusion could be described from different perspectives: porous and non-porous. In the porous consideration, polymeric chains are thought to construct randomly oriented channels, which allow solvent and solute to flow through. In the non-porous consideration, solute molecules dissolve in the membrane and diffuse through it [65]. As more dragging forces exist, the diffusion coefficient through polymers is generally smaller than that in pure solvent.

In most polymeric membranes, no distinct pores could be identified; hence drug release behavior can be better understood by the non-porous description. The permeation process through non-porous polymeric membrane could be more realistically described by a two-step process: solute dissolves in the membrane material and then diffuses through it down a concentration gradient. Two factors limit solute permeation: solubility and diffusivity. Membranes made of porous substrate and filled by selective soluble polymer were successfully applied to organic liquid separation

based on this two step-mechanism [66]. Models based on this description are therefore called solution-diffusion models. The fundamental assumption of solute-diffusion model is that the chemical potentials of the fluid at both sides of the membrane are in equilibrium with the adjacent membrane surfaces [67]. For passive diffusion where no chemical reaction is involved, this assumption is valid. Here, chemical potential is used because all the common driving forces of diffusion can be reduced to chemical potential gradient. The chemical potential of i th component in a system is given by [65]

$$u_i = u_i^0 + RT \ln(\gamma_i C_i) + v_i(p - p_{i,sat}) \quad \text{Equation 14}$$

μ_i^0 is the chemical potential of pure I at pressure $p_{i,sat}$, γ_i is the activity coefficient v_i is the molar volume of i th component and R is ideal gas constant

Because of the equilibrium between solution and the membrane, the chemical potential of the bulk solution and solution in the membrane is equal:

$$\mu_i^0 + RT \ln(\gamma_i C_i) + v_i(p - p_{i,sat}) = \mu_i^0 + RT \ln(\gamma_{i,m} C_{i,m}) + v_i(p - p_{i,sat}) \quad \text{Equation 15}$$

Therefore

$$C_m = \frac{\gamma_i}{\gamma_{i,m}} C_i = K_p C_i \quad \text{Equation 16}$$

Substitute this relation to the Fick's first law, where effective diffusion coefficient is used:

$$J = \frac{DK_p}{L} (C_A - C_B) \quad \text{Equation 17}$$

D measures the diffusivity of the solute in the membrane. Activity coefficient measures solute-solvent interaction, which will be discussed in more detail in the subsequent

section. For the sake of discussion, in the following sections, the effective diffusion coefficient is defined as the product of partition coefficient and diffusivity of the solute.

In the non-porous description of membrane, diffusion is a result of random movement through free volume in the membrane. Free volume is defined as the difference between the actual volume of a liquid and the minimum volume of its molecules packed firmly in contact with each other [68]. It could also be interpreted as the volume difference between temperature T and absolute zero. Molecule in the liquid at temperature T is moving at average thermal velocity but most of the time is confined to a cage bounded by their immediate neighbors. Fluctuation in molecule arrangement opens up a hole in the cage big enough to permit a considerable displacement of the molecule contained in the cage. Net-movement of the displaced molecule could only be resulted if another molecule jump into the hole before the displaced molecule returns to its original position. Diffusion occurs as a result of re-distribution of the free volume within the liquid. This description of diffusion by Cohen and Turnbulli [69] was originally derived to describe self-diffusion of liquid with impurities (solute). Because another molecule has to fill the void left by the displaced molecule, the free volume (difference between the cage volume and volume of the molecule) has to be large enough to permit another molecule to jump in after displacement.

By assuming immobile, impenetrable polymer chains suspended in a mobile solvent continuum, diffusion of solute may only proceed through the water phase permeating the whole membrane. The polymer molecules of the hydrated membrane are forming a network immersed in water and exhibiting a wide spectrum of holes. The solute can diffuse through the membrane if it finds a hole larger than the solute molecules. Average free volume in the membrane is contributed by water molecules and polymer

chains:

$$v_f = (1 - \phi_p)V_{f,water} + \phi_p V_{f,polymer} \quad \text{Equation 18}$$

By assumption, free volume contributed by polymer chains is not permeable to solutes, therefore:

$$v_f \cong (1 - \phi_p)V_{f,water} \quad \text{Equation 19}$$

Where ϕ_p is polymer volumetric fraction. Volume degree of swelling is

$$Q = \frac{1}{\phi_p} \quad \text{Equation 20}$$

In a solvent-solute binary system, solute diffusion coefficient is therefore proportional to the probability of finding a free volume, which is greater than its own volume. The total probability of finding a hole of volume exceeding v^* is [69]

$$P(v^*) = \exp[-\kappa v^* / v_f] \quad \text{Equation 21}$$

Where κ is a factor to correct for overlap of free volume ($0.5 \leq \kappa \leq 1$ [70]), and v_f is the average free volume. κ is introduced because total free volume (V_f) is not the summation of individual free volume v

In membranes, Peppas *et al.* [71] considered free volume as a permeating parameter and expressed the normalized diffusion coefficient as a function of polymer volume ratio and solute size:

$$\frac{D}{D_0} = f(\bar{M}_c) \exp\left(\frac{-v_{solute}}{V_{solvent} \left(\frac{1}{\phi_p} - 1\right)}\right) \quad \text{Equation 22}$$

$\bar{M}_c$ is the number average molecular weight between crosslinks. $f(\bar{M}_c)$ is the

probability function of finding a void big enough to allow diffusion, v_{solute} is solute volume, $v_{solvent}$ is the free volume of solvent. Molar weight between crosslinks $\overline{M_c}$ and crosslinking density ρ_x is determined using [72]:

$$\frac{1}{\overline{M_c}} = \frac{2}{\overline{M_n}} - \frac{(\overline{v}/\overline{V_1})[\ln(1-v_{2,s}) + v_{2,s} + \chi v_{2,s}^2]}{v_{2,r} \left[\left(\frac{v_{2,s}}{v_{2,r}} \right)^{1/3} - \frac{1}{2} \left(\frac{v_{2,s}}{v_{2,r}} \right) \right]}$$

$$\rho_x = \frac{1}{\overline{v} \overline{M_c}}$$

$$v_{2,r} = \frac{m_p / \rho_p}{(m_{membrane-air} - m_{membrane-hep}) / \rho_h}$$

$$v_{2,s} = \frac{m_p / \rho_p}{(m'_{membrane-air} - m'_{membrane-hep}) / \rho_h}$$

$$N = 2 \frac{\overline{M_c}}{M_r}$$

Equation 23

Volume of the polymer could be obtained by considering buoyancy. Conventionally, heptanes and air were chosen as the weighting medium because heptane does not induce swelling. After synthesis, sample was weighed in air $m_{membrane-air}$, and in heptane $m_{membrane-hep}$, after swelling, sample was weighed in air $m'_{membrane-air}$ and heptane $m'_{membrane-hep}$ again. Finally, sample was dried to a constant weight m_p . ρ_p and ρ_h are density of polymer and heptane respectively. $\overline{v}$ is the specific volume of the membrane (cm^3/g), $\overline{V_1}$ is the molar volume of water (volume per water molecule is $30\text{\AA}^3$ [73], therefore $\overline{V_1} = 18cm^3/mol$), $\overline{M_n}$ is number average molecular weight of the uncrosslinked polymer [74], and M_r is the molecular weight of the repeating unit, N is number of repeating units between two crosslinks. χ is Flory thermodynamic

interaction parameter χ varies between 0.49-0.51 for PVA [74]. For HEMA [78]:

$$\chi = 0.22 + 0.904v_{2,s} \quad \text{Equation 24}$$

The empirical relationship between glass transition temperature and $\overline{M}_c$ is also available [75]

$$\overline{M}_c = \frac{3.9 \times 10^4}{T_g - T_{g0}} \quad \text{Equation 25}$$

T_g is the glass transition temperature of the crosslinked polymer and T_{g0} is the glass transition temperature of the uncrosslinked polymer with the same chemical composition.

Instead of number average molecular weight between crosslinks, it is more physically sound to use a length scale in describing structure of the polymer for diffusion, ξ , which is defined as the radius of gyration of a chain between crosslinks or mesh size[76, 77]. Mesh size is proportional to the end-to-end distance of polymer chain:

$$\overline{r_0^2} = Nl^2C_n \quad \text{Equation 26}$$

l is the C-C bond length $1.54 \text{ \AA}$, C_n is the Flory characteristic ratio, or rigidity factor (6.95 for poly(methyl methacrylate) and poly(2-hydroxyethyl methacrylate) [78], whereas 8.9 for poly(vinyl alcohol) [74], and 14.4 in case of a methacrylate chain [79])
Mesh size is

$$\xi = Q^{1/3}(\overline{r_0^2})^{1/2} \quad \text{Equation 27}$$

In a highly swollen hydrogel, diffusion coefficient can be calculated from [76, 79] :

$$D \cong (1 - \frac{a}{\xi}) \exp(-\frac{Y}{Q-1}) \quad \text{Equation 28}$$

Y is a structural parameter, near unity. For most polymers, Y=1 is a good approximation [76]. In the case where mesh size is unknown, another form of Equation 28 was derived for a highly swollen hydrogel

$$D \cong aQ^{-3/4} \exp\left(-\frac{Y}{Q-1}\right) \quad \text{Equation 29}$$

2.2.2.2 Partition: Thermodynamics of mixture

In the non-porous description, diffusion and partition are considered two separate steps. Prior to diffusion step, the very first step in release mechanism is a partition step, whereby drug dissolves in the membrane phase. Although partition behavior of protein is an intensively studied topic in protein purification, partition behavior does not draw much attention so far in the area of controlled drug release. However, as the solution-diffusion model suggests, both partition and diffusion contributes to the effective diffusion coefficient of the solute hence both determine the rate of release through the membrane. Since partition coefficient is a measure of drug relative solubility in membrane as compared to the aqueous solution, the key of partition control is to control the solubility of drug in the membrane phase. Therefore, a solid understanding of the thermodynamics of solution/mixture is a must. A comprehensive review of basic thermodynamics is out of the scope of this thesis, only information relevant to this thesis is reviewed in this session. A more comprehensive introduction of basic thermodynamics can be found in some standard text books [80].

Thermodynamics, originated during 19th century, was originally applied to describe heat energy. After J. Willard Gibbs, who generalized the thermodynamics, a wide variety of phenomena both physical and chemical were successfully described by thermodynamic approach. Thermodynamic description is eventually an energy

approach. To discuss thermodynamic involved in solution/mixture, we start the discussion by defining a system under consideration. A solution could be considered a homogeneous open system, in which matter and energy are allowed to exchange with the surrounding. In a homogeneous open system, the total energy change dU is given by

$$dU = TdS - PdV + \sum_i \mu_i dn_i$$

$$\mu_i = \left(\frac{\partial U}{\partial n_i} \right)_{S,V,n_j}$$

Equation 30

n_i refers to all mole numbers of the component in the system.

The first term describes energy change due to heat exchange, the second term is energy change due to the work done to the system, and the last term accounts for the change in energy from matter exchange. μ_i is defined as chemical potential of component i.

By rearrangement of T, S, P and V in the above equation, we have different energy terms:

$$dH = TdS + VdP + \sum_i \mu_i dn_i$$

$$dA = -SdT - PdV + \sum_i \mu_i dn_i$$

$$dG = -SdT + VdP + \sum_i \mu_i dn_i$$

Equation 31

H is the enthalpy of the system, A is the Helmholtz energy and G is the Gibbs energy.

By definition

$$H = U + PV$$

$$A = U - TS$$

$$G = U + PV - TS = H - TS = A + PV$$

Equation 32

So that

$$\mu_i \equiv \left(\frac{\partial U}{\partial n_i} \right)_{S,V,n_j} = \left(\frac{\partial H}{\partial n_i} \right)_{S,P,n_j} = \left(\frac{\partial A}{\partial n_i} \right)_{T,V,n_j} = \left(\frac{\partial G}{\partial n_i} \right)_{T,P,n_j} \quad \text{Equation 33}$$

In all homogenous open systems

$$SdT - VdP + \sum_i \mu_i dn_i = 0 \quad \text{Equation 34}$$

To obtain the expression for chemical potential, we first start from chemical potential for pure, ideal gas.

$$\left(\frac{\partial \mu_i}{\partial P} \right) = v_i \quad \text{Equation 35}$$

v_i is the molar volume of component i. and by ideal gas law:

$$v_i = \frac{RT}{P} \quad \text{Equation 36}$$

And integrate at constant temperature:

$$\mu_i - \mu_i^0 = RT \ln \frac{P}{P_0} \quad \text{Equation 37}$$

This is the chemical potential change from isothermal process (P to P_0). For ideal gas, its partial pressure is proportional to its mole fraction in the system.

For a non-ideal substance (gas, liquid, solid), whose intermolecular interaction is not negligible, at constant temperature:

$$\mu_i - \mu_i^0 = RT \ln \frac{f_i}{f_i^0} \quad \text{Equation 38}$$

f is called fugacity. For ideal gas, f is equal to pressure, and for ideal gas mixture,

f_i is equal to its partial pressure. The ratio f / f_0 is the activity of the component denoted as a . the physical meaning of activity is an indication of how active a substance is relative to its standard state.

The change in Gibbs free energy of ideal solution is

$$\Delta G_{mix} = \Delta H_{mix} - T \Delta S_{mix} \quad \text{Equation 39}$$

In order to extend Gibbs free energy to real solution, an excess term is added to the ideal equation. The excess term is defined by the difference of real Gibbs free energy in mixing minus the Gibbs free energy predicted by ideal equation.

$$\mu_i - \mu_i^0 = RT \ln \gamma_i x_i = RT \ln x_i + RT \ln \gamma_i \quad \text{Equation 40}$$

Compare it with ideal solution; the last term is an excess chemical potential, since the partial molar Gibbs free energy is the chemical potential, partial molar excess Gibbs free energy can be expressed as:

$$\left(\frac{dg^E}{dn_i} \right)_{P,T,n_{j \neq i}} = RT \ln \gamma_i \quad \text{Equation 41}$$

$$\gamma_i \equiv \frac{a_i}{x_i}$$

And total excess Gibbs free energy is given by

$$g^E = RT \sum x_i \ln \gamma_i \quad \text{Equation 42}$$

2.2.2.2.1 Equilibrium condition of partition

The governing condition of partition at an equilibrium state is equal in chemical potential of all the components. Chemical potential equilibrium can be illustrated in aqueous two-phase system, which is widely used for protein purification. An aqueous

two-phase system (ATPS) will be formed if a thermodynamically incompatible substance is added into an aqueous medium and it is above some minimum concentration [81]. Protein separation can be achieved by adding proteins in ATPS because they will prefer to accumulate in one of the two phases (partitioning into one phase). The protein partition behavior is influenced by a few factors: pH, type of buffer, ionic strength, temperature and the phase forming solution.

Since ion in the protein solution redistributes until equilibrium is established, an electro-static potential exists at the interface. The chemical potential of protein at constant temperature and pressure is therefore also a function of ions in the solution

$$\mu_p = \mu_p^0 - \frac{z_p}{z_r} \mu_r^0 + RT \ln x_p \gamma_p - RT \frac{z_p}{z_r} \ln \gamma_r + z_i F \phi \quad \text{Equation 43}$$

F is Faraday's constant, z is net surface charge and ϕ is electric potential. Subscript p denotes protein, and r denotes a reference ion in the solution. At equilibrium, the partition of protein from top to the bottom is

$$\ln K_p = \ln \frac{x_B}{x_A} = \ln \frac{\gamma_p^A}{\gamma_p^B} - \frac{z_p}{z_r} \left[\ln \left(\frac{a_r^A}{a_r^B} \right) \right] \quad \text{Equation 44}$$

And partition coefficient of reference ion is

$$\ln K_{p,r} = \ln \frac{x_{r,B}}{x_{r,A}} = - \frac{z_r F}{RT} \Delta \phi \quad \text{Equation 45}$$

2.2.2.2.2 Partition of protein in hydrogel membrane

In ATPS, pressure is equal in the two phases. However in the hydrogel membrane, a higher pressure is expected in it because of the rigidity of the polymer structure, and

therefore the reference state of chemical potential in the membrane is at a higher pressure compared to aqueous phase

$$\mu_p^B = \mu_{pure\ p}^B(T, P + \pi) + RT \ln a_p^B \quad \text{Equation 46}$$

$$\mu_p^A = \mu_{pure\ p}^A(T, P + \pi) + RT \ln a_p^A \quad \text{Equation 47}$$

For a pure incompressible liquid $\left(\frac{\partial \mu}{\partial P}\right)_T = v$, so

$$\begin{aligned} \mu_p^B &= \mu_{pure\ p}^B(T, P) + \pi v_p + RT \ln a_p^B \\ \text{and} \\ \mu_{pure\ p}^B &= \mu_{pure\ p}^A \end{aligned} \quad \text{Equation 48}$$

By applying equilibrium condition

$$\mu_p^A = \mu_p^B \quad \text{Equation 49}$$

Therefore

$$\pi = (RT \ln \frac{a_p^A}{a_p^B}) / v_p \quad \text{Equation 50}$$

Thus partition coefficient is

$$\ln K_p = \ln \frac{\gamma_p^A}{\gamma_p^B} - \frac{\pi v_p}{RT} \quad \text{Equation 51}$$

As this equation suggests, higher the osmotic pressure in the membrane, lower the partition coefficient from aqueous phase to the membrane.

To figure out what are the factors that affect activity coefficient and how they affect it, we need the mathematical models to express activity coefficient in solution.

2.2.2.2.3 Excess function and activity coefficient (for non-electrolyte)

In the previous section, it can be seen that if activity coefficient of a component in a system can be calculated, its partition coefficient will be known. Moreover, in order to calculate activity coefficient, excess Gibbs' free energy (excess function) is needed. In this section, models of excess function and activity coefficient will be reviewed; therefore factors that affect activity coefficient, thus partition coefficient, can be understood.

For nonrandom solution containing non-electrolyte molecules of different size, the model Universal QUasi-Chemical theory (UNIQUAC) was developed based on the local composition description[82]. In UNIQUAC equation, excess Gibbs free energy has two parts, a combinatorial part that describes the dominant entropic contribution, and a residual part that is due primarily to short-range intermolecular forces that induce enthalpy contribution. The combinatorial part is affected only by the composition, sizes and shapes of the molecules of pure component.

$$\frac{g^E}{RT} = \left(\frac{g^E}{RT} \right)_{\text{combinatorial}} + \left(\frac{g^E}{RT} \right)_{\text{residual}} \quad \text{Equation 52}$$

For binary:

$$\left(\frac{g^E}{RT}\right)_{\text{combinatorial}} = x_1 \ln \frac{\Phi_1^*}{x_1} + x_2 \ln \frac{\Phi_2^*}{x_2} + \frac{z}{2} \left(x_1 q_1 \ln \frac{\theta_1}{\Phi_1^*} + x_2 q_2 \ln \frac{\theta_2}{\Phi_2^*} \right)$$

$$\left(\frac{g^E}{RT}\right)_{\text{residual}} = -x_1 q_1' \ln (\theta_1' + \theta_2' \tau_{21}) - x_2 q_2' \ln (\theta_2' + \theta_1' \tau_{21})$$

coordination number $z=10$

segment fraction

$$\Phi_1^* = \frac{x_1 r_1}{x_1 r_1 + x_2 r_2}$$

$$\Phi_2^* = \frac{x_2 r_2}{x_1 r_1 + x_2 r_2}$$

Equation 53

area fraction

$$\theta_1 = \frac{x_1 q_1}{x_1 q_1 + x_2 q_2}$$

$$\theta_2 = \frac{x_2 q_2}{x_1 q_1 + x_2 q_2}$$

$$\theta_1' = \frac{x_1 q_1'}{x_1 q_1' + x_2 q_2'}$$

$$\theta_2' = \frac{x_2 q_2'}{x_1 q_1' + x_2 q_2'}$$

R , q , and q' are pure-component molecular-structure constants. q' is surface of interaction and q is geometric external surface [82]. In the original derivation of Abrams [83], q' and q were not distinguished, but for water and lower alcohols (for water $r=0.92$, $q=1.4$, $q'=1$ [84]), they are different. Only for fluids other than water and lower alcohols, $q=q'$.

$$\tau_{12} = \exp\left(-\frac{g_{12} - g_{22}}{T}\right)$$

$$\tau_{21} = \exp\left(-\frac{g_{21} - g_{11}}{T}\right)$$

Equation 54

τ_{ij} is a adjustable parameter that accounts for energy interaction

Activity coefficients are given by

$$\begin{aligned}
 \ln \gamma_1 &= \ln \frac{\Phi_1^*}{x_1} + \frac{z}{2} q_1 \ln \frac{\theta_1}{\Phi_1^*} + \Phi_2^* \left(l_1 - \frac{r_1}{r_2} l_2 \right) \\
 &\quad - q_1' \ln (\theta_1' + \theta_2' \tau_{21}) + \theta_2' q_1' \left(\frac{\tau_{21}}{\theta_1' + \theta_2' \tau_{21}} - \frac{\tau_{12}}{\theta_2' + \theta_1' \tau_{12}} \right) \\
 \ln \gamma_2 &= \ln \frac{\Phi_2^*}{x_2} + \frac{z}{2} q_2 \ln \frac{\theta_2}{\Phi_2^*} + \Phi_1^* \left(l_2 - \frac{r_2}{r_1} l_1 \right) \\
 &\quad - q_2' \ln (\theta_2' + \theta_1' \tau_{12}) + \theta_1' q_2' \left(\frac{\tau_{12}}{\theta_2' + \theta_1' \tau_{12}} - \frac{\tau_{21}}{\theta_1' + \theta_2' \tau_{21}} \right)
 \end{aligned} \tag{Equation 55}$$

where

$$l_1 = \frac{z}{2} (r_1 - q_1) - (r_1 - 1)$$

$$l_2 = \frac{z}{2} (r_2 - q_2) - (r_2 - 1)$$

Multi-component equations:

$$\begin{aligned}
 \frac{g^E_{\text{combinatorial}}}{RT} &= \sum_{i=1}^m x_i \ln \frac{\Phi_i^*}{x_i} + \frac{z}{2} \sum_{i=1}^m q_i x_i \ln \frac{\theta_i}{\Phi_i^*} \\
 \frac{g^E_{\text{redidual}}}{RT} &= - \sum_{i=1}^m q_i' x_i \ln \left(\sum_{j=1}^m \theta_j' \tau_{ji} \right)
 \end{aligned}$$

where

$$\begin{aligned}
 \Phi_i^* &= \frac{r_i x_i}{\sum_{j=1}^m r_j x_j} & \theta_i &= \frac{q_i x_i}{\sum_{j=1}^m q_j x_j} & \theta_i' &= \frac{q_i' x_i}{\sum_{j=1}^m q_j' x_j} \\
 \tau_{ij} &= \exp \left(- \frac{g_{ij} - g_{jj}}{T} \right) & \text{and} & & \tau_{ji} &= \exp \left(- \frac{g_{ji} - g_{ii}}{T} \right)
 \end{aligned} \tag{Equation 56}$$

coordination number z is set to 10

$$\begin{aligned}
 \ln \gamma_i &= \ln \frac{\Phi_i^*}{x_i} + \frac{z}{2} q_i \ln \frac{\theta_i}{\Phi_i^*} + l_i - \frac{\Phi_i^*}{x_i} \sum_{j=1}^m x_j l_j \\
 &\quad - q_i' \ln \left(\sum_{j=1}^m \theta_j' \tau_{ji} \right) + q_i' - q_i' \sum_{j=1}^m \frac{\theta_j' \tau_{ij}}{\sum_{k=1}^m \theta_k' \tau_{kj}}
 \end{aligned} \tag{Equation 57}$$

where

$$l_j = \frac{z}{2} (r_j - q_j) - (r_j - 1)$$

The first three terms are due to combinatorial contribution and the rest three terms are

from the residual part of the excess free energy.

Although r does not correspond to the exact size of molecules, it measures the relative size of the solute over solvent. Relative size of the solute dramatically affects solute activity coefficient. The solute with bigger size is more sensitive to composition, whereas solvent activity coefficient is independent of solute size as long as protein solute is concerned.

2.3 Comment on the reviewed literatures

Different release profiles can be realized by various designs based on different materials and fabrication approaches. It is usually satisfied by observing a change in release profile after a new drug delivery system is proposed. However, quantification or precise control of release rate is still a challenging topic

In the attempt to control release rate quantitatively, diffusion and partition can be viewed as two distinct steps. However they are affected by the same factors: size of the solute, crosslinking density of the membrane, solute-membrane interaction and swelling ratio. Among those factors, crosslinking density and solute-membrane interaction can be controlled in different designs by using different crosslinking agents and different constitutional materials of the hydrogel; whereas swelling ratio is usually determined by the crosslinking density and the hydrophilicity of the material. For different designs, some fabrication variables (e.g. crosslinking density and composition) are changed to alter release profiles, however it is unclear to what extent does a modification affect diffusivity and partition coefficient respectively (does crosslinking density affect diffusivity more than partition coefficient?). There is no sign yet to control diffusivity and partition coefficient separately for a more precise control over release rate.

Chapter 3 Problem statement and proposed approach

We have reviewed different types of hydrogel based drug delivery systems and their release rate control strategies. The underlying principles for those strategies have been reviewed as well. Among different control principles, diffusion oriented methods apparently overweigh the partition oriented methods. This is partially because diffusivity and partition coefficient are affected by some common factors. Since diffusion is better understood, it is easier to target at diffusion control while taking any variation in partition coefficient as a byproduct and simply accept it.

Given the current research trend, even if diffusivity can be precisely controlled, the overall release rate remains problematic because partition coefficient is not purposefully controlled. Therefore it is important to develop new control methods that can tailor diffusivity and partition coefficient separately. The ultimate question is: how to control diffusivity and partition coefficient separately, or in another word: how to control partition coefficient without affecting diffusivity and vice versa.

From thermodynamic analysis, there is a possibility that the partition coefficient might be separately controlled while diffusivity might not be affected, that is total solute mole fraction in membrane. As discussed in the review section; solute will partition into the membrane from donor until the chemical potential of the solute in the membrane is equal to the chemical potential of the solute in the donor. It is hypothesized that partition coefficient might be controlled by immobilizing a certain amount of the solute molecule in the membrane, contributing to the overall chemical potential of solute in the membrane; hence the amount of free solute molecule from

donor that could partition into the membrane is reduced (Figure 16). The reduction in partition coefficient is because less amount of solute molecule is needed to balance its chemical potential in the donor due to the contribution of immobilized ones. On the other hand, although the net flux of diffusion is a function of chemical potential gradient, the diffusivity itself is not affected by the chemical potential. Therefore partition coefficient and diffusivity might be controlled separately.

Figure 16 Drug partition into the membrane without immobilized drug (left) and with immobilized drug ○ free drug coming from donor ● immobilized drug of the same type as free drug

The practical foundation behind this proposal is similar to the so-called ‘common ion effect’ in general chemistry. In a solution at a given condition (temperature, pressure), there is a solubility product, which is the product of anions and cations concentration of a solute at saturation. If the real concentration product is more than the solubility product, the solute will precipitate. It has been well documented that at a given condition, the solubility of NaCl will be reduced by the existence of AlCl₃ for example, because the contribution of the [Cl]⁻ from the AlCl₃ will only allow a smaller [Na]⁺ to exist in the solution before saturation. Since partition is essentially dissolution of a solute in the membrane, the same laws governing solubility should be applicable to the partition step. Hence by artificially introducing drug molecules in the membrane (as ‘common ion’), the ‘solubility’ of the drug coming from the donor shall be reduced. But a membrane is different from a beaker of solution in a way that the solute being artificially introduced to the membrane will be lost due to diffusion; hence their mobility shall be restricted to retain them inside the membrane. This can be done by

covalent binding of the solute molecule to the polymer chains, or immobilization.

From theoretical point of view, it is clear that current understanding of partition behavior in polymer solutions is based on interactions among solute, solvent and polymer chains, which is affected by: relative size of the solute and solvent, geometry of the molecules, spatial distribution and intermolecular forces; but ‘common ion’ effect is not caused by the factors described above, rather it is simply because of the presence of the common ions, which moves the chemical potential equilibrium according to Le Chatelier's Principle. The effect of interactions among different species on chemical potential equilibrium can be studied by the activity coefficient in the chemical potential equation (Equation 40), however the effect of ‘common ion’ on chemical potential equilibrium is not considered in the same equation. This is because in the governing equation of chemical potential, all the species in the system have to be independent (Equation 33): the amount of one species does not affect the amount of another. So the theoretical question that needs to be answered is how to take ‘dependence’ among different species in a system into consideration.

To prove the hypothesis, protein is going to be immobilized by reacting acryloyl chloride with the nitrogen of amine groups in the protein. Thermodynamic studies are to be carried out to deal with ‘dependent’ case and to establish a quantitative relation between partition coefficient and the amount of immobilization for a more precise control of overall release rate.

Chapter 4 Experimental details

Experimental procedure to realize the proposed partition control principle is detailed in this section. The principle is first tested in a pH-sensitive pulsatile drug delivery system and then applied to a hydrogel incorporated BioMEMS drug delivery device. In order to apply the principle in sustained drug delivery, microsphere embedded polymeric film is chosen because in this configuration, drug has to partition into the film before being released, hence partition control principle can be applied.

4.1 Drug Immobilization in hydrogel

In order to test the proposed partition control principle in pulsatile drug delivery systems, drug molecules need to be immobilized in the membrane so that they are not able to escape from it. A pH-sensitive hydrogel is chosen to be the membrane under investigation and Bovine Serum Albumin (BSA) is chosen as the model drug in this study.

4.1.1 Synthesis of hydrogel membrane

The monomers used were polyethylene glycol monomethacrylate (PEGMA, EG repeating unit molecular weight 400; Polysciences, Warrington, PA, pharmaceutical grade), 2-(diethylamino) ethyl methacrylate (DEAEMA, Sigma, analytical grade), tetraethylene glycol diacrylate (TEGDA, Sigma, analytical grade) and 2,2-dimethoxy-2-phenyl acetophenone (DMPA, Sigma, analytical grade). All the chemicals were used as received.

The synthesis follows the method described by Peppas *et al.* [85]: hydrogel without

drug immobilization was synthesized by adding 1mL Deionized (DI) water into 1mL ethanol drop-wise; followed by 4g PEGMA, and 2mL DEAEMA and followed by another 1mL DI. Crosslinking density was set to be 1 mol% TEGDA per mole of monomer (PEGMA+DEAEMA). The monomer mixture was stirred over night in a glass bottle. Just before synthesis, 3% w/w (total monomer) DMPA powder was added and stirred for two hours by a magnetic stirrer. The monomer mixture was put in an ultrasonic bath for 10 min in order to remove dissolved air, which acts as a free radical scavenger [86]. 100 μ L monomer was then pipetted between two glass slides (pre-cleaned by ethanol and then followed by DI water), which were separated by 200 μ m spacer (placed near the edge of the glass slides). The system was then exposed to UV light (Crosslinker, 365nm) for 2 min on each side at an intensity of 3 mW/cm^2 . The distance between the UV light source and the glass slide is 35cm, which is the height of the chamber of the UV crosslinker. The whole preparation was done at room temperature.

Since drug delivery systems are mainly designed for protein/peptide drugs, to demonstrate the effect of drug immobilization on drug release properties of pH-sensitive hydrogel, a well studied protein: Bovine Serum Albumin (BSA, Sigma, reagent grade) was chosen as a model protein drug (properties are summarized in Table 1).

Table 1 BSA properties

Property	Value	Data source
Molecular Weight (MW)	66430	Sigma
Number of amino groups per BSA molecule	583	Sigma
Number of disulfide bridges per BSA molecule	17	Sigma
Stoke radius	3.48nm	[87]
Diffusion coefficient in water at 25 °C	$6 \times 10^{-7} \text{ cm}^2 / \text{s}$	[88]
Surface charge	Refer to Table 2	

Table 2 Surface charge of BSA at various ion concentration and pH values [89]

Parameter	Ion Concentration	Value (mV)
Net Charge	I=1.0mol/L	+20 at pH 4.0; -13.5 at pH 6.0; -18.4 at pH 7.0; -22.9 at pH 8.0
	I=0.15mol/L	-20.4 at pH 7.4; -9.1 at pH 5.4; +4.5 at pH 4.7;

4.1.2 Drug immobilization in hydrogel

In order to immobilize BSA in the membrane, C=C was first introduced to BSA by covalent binding, which is the drug functionalization step. Functionalized BSA was immobilized in the membrane by two different methods: simultaneous immobilization and two-step immobilization. In the simultaneous immobilization method,

functionalized BSA was added into the monomer solution directly and they were immobilized while photo-crosslinking of the hydrogel took place. However because the solubility of BSA in organic solvent is lower than water, the amount of BSA can be immobilized in the membrane is limited by its organic solubility in simultaneous immobilization method. Another immobilization method (two-step immobilization) was used to immobilize BSA up to its water saturation concentration (40mg/mL): after synthesis of pH-sensitive hydrogel, C=C was bound to the membrane again to functionalize the membrane; soaking the functionalized membrane in functionalized BSA solution of various concentration so that functionalized BSA can be loaded into the membrane and then induce photo-polymerization. After polymerization BSA can be immobilized in the membrane.

4.1.2.1 Drug functionalization

The functionalization of BSA was done by first adding a certain amount of acryloyl chloride (Merck) into BSA solution (dissolve in pH=7.4 PBS) of various concentrations and reacted at 4 °C for 2 hr (Reaction 2) as described by Peppas *et al.* [85]. For simultaneous immobilization, 2 µL acryloyl chloride was added into 1 mL 20 mg/mL BSA solution. For two-step immobilization, different amount of acryloyl chloride was added into 50 mL BSA solution of different concentration according to Table 3.

Table 3 Reactant ratio between Bovine Serum Albumin and Acryloyl Chloride

Bovine Serum Albumin (mg/mL)	Acryloyl chloride (μ L)
5	40
10	80
20	160
40	320

4.1.2.2 Simultaneous immobilization

After functionalization of BSA, another bottle of monomer mixture was prepared as before, with the second 1mL DI being replaced by 1mL functionalized BSA, and added drop wise with constant stirring to prevent precipitation.

4.1.2.3 Two-step immobilization

To introduce C=C bonds to the synthesized membrane for protein immobilization, 100 μ L Acryloyl chloride was added into 50 mL DI water and 10 pieces of synthesized membranes were soaked in it. The beaker was kept at 4 °C for 24 hr. After functionalization of BSA and the membrane, the functionalized membranes were soaked in the functionalized BSA solution for three days to allow the functionalized BSA to load into the membranes. In the chamber of UV crosslinker, 100 μ L of the 20mg/mL DMPA solution (dissolved in ethanol) was applied to the surface of F-BSA

loaded membranes and then the UV polymerization was initiated. The procedure was repeated on both sides of the membrane, so that BSA was immobilized.

All the membranes were soaked in DI water at 4°C for a week with water changed daily to remove unreacted monomer or BSA.

4.2 Drug immobilization in hydrogel incorporated BioMEMS membrane

A ‘positively triggered’ drug delivery system is fabricated by incorporating the hydrogel into a microfabricated silicon membrane (Figure 17). The driving force of current design is diffusion, and the working mechanism is as follows: when the hydrogel swells, drug is released by diffusion; when the hydrogel shrinks, drug is retained in the reservoir due to restricted diffusion. Lastly, cytotoxicity study is conducted to ensure that the design is cell friendly, hence can be potentially used in physiological systems.

Figure 17 Positive (right) and negative (left) hydrogel incorporated membrane

The fabrication of the final device is accomplished by a three-step process: 1, silicon membrane fabrication; 2, silane functionalization; 3, hydrogel incorporation. In step 1, uniformly distributed holes were fabricated. To promote adhesion between silicon membrane and the hydrogel, C=C was chemically bound to the membrane, which

would be used to copolymerize with hydrogel. In the last step, liquid monomer mixture of the hydrogel was applied to the membrane followed by UV curing by conventional photolithography technique.

4.2.1 Membrane microfabrication

Figure 18 summarizes the silicon membrane fabrication process. Holes of 10 μm in diameter were etched to the desired depth by a multi-step Deep Reactive Ion Etching (DRIE, STS) process. The DRIE parameters are given in Table 4. The coil power and platen power were gradually increased to reduce the degree of undercut and preserve vertical profile for long-hours etching. After frontside etching, backside etching was conducted to release the membrane. The final membrane thickness is controlled by both front and backside etching depth.

Figure 18 Silicon membrane fabrication flowchart in crosssectional view: (a) bare silicon; (b) front side patterning and DRIE etching; (c) back side patterning and DRIE etching

Table 4 Multi step DRIE etching parameters for front side etching

Step	Etching Time (min)	Coil Power (W)	Platen Power (W)	Etching cycle			Passivation Cycle	
				SF6 flow rate (sccm)	O2 flow rate (sccm)	Etching cycle time (s)	C4F8 flow rate (sccm)	Passivation cycle time (s)
1	30	600	12	120	13	12	100	8
2	90	600	12	130	13	14	100	8
3	60	800	14	130	13	14	85	8

4.2.2 Surface functionalization

The fabricated membrane was first cleaned by a similar process as described in [53]. In short, the membrane was cleaned using $\text{H}_2\text{O}:\text{H}_2\text{O}_2:\text{NH}_4\text{OH}$ and $\text{H}_2\text{O}:\text{H}_2\text{O}_2:\text{HCl}$ both in (4:1:1) proportions at 80°C for 10 min followed by rinsing in deionized water (DI). After this process, the membrane was terminated by $-\text{OH}$ groups. A bi-functional silane coupling agent, γ -methacryloxypropyltrimethoxysilane (Sigma) was dissolved in 95% ethanol at 0.25% v/v and stirred for 5 min (functionalization step is reviewed in section 2.1.2.2). Membrane functionalization was done by treating the $-\text{OH}$ terminated membrane in the silane solution for 45 min at room temperature, and then dried by nitrogen followed by curing in air at 110°C for 30 min [58].

4.2.3 Hydrogel Incorporation

The monomer mixture of a pH-sensitive hydrogel was adjusted according to the same composition as described in section 4.1.1. The mixture was dipped onto the functionalized surface of the membrane. The dip-coated membrane was then placed vertically onto lab tissue paper so that excess monomer mixture could flow down to the tissue due to gravity. Because the mixture will flow through the membrane channels to the other side of the surface, dip-coated membrane was cured in a mask

aligner (Karl Suss, 365nm) for 2 min at both sides. The thickness of the membrane equilibrated at pH 7.4 was $4995 \pm 17.7 \mu\text{m}$ and the thickness of the membrane equilibrated at pH 6.5 was $6505 \pm 10.6 \mu\text{m}$ as measured by micrometer.

4.2.4 Drug immobilization in the BioMEMS membrane

Drug immobilization in the hydrogel incorporated membrane is the same as described in section 4.1.2.3.

4.3 Embedding microsphere in drug immobilized poly (ethylene glycol) film

Alginate (Sigma-Aldrich) was chosen to be the material for microsphere synthesis because it is one of the most common used polymers in drug delivery research: natural source, biocompatible, abundant and low price. Alginate microspheres were synthesized by a water/oil/water double emulsion method. Drug loading into the microsphere was achieved by two different methods: simultaneous loading and soaking. Drug loaded microspheres were re-dispersed in the monomer mixture of PEG before photo crosslinking.

4.3.1 Synthesis of alginate microspheres

Alginate was dissolved in 10 ml NaCl solution (9mg/mL) at a concentration of 20 mg/mL. 2mL alginate solution and 0.2 mL Span 85 were added into 10 mL isopropyl myristate. The mixture was processed by an ultrasonic processor for 5 min (5 W) (first emulsion). 0.1 mL Tween 80 was added into 1 mL isopropyl myristate and the mixture was added into the first emulsion and ultrasonically processed for another 5 min at the

same power (final emulsion). 7mL CaCl₂ solution (100mg/mL) was added into the final emulsion using a syringe and the mixture was stirred for 30 min at room temperature using a magnetic stirrer. The final mixture was centrifuged at 6000 rpm for 10 min to collect the synthesized microspheres. The collected microspheres were rinsed for three times in DI water followed by acetone and finally DI water.

4.3.2 Drug loading into the microspheres

Various concentration of BSA was dissolved in the Alginate solution so that BSA can be simultaneously loaded into the microsphere during synthesis. As for the soaking method, synthesized microspheres were dispersed into 20mg/mL FITC-BSA solution for two days in darkness for the FITC-BSA to diffuse into the microspheres.

4.3.3 UV polymerization of microsphere embedded poly(ethylene glycol) film

1mL ethanol was added into 8 g PEGMA. 450 μ L TEGDA was added into the PEGMA solution (10% crosslinking density). 3% w/w DMPA was added into the monomer solution and stirred for two hours to dissolve the photo initiator. The final monomer solution was equally divided into 6 parts. Microspheres were re-dispersed in different solvent and the dispersion was then added into the monomer mixture at constant stirring according to the composition in Table 5. It is worth noting that BSA loaded in the alginate is free to be released whereas the BSA in PEG precursor is to be immobilized in the film. The samples were named according to the solvent used for the synthesis, for example samples in part 3 was called 5 mg/mL immobilization in PEG, in short 5 mg/mL immobilization. The concentration of 5 mg/mL does not reflect the actual concentration of the immobilized BSA in the membrane.

Table 5 Composition of microsphere embedded film

Part	Solvent	Microspheres (mg/mL)
1	0.5 mL 0.1 mg/mL FITC-BSA solution	No
2	0.5 mL DI water	5
Part	Solvent: 0.5 mL functionalized BSA of different concentration (mg/mL):	Microspheres (mg/mL)
3	5	5
4	10	5
5	20	5
6	40	5

Finally, the microsphere containing monomer mixture was poured into a 24-well culture plate and cured by UV crosslinker (365 nm) for four minutes at both sides.

4.4 Characterization

Experimental procedures to characterize the structure of the drug immobilized membrane and its swelling and drug release profile are summarized in this section.

4.4.1 Swelling experiment

The hydrogel membranes were dried in a vacuum oven (1mBa) at 37 °C for two days. The pH response of the membrane was studied in phosphate buffer solutions with pH values between 4.5 and 7.4. To study the dynamic swelling behavior of the membrane, samples were weighted and immersed into PBS of pH 2.4 and pH 7.4 at room temperature for ten min. The sample was taken out every minute and weighted after carefully removing surface water. The equilibrium swelling ratio was obtained by leaving the membrane in the PBS for 24 hr. Both static and dynamic experiments were duplicated and student t test was used for statistical analysis. Swelling ratio was

calculated according to

$$swelling_ratio = \frac{m_{swollen} - m_{dry}}{m_{dry}}$$

$m_{swollen}$ is the membrane mass at swollen state and m_{dry} is the mass when the membrane is dry.

The dynamic swelling behavior was characterized by fitting the data into an empirical relationship as shown in Equation 3.

4.4.2 Particle size and Florescence microscopy

Particle size of alginate microspheres were characterized by a particle size analyzer. Florescence microscopy was used in two different situations: 1, verify that drug can be immobilized in the membrane by the method described above (using FITC-BSA as the model drug for immobilization); 2, visualize the microsphere embedded PEG film.

4.4.2.1 Florescence of drug immobilized hydrogel

In order to prove that protein can be retained in the hydrogel by simultaneous immobilization method, the fluorescein isothiocyanate labeled BSA (FITC-BSA) was functionalized and immobilized according to the presented procedure. After soaking the FITC-BSA immobilized membrane in DI water for a week with water changed daily (stored at 4°C), the membrane was imaged by a fluorescence microscope (Carl Zeiss, Germany), with 10X objective lens and 40ms exposure time.

4.4.2.2 Florescence of microsphere embedded PEG film

The PEG monomer containing FITC-BSA loaded microsphere was dipped onto a piece

of glass slide and then induce photo-polymerization in a UV Crosslinker (365nm). The cured film was observed by a fluorescence microscope (Carl Ziess, Germany).

4.4.3 X-ray Photoelectron Spectroscopy (XPS)

XPS was conducted to 1, qualitatively differentiate the amount of drug immobilization in the pH-sensitive hydrogel by different soaking concentration; 2, prove surface functionalization of the silicon membrane was properly done.

4.4.3.1 XPS of drug immobilized pH-sensitive hydrogel

Washed pH-sensitive hydrogel membranes were dried in air for 8 hours and then put into a vacuum oven at 37 °C for three days to completely dry the samples. Vacuum dried samples were analyzed by X-ray Photoelectron Spectroscopy (XPS, Kratos AXIS Ultra) with monochromatic Al K α (1486.71 eV) X-ray radiation. The surface scan was done with a pass-energy of 160 eV, whereas narrow scan was done with a pass-energy of 40 eV. Two different points were analyzed for each sample.

4.4.3.2 XPS of functionalized silicon membrane

Functionalized silicon membrane was dried in air and transferred to the transfer chamber of XPS equipment. Same analysis parameter was used as for pH-sensitive hydrogel membranes. Two points were analyzed for each sample.

4.4.4 Scanning electron microscopy (SEM)

SEM (JEOL JSM-5600L) was used to observe the structure of the fabricated BioMEMS drug delivery device and the internal structure of alginate microsphere embedded PEG film. Hydrogel incorporated BioMEMS device and the microsphere embedded film were first vacuum dried for 24 hr. A layer of gold was sputtered onto

sample surface before SEM observation (secondary electron mode). Samples were mounted on the sample holder by conductive double-sided tape. The accelerating voltage was 10 keV.

4.4.5 Drug loading efficiency determination

Loading efficiency of alginate microspheres were studied by unloading method: 5mg drug loaded microspheres were suspended in 3mL PBS buffer (pH 7.4) for 3 days. The unloading medium was centrifuged at 6000 rpm for 10 min and the aqueous phase was measured by UV spectrophotometer to determine the concentration of the protein.

Loading efficiency was calculated according to

$$\text{Loading Efficiency} = \frac{m_{\text{protein}}}{m_{\text{microsphere}}}$$

Where m_{protein} is the mass of protein that is unloaded and $m_{\text{microsphere}}$ is the amount of microsphere used for unloading experiment. Student t test was used for statistical analysis.

4.4.6 Release experiment

Two different types of release experiment were conducted according to the nature of the delivery system (all release experiments were conducted at room temperature of 25 °C):

1. Membrane based system: membrane was clamped between two glass cells, one of which serves as donor and the other serves as receptor (Figure 19). Donor concentration was kept constant to ensure infinite donor condition and receptor solution was taken out at regular intervals to measure concentration of the drug being released.

Figure 19 Home made side-by-side diffusion cell

2. Matrix based system: drug loaded microsphere embedded PEG film or drug immobilized PEG film was soaked in PBS buffer (pH 7.4) of known volume. The soaking buffer was taken out completely at regular intervals and drug concentration in the buffer was measured.

4.4.6.1 Release experiment for membrane based systems

Release experiment of membrane based system was conducted by a pair of home-made side-by-side diffusion cell with inner diameter of 1cm. The diffusion cell consists of two glass chambers with the volume of 3mL. Precisely weighed BSA was dissolved in PBS of desired pH and stored at 4 °C before use. Samples that were equilibrated at that pH were used for diffusion experiment. 2mL 5mg/mL BSA solution was loaded into the donor chamber and 2mL fresh PBS of the same pH was loaded into the acceptor chamber. Acceptor solution was completely taken out at predetermined interval for measurement and fresh solution of the same volume was refilled into the acceptor and restart timing. The sampling interval chosen was 0.5hr, 1hr, 1.5hr, 2hr, 2.5hr and 3hr.

Collected acceptor solution was measured using a UV-Vis spectrophotometer (Shimadzu UV-2450) at 278.5nm, the concentration was obtained from a standard curve, which was calibrated at each pH. The diffusion experiment for each type of membrane at a given pH value was repeated for 2-5 times: release experiment for simultaneous immobilized membrane (section 4.1.2.2) was repeated for 5 times and release experiment for two-step immobilized membrane (section 4.1.2.3) was repeated twice. Diffusivity and partition coefficient were calculated by Equation 12. Student t test was used for statistical analysis.

4.4.6.2 Release experiment for matrix based systems

Matrices were placed in 12-well culture plate. 3m PBS buffer (pH 7.4) was added to each well. Drug immobilized microsphere embedded system cannot be pre-rinsed to remove un-reacted monomers or un-reacted functionalized BSA. In order to measure the amount of BSA that is released from the microsphere, the loaded BSA has to be differentiated from the immobilized ones. FITC-BSA was used for loading and normal BSA was used for immobilization. The buffer was taken out at pre-determined time for measurement and fresh buffer was re-filled. The measurement of the buffer was done by UV-Vis at 491 nm, and the concentration was calculated according to a standard line. All the experiment was done at room temperature and was duplicated.

For release experiment of drug loaded microsphere alone, FITC-BSA loaded microspheres were suspended in 3mL PBS (pH 7.4) in a centrifuge tube. The tube was gently shaken during the course of the experiment. 10 min before pre-determined time, the tube was centrifuged at 6000 rpm for 10 min and the concentration of FITC-BSA in the aqueous phase was measured. Fresh buffer was refilled to the tube after each

measurement. The experiment was repeated twice.

4.4.7 Partition coefficient Measurement

To double confirm the partition coefficient calculated from release experiment, membranes after each diffusion experiment were rinsed once by dipping into DI water to remove the absorbed BSA on the membrane surface, followed by removing the surface water of the membrane using lab tissue; the membrane was then immersed into 3 mL PBS solution (un-loading medium) at the same pH level as the diffusion experiment. After 12 hr (the entire drug will be unloaded, predicted by simulation shown in appendix A), the BSA concentration in the un-loading medium was measured by UV-Vis spectrophotometer to determine the total amount of protein dissolved in the membrane during diffusion experiment. As for the diffusion experiment, the partition coefficient measurement was done 5 times and student t test was used for statistical analysis.

The average BSA concentration in the membrane was calculated by dividing the total amount of BSA dissolved it by the total solvent volume in the membrane. Under steady-state, assuming infinite source (donor concentration is constant) and perfect sink condition (acceptor concentration is zero) as in the diffusion experiment, a linear concentration gradient (assuming constant diffusivity in the membrane) is established in the membrane; hence the solute concentration at the donor side of the membrane is twice of the average solute concentration in the membrane.

4.4.8 Cell viability

The NCTC clone 929 (Connective tissue, mouse) clone of strain L or L929 were obtained from American Type Culture Collection (ATCC). Cell lines were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated Premium Fetal Bovine Serum (FBS) (lonza) and 1% Penicillin-Streptomycin-Glutamine (Antibiotic) (Gibco) maintained at 37 °C in 5% CO₂.

The Cell Titer 96 Aqueous One Solution Assay (Promega) is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays. The One Solution contains a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and an electron-coupling reagent (phenazine ethosulfate; PES). The MTS compound is bio-reduced by living cells into a colored formazan product that is soluble in tissue culture medium. The quantity of formazan product as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture.

Hydrogels were soaked in distilled water for a week to unload un-reacted PEG monomers. The gel without BSA immobilization was then soaked in acetone for another week. They were sterilized by washing in 70% ethanol, DMEM medium and Phosphate Buffered saline (PBS without Calcium and Magnesium ions) and finally with sterile distilled water before the cell culture. L929 cells were cultured on the samples with the seeding density of 2×10^3 cells/well/100 μ L in a 24-well tissue culture plate for 24hours. They were then transferred in sterile conditions to a new 24-well tissue cultured plate and fresh 500 μ l of DMEM medium was added. Then, 100 μ l of Cell Titer 96 Aqueous One Solution (Promega) were added into the medium and incubated at 37 $^{\circ}$ C for 4 hours. After 4 hours of incubation, the absorbencies of samples were measured at 490nm in a Micro plate Reader. For reference purposes, cells were cultured at the same density on the bare culture plate (control). The cell viability was calculated as a ratio of sample absorbance versus the absorbance of the control. Each experiment was done in triplicate.

Chapter 5 Results

Results of the designed experiments are presented in this chapter. Detailed analysis and discussion are provided in the subsequent chapter. In this chapter, proof of concept results of the drug immobilized hydrogel are presented first to prove that the proposed control method works. Since the control principle has been applied to two different systems: pulsatile and sustained, results of each of the systems are then presented.

5.1 Proof of concept: drug immobilized hydrogel

The results of drug immobilized hydrogel are presented in this section. XPS and fluorescence are used to prove the success of immobilization; swelling behavior and release profile as a function of immobilized drug are also presented to prove the concept: drug immobilization in the membrane could control partition behavior of the free drug whereas diffusivity of the free drug is not affected.

5.1.1 X-ray photoelectron spectroscopy (XPS) and Florescence

Before the drug release results after drug immobilization is reported, the first question to be addressed is whether the BSA can be successfully immobilized in the dydrogel using the experimental procedure described. If BSA is not immobilized, all of them will be released from the membrane after one week rinsing. The florescence image of FITC-BSA un-immobilized and immobilized membranes are shown in Figure 20. Uniform green luminance proves that BSA is successfully immobilized in the membrane according the simultaneous immobilization procedure described in section 4.1.2.2 and 4.4.2.1.

Figure 20 Florescence image of simultaneously drug immobilized hydrogel: no FITC-BSA immobilized (upper); with FITC-BSA immobilized (bottom)

XPS was used to characterize the two-step immobilized hydrogels (section 4.1.2.3). There are three carbon peaks observed in all samples (Figure 21): C1 at 285 eV corresponds to C-H, C2 at 288.6 eV corresponds to C-O and C3 at 288.3 corresponds to amide or carboxyl group [90]. The percentage of C3 in all carbon can be used as fingerprint of protein immobilization because 23.55% of carbon in BSA has binding energy of 288.6 whereas only 8.33% of carbon in the membrane has the same binding energy. If BSA is successfully immobilized in the membrane the percentage of C3 should increase. In the two-step immobilization method, functionalized membrane (f-membrane) is soaked in functionalized BSA solution of various concentrations so that

different amount of functionalized BSA can be loaded into the membrane. It can be seen from Figure 21 that percentage of C3 in the membrane is increased as the drug concentration used for soaking is increased. It means that more BSA can be immobilized in the membrane by more concentrated BSA solution. Therefore the amount of immobilization can be controlled by soaking concentration. However because XPS is only valid for relative ratio calculation, the exact amount of BSA immobilization cannot be calculated based on XPS result. Thus XPS verification can only establish a qualitative relation between the amount of immobilization and the soaking concentration.

Figure 21 XPS spectrum of C 1s narrow scan of two-step drug immobilized hydrogel (upper); C3 content as a function of immobilization level (bottom)

5.1.2 Swelling behavior

Both static and dynamic swelling behaviors of BSA immobilized hydrogel were tested. In each of the category, results are presented according to the sample preparation

procedure: simultaneous immobilization and two step immobilization.

5.1.2.1 Static swelling result

Two different methods are used to immobilize BSA in pH-sensitive hydrogels: simultaneous immobilization and two-step immobilization. For simultaneously immobilized hydrogel, the swelling ratio at each pH is not affected by BSA immobilization expect for pH 7 as no significant difference was observed using a paried student t test at each pH ($p=0.05$), expect for pH 7(Figure 22). Therefore swelling transition with respect to pH (transition pH value) is sharpened with BSA immobilization.

Figure 22 Static swelling of simultaneously drug immobilized hydrogel

As for two-step immobilization, the amount of immobilization can be easily changed by the soaking concentration; therefore a series of swelling experiment with different amount of immobilization is conducted. At each immobilization level, transition pH values are all about the same at around pH 7 (Figure 23). Same transition sharpening effect can be observed after BSA immobilization, which is indicated by the swelling ratio at pH 6.8: without immobilization, the swelling ratio at pH 6.8 is lower than that

with BSA immobilization (Figure 24). It means that BSA immobilized pH-sensitive hydrogel is more sensitive to pH changes at the transition pH.

Figure 23 Static swelling of two-step drug immobilized hydrogel synthesized at different soaking concentration

Figure 24 Swelling ratio comparison of two-step drug immobilized hydrogel at different pH values

5.1.2.2 Dynamic swelling results

The dynamic swelling result of hydrogel by simultaneous immobilization method is shown in Figure 25. Hydrogels with and without BSA immobilization show rapid swelling profiles at lower pH values. But the swelling rate at each pH value is not significantly affected by BSA immobilization using a student t test ($p=0.05$), especially during early stage of swelling.

Figure 25 Dynamic swelling of simultaneously drug immobilized hydrogel at pH 2.4 and pH 7.4

Equation 3 is used to fit the dynamic swelling result for both simultaneous and two-step immobilization methods. Fitting parameters are obtained by fitting the data using a power law function in the range of $0 < M_t / M_0 < 0.6$. The fitting was performed by a Scaled Levenberg-Marquardt algorithm without weighting. A typical fitting curve is given in Figure 26. The results are summarized in Table 6. Value of n indicates the mechanism of swelling: 0.5 means diffusion dominates and 1 means network relaxation dominates.

Figure 26 Curve fitting of the dynamic swelling ratio

Table 6 Order of swelling kinetics before and after drug immobilization

Immobilization method	Sample	pH	k	n	R^2
Simultaneous	Without BSA	7.4	0.257 ± 0.0356	0.671 ± 0.0209	0.992
		2.4	0.200 ± 0.0259	0.813 ± 0.0138	0.995
	With BSA	7.4	0.275 ± 0.0141	0.601 ± 0.0224	0.993
		2.4	0.207 ± 0.0139	0.655 ± 0.0412	0.999
Two-step	5 mg/ml	7.4	0.303 ± 0.0202	0.516 ± 0.0202	0.953
		2.5	0.234 ± 0.0279	0.571 ± 0.0279	0.97
	10 mg/mL	7.4	0.269 ± 0.0378	0.565 ± 0.0378	0.996
		2.5	0.222 ± 0.00906	0.622 ± 0.00906	0.991
	20 mg/mL	7.4	0.429 ± 0.0154	0.571 ± 0.0402	0.996
		2.5	0.32 ± 0.0628	0.671 ± 0.0628	0.938
	40 mg/mL	7.4	0.343 ± 0.0067	$0.612 \pm 0.022.17$	0.999
		2.5	0.306 ± 0.0163	0.703 ± 0.0163	0.995

For each sample, n is higher at lower pH compared to high pH, indicating that extra force is available to expand polymer network at lower pH (Figure 27). This is because of the charge carried by nitrogen at lower pH value, which provides electrostatic repulsion force for swelling. After immobilization, n values at each pH are smaller compared to the corresponding gel without BSA immobilization (Figure 27), which means drug immobilization generally restricts network relaxation. However among all the BSA immobilized samples, n increases as more BSA is immobilized. It suggests that BSA immobilization in the hydrogel has two competing effects: relaxation restriction and extra driving force for expansion.

Figure 27 Comparison of order of release kinetics at pH 7.4 and pH 2.5

5.1.3 Release rate

The release rate of membrane based system can be calculated according to an ideal condition: infinite source and perfect sink. Infinite source of the donor is ensured by refilling the donor chamber with drug solution of known concentration in the end of each measurement interval; whereas the perfect sink condition is maintained by refilling the receptor chamber with fresh buffer. Under this condition, flux of drug release from donor to receptor is constant once concentration equilibrium in the membrane is established, which can be used for release rate comparison. However because both diffusion and partition play a role in the final flux (section 2.2.1.2), diffusion coefficient and partition coefficient are extracted from release result (partition coefficient is also separately measured from drug loading and unloading experiment) so that the underlying course of flux difference can be studied.

5.1.3.1 Flux of BSA

By simultaneous immobilization method, the flux of BSA release at pH 7.4 is reduced by 35.5% compared to the case without BSA immobilization in the membrane (Figure 28).

Figure 28 Flux of simultaneously drug immobilized hydrogel at pH 7.4

To further examine the effect of BSA immobilization on BSA release rate, a series of membranes with different amount of immobilization is studied with respect to pH changes (Figure 29): as immobilization level increases, flux at both low and high pH decreases.

Figure 29 Flux of two-step drug immobilized hydrogel

At each immobilization level, the changes in the flux at different pH values are different (Figure 30). The reduction in flux is increased with more drug immobilization, which is desirable in pulsatile drug delivery systems because a big change in flux between on and off state enables an effective switching.

Figure 30 Change in flux with respect to pH variation at different soaking concentrations (two-step immobilization method)

5.1.3.2 Diffusion coefficient

Although the overall flux was reduced after BSA immobilization, the diffusion coefficient of free BSA in the membrane is not significantly affected by immobilization (Figure 31). Rather than immobilization amount, pH is a dominant factor that determines the diffusivity of the free BSA in the membrane.

Figure 31 Comparison of diffusion coefficient between pH 7.4 and pH 6.5 at different soaking concentrations (two-step immobilization method)

5.1.3.3 Partition coefficient

Since diffusivity is not affected by immobilization, another factor (partition coefficient) that determines the overall flux has to be assessed against different immobilization levels. It can be seen from Figure 32 that partition coefficient is not just sensitive to pH changes; it is also a function of immobilization levels. The trend of partition coefficient as a function of immobilization level is similar to that of flux. The sensitivity of partition coefficient with respect to immobilization level is gradually reduced as

immobilization level increases, as suggested by the reducing slope of the curve. Since diffusivity is not affected by drug immobilization in the membrane, whereas partition coefficient reduces after immobilization, it can be concluded that the reduction in the flux with immobilization is resulted from the partition perspective rather than diffusion perspective. Hence, the proposed partition control method works.

Figure 32 Comparison of partition coefficient between pH 7.4 and pH 6.5 at different soaking concentrations (two-step immobilization method)

Once a certain amount of BSA is immobilized, its mass is constant regardless of the swelling ratio of the membrane. But the amount of solvent in the membrane is different at different swelling ratios, which translates to different concentration of the immobilized BSA in the membrane. Based on the swelling data, the concentration of the immobilized drug in the membrane at different pH values can be calculated and a plot (Figure 33), which correlates partition coefficient and concentration of the immobilized drug in the membrane, can be established.

Figure 33 Partition coefficient as a function as immobilization levels

5.1.4 Cell viability

It can be seen from Figure 34 that hydrogel without drug immobilization has the highest cell viability and it decreases as BSA loading concentration increases. Although cell viability decreases after drug immobilization, it is still about 70% viable at 40mg/mL loading level, suggesting that BSA immobilization does not cause severe toxicity to epithelial tissues.

Figure 34 Cell viability with different amount of immobilization

5.2 Application of partition control principle in hydrogel incorporated BioMEMS membrane

Swollen hydrogel based on the materials and composition in this project is very fragile, which limits its use in practical situations. In order to bring it one step closer to application, a hybrid device which combines silicon membrane and hydrogel is proposed. The fabrication of the device is presented first followed by the characterization results.

5.2.1 X-ray Photoelectron Spectroscopy

Surface functionalization by γ -methacryloxypropyltrimethoxysilane will introduce new elements to the silicon surface; most noticeably oxygen, which can be verified by the strong O 1s peak (Figure 35). However it is known that silicon is easily oxidized at room temperature, therefore a native oxide layer is present on the surface. Moreover – OH group is introduced to silicon prior to silane functionalization, therefore O 1s peak alone does not confirm the silane functionalization. The same argument can be applied to C 1s since natural contaminations in air could easily introduce carbon to the sample surface. But natural contaminants are usually absorbed gas molecules such as CO₂ or CO, the existence of C3 peak, which corresponds to carboxyl group can be used as the fingerprint of silane functionalization since it cannot be introduced by natural contaminants.

Figure 35 XPS spectra of silane functionalized silicon membrane (upper: surface scan; bottom: C 1s narrow scan)

5.2.2 Scanning electron microscopy observation

Arrays of micro-channels are etched with spaces in between the channel arrays to maintain its mechanical integrity (Figure 36 (a) and (b)). The channels are released by backside etching, which can be confirmed by microscopic inspection from the back (Figure 36 (c)) and cross-section (Figure 36 (e)) of the device. The length of the final channel is determined by both front and back side etching. The diameter of the channel is larger than the pattern due to long hour's ion etching. However since the function of

the membrane is to provide mechanical support for the hydrogel while allowing the drug to pass through, as long as the walls separating each channel are not fully etched away, the device functionality will not be compromised.

The hydrogel incorporated membrane is shown in Figure 37. The monomer mixture of hydrogel exhibits both hydrophilic and hydrophobic properties; hence it is easy for the mixture to flow into the channel during incorporation procedure. The final device thickness is defined by the total thickness of the hydrogel and silicon, which is found to be 494 ± 25 μm at pH 7.4 and 647 ± 18 μm at pH 6.5.

Figure 36 SEM of fabricated silicon membrane

Figure 37 SEM of hydrogel incorporated silicon membrane

5.2.3 Release rate

As hydrogel is incorporated into the membrane, the flux of the membrane shows pH dependence (Figure 38). Similar flux reduction effect can be observed after BSA immobilization in the hydrogel. However the flux changes with respect to pH changes at each immobilization level is minor due to big variations of membrane thickness at different pH values ($494 \pm 25 \mu\text{m}$ at pH 7.4 and $647 \pm 18 \mu\text{m}$ at pH 6.5).

Figure 38 Flux of BSA from hydrogel incorporated membrane at different soaking levels: pH 7.4 for on state and pH 6.5 for off state

Compared to hydrogel membrane, it was observed (by visual inspection and micrometer measurement) that volume expansion direction of incorporated hydrogel upon pH changes is different from free hydrogel. The major expansion axis of free membrane is planar (thickness does not change much after swelling), whereas the major expansion axis of incorporated hydrogel is vertical to the planar direction (Figure 39).

Figure 39 Illustration of expansion direction of hydrogel

It is because the difference in the synthesis condition: for free hydrogel membrane

synthesis, two glass slides are used to clamp the gel precursor, so that gel formation along planar direction is less restricted by external forces therefore it is easier for gel to expand its volume along the direction with less restriction; whereas for incorporated hydrogel synthesis, gel precursor is simply applied to the silicon membrane, there is no external force being applied to the vertical direction, but horizontal direction (or planner direction) of the gel formation is restricted by the channel walls of the silicon membrane, which serve a similar function as the clamping glass slides in the free membrane case, thus gel tends to expand its volume along vertical direction.

5.3 Application of partition control principle in microsphere embedded polymeric film

To demonstrate the applicability of the partition control principle in sustained drug delivery, a microsphere embedded polymeric system is developed (section 2.1.1.1.2). In such systems, drug has to dissolve in the polymeric film first (partition step) and then diffuse out of the system. Therefore the overall drug release rate can be controlled by the partition control scheme previously used in pulsatile systems.

5.3.1 SEM and florescence observation

In drug-loaded films/matrices, drug molecules are evenly distributed everywhere in the film. The drug near the surface will be release quickly, which gives rise to burst release. As discussed in section 2.1.1.1.1, surface crosslinking of the matrices or films can be a feasible approach to reduce burst release and to achieve a delayed formulation. Another approach is to isolate drug molecules from the boundary of the system before release, which can be done by loading the drug in the microsphere and then dispersing drug loaded microsphere into the film/matrix. The SEM images show that micron-sized particles ($25 \pm 4.5 \text{ }\mu\text{m}$) are distributed in the film and from the florescence image

(Figure 40), it can be seen that drug molecules are localized as compared to Figure 20.

Figure 40 Florescence and SEM images of microsphere embedded film, and the particle size distribution curve

5.3.2 Loading efficiency

Drug loading efficiency at different concentration is shown in Figure 41. It is observed that loading efficiency increases first with higher concentration, but decreases when drug concentration exceeds 25mg/mL. The increase in the loading efficiency at higher

drug concentration is because more drugs are distributed in a unit volume of precursor solution so that more drug molecules can be trapped during microsphere formation. Whereas when drug concentration further increases, electrostatic repulsion between the drug molecules and forming microspheres dominates, which prevents more drug to be trapped in the microsphere.

Figure 41 Loading efficiency of BSA in alginate microspheres at different drug concentration

5.3.3 Release

Drug loaded microsphere releases its content in six hours (Figure 42). By simply embedding the microsphere in PEG film, drug can be released in a linear manner for 70 hours, which is 10-fold longer. The duration can be further extended to 170 hours (7 days) by 5mg/mL drug immobilization in the PEG matrix.

Figure 42 Accumulative release of FITC-BSA from microsphere embedded film within 15 days

With different amount of immobilization in the matrix, the duration of release can be further extended to two weeks (Figure 43). Moreover a silent period is observed at high immobilization levels, which is similar to surface crosslinked gels as discussed in section 2.1.1.1.1. The silent period is resulted from the fact that drug has to partition into the matrix and diffuse to the surface before it can be released. As long as the overall transport in the matrix is slower and drug molecules are not distributed near the surface, a silent period can be realized. In the surface crosslinked case, both partition and diffusion are reduced due to the existence of the highly crosslinked phase. In the drug immobilized case, it has been proved in the previous section that partition coefficient is reduced when free drug moves from the microsphere to the matrix whereas the diffusion coefficient of the free drug in the matrix is not affected, but the overall transport rate of the free drug in the matrix is smaller.

Figure 43 Accumulative release of FITC-BSA from microsphere embedded film at different immobilization levels within 30 days

From release mechanism point of view, it can be seen that the order of the release is increased after drug immobilization (Table 7), hence it is possible to tune the order of release by the drug immobilization method. Zero-order release can be realized by a suitable amount of drug immobilization (5mg/mL immobilization).

Table 7 Order of the release profile

	m.s	w/o	5mg/mL	10mg/mL	20mg/mL	40mg/mL
n	N.A.	0.87 ±0.01	1.08 ±0.02	1.48 ±0.02	2.64 ±0.04	1.59 ±0.03

Chapter 6 Analysis and discussion

Drug immobilization has been proved to be an effective method in release rate control. Although the working mechanism of the method is proposed to be due to chemical potential contribution of the immobilized drug; theoretical analysis has to be carried out to prove the hypothesis. Structure of the drug immobilized hydrogel is first discussed in this section followed by mechanism study of the partition control principle. Application prospects of the partition control principle in both pulsatile and sustained drug delivery systems are discussed as well.

6.1 Structure of the drug immobilized hydrogel

Molecular structure of the drug immobilized hydrogel can be inferred from its dynamic and static swelling behavior. Two mechanisms contribute to hydrogel swelling characteristics: diffusion and network relaxation. The value of n indicates which mechanism dominates the swelling process. A value of 0.5 means that swelling is dominated by diffusion; whereas $n=1$ means that swelling is dominated by network relaxation.

Before BSA immobilization, n is closer to unity; whereas after BSA immobilization, n values are smaller compared to the un-immobilized case (Figure 27), which means that the immobilized BSA tends to restrict relaxation in general. This effect can be explained by re-examining the BSA functionalization step. More than enough quantity of acrylate groups are introduced to the reaction vessel so that most NH_2 of BSA can be functionalized, which makes a single functionalized BSA molecule contain more than one acrylate group. In another words, functionalized BSA becomes a multi-

functional molecule, which will behave as a crosslinker in UV crosslinking reaction. Therefore by introducing functionalized BSA into hydrogel, it is equivalent to increase crosslinking density of the gels.

On the other hand, the n values gradually increases with more immobilization (Figure 27). This contradiction can be explained by a combined effect of immobilization method and immobilization level. The BSA immobilization at higher levels uses a two-step method (section 4.1.2.3). By introducing functionalized BSA into the drug-free gel, secondary crosslinking takes place. Secondary crosslinking of the gel may proceed according to different schemes:

- 1, at low concentration of functionalized BSA, single functionalized BSA could crosslink the active sites of the gel from adjacent chains which increases the crosslinking density of the gel;

- 2, at moderate concentration of functionalized BSA, multiple functionalized BSA might crosslink themselves first to become a giant crosslinker, which then crosslink the active sites of the gel. In this way the original network structure of the gel is less tailored;

- 3, at very high concentration of functionalized BSA, self-crosslinking of BSA dominates. Giant BSA molecules are attached to the polymeric chains

In another word, crosslinking density does not increase linearly with more functionalized BSA, and in fact, the crosslinking density at higher immobilization levels might be even smaller than lower immobilization levels, due to self-reaction of

BSA. Therefore n value increases slightly when immobilization level increases.

Another reason behind the increase in n values is due to osmotic pressure and electrostatic repulsion caused by the immobilized BSA. The osmotic pressure will introduce extra force for network expansion/relaxation, which will increase the value of n . And since BSA are charged at physiological pH, the electrostatic repulsion among BSA molecules serves as another driving force for network relaxation.

In summary, on one hand, functionalized drug restricts network relaxation by increasing crosslinking density; on the other hand, immobilized drug molecules provide extra driving force for network relaxation. The net result is that although dynamic swelling behavior is affected by drug immobilization, static or equilibrium swelling ratio is not affected. It has been shown that diffusivity of the free drug is not affected by drug immobilization either. Because the diffusivity of the drug in membrane is largely determined by the swelling ratio and mesh size of the polymer, the final mesh size of the hydrogel is not significantly reduced by drug immobilization. Therefore, the third crosslinking scheme is more appropriate to describe how the drug molecules are immobilized in the membrane.

6.2 Control of drug release rate by partition effect via drug immobilization

It has been shown experimentally that release rate reduction with drug immobilization is caused by partition effect rather than diffusion. But question remains: why does the immobilized drug in the membrane affect the partition behavior of the free drug from

the donor?

6.2.1 Classical partition coefficient study

Partition of a solute could be contributed by non-electrostatic or electrostatic forces between solutes and the membrane. By considering only steric effect for non-electrostatic effect a more rigorous model describing solute partition in hydrogel was established [91, 92]. The total partition coefficient is

$$K_p = K_{p-non} + K_{p-elec} \quad \text{Equation 58}$$

6.2.1.1 Steric effect

Steric effect refers to size exclusion of solute by highly swollen hydrogels. Schnitzer's uniform-pore model agrees well with experimental data [92]

$$\begin{aligned} K_{p-non} &= (1 - \phi_p) \left(1 - \frac{r_s}{r_p} \right)^2 & r_s \leq r_c \\ K_{p-non} &= 0 & r_s > r_c \end{aligned} \quad \text{Equation 59}$$

The pore radius of the nonporous membrane is taken to be one-half of the mesh size. Because the diffusivity is not affected by drug immobilization, hence mesh size can be reasonably assumed unchanged according to Equation 28 and therefore steric effect is not changed.

6.2.1.2 Chemical potential equilibrium

The contribution by electro-static interaction can be derived from chemical potential equilibrium condition

Chemical potential of all the components in the solution and hydrogel must be equal

$$\mu_{i,membrane} = \mu_{bulk} \quad \text{Equation 60}$$

The equilibrium equation can be re-written as before

$$\mu_i^0 + RT \ln(\gamma_{i,bulk} x_{i,bulk}) = \mu_i^0 + v_i \pi + RT \ln(\gamma_{i,membrane} x_{i,membrane}) \quad \text{Equation 61}$$

The osmotic pressure in the membrane is calculated based on gel elasticity

$$\pi_{elastic} = -C_{cross} RT \left(\frac{\phi_p}{\phi_{p,synthesis}} \right)^{1/3} \quad \text{Equation 62}$$

C_{cross} is the concentration of crosslinks (mol / m^3)

Therefore the partition coefficient is

$$\ln K_{p-elec} = \ln \frac{x_{p,membrane}}{x_{p,bulk}} = \ln \frac{\gamma'_{p,bulk}}{\gamma'_{p,membrane}} - \frac{v_p \pi}{RT} \quad \text{Equation 63}$$

Since the only intermolecular interaction in consideration of this model is electrostatic interaction between charged species, and the activity coefficient of charged ion in the membrane was derived by ignoring interaction between mobile ions (consider only ion-polymer interaction), therefore the activity coefficient of charged ion in the bulk solution should also ignore mobile ion interactions, which implies the bulk solution shall be treated as an ideal solution ($\gamma'_{p,bulk} = 1$). So the partition caused by protein-polymer interaction can be simplified as

$$\ln K_{p-elec} = \ln \frac{x_{p,membrane}}{x_{p,bulk}} = -\ln \gamma'_{p,membrane} + C_{cross} v_p \left(\frac{\phi_p}{\phi_{p,synthesis}} \right)^{1/3} \quad \text{Equation 64}$$

6.2.1.2.1 Osmotic pressure

Since osmotic pressure affects the partition coefficient, osmotic pressure in the membrane has to be calculated based on Equation 62 (Matlab code of calculation details are given in Appendix B). The theoretical osmotic pressure value with different

amount of BSA immobilization is presented in Figure 44. Note that the BSA concentration values are the concentration of immobilized BSA in the membrane other than the concentration of solution used in synthesis. A more negative osmotic pressure should increase the partition coefficient (Equation 64).

Figure 44 Osmotic pressure in the membrane as a function of BSA immobilization levels

6.2.1.2.2 Activity coefficient

To find out the influence of protein immobilization on solute activity coefficient, the UNIQUAC model (session 2.2.2.2.3) was applied to predict the changes of solute activity coefficient. From the UNIQUAC model (Matlab code of UNIQUAC calculation is shown in Appendix C and D); even 200mg/mL BSA was used during synthesis (Figure 45), the change in solute activity coefficient is less than 1%. Furthermore, the model predicts that solute activity coefficient in the membrane decreases after immobilization, which causes an increase in partition coefficient.

Figure 45 BSA activity coefficient with different amount of immobilization ■ in bulk solution; ■ in membrane; ■ in membrane with 200mg/mL added during polymerization in membrane

6.2.1.3 Summary of classical partition analysis

Our experiment shows that partition coefficient is reduced after drug immobilization whereas theoretical analysis based on classical partition study suggests that partition coefficient should increase after drug immobilization because of its effect on activity coefficient and osmotic pressure. The problem of classical thermodynamic analysis is that the chemical potential contribution by the immobilized drug is not considered in the classical theory.

6.2.2 Thermodynamic modeling study

From activity coefficient and osmotic pressure analysis, experimental results seem to

be contradictory to the theory, but the major problem with the classical partition coefficient theory is that it ignores the chemical potential contribution by any other components in the system. A generalization of the classical theory is needed so that three effects due to a third component in a system could be considered:

- (a) Effect on activity coefficient;
- (b) Effect on osmotic pressure;
- (c) Contribution to the total chemical potential;

The chemical potential contribution can only be considered if the governing equilibrium condition of the classical theory is re-examined (in the classical theory, chemical potential of the free BSA in the membrane is equal to the chemical potential of free BSA in the donor). In the drug immobilized membrane, there are two types of BSA, namely free BSA coming from the donor and immobilized BSA. Therefore, it is the total chemical potential due to both free and immobilized BSA in the membrane that balances the BSA chemical potential in the donor. Therefore in order to consider chemical potential contribution, an expression for the total chemical potential is needed.

The definition of chemical potential of a component in a system is given by differentiating total Gibbs free energy of the system with respect to the amount of that component, under the condition that temperature, pressure and the amount of all other components are constant. In another words, the existence of one component in a system shall be independent on other components if a chemical potential is to be defined mathematically.

We propose that dependence shall be considered in some practical situations whereby a

change in one component inevitably causes a change in the other. The infinitesimal expression of Gibb's free energy equation (Equation 31) is re-configured in the following way to handle the 'dependent' case: in a general scenario two dependent substances (A and B) in the membrane are grouped together, represented by a combined chemical potential ($\mu_{M,grouping}$), and total concentration in the membrane ($C_{M,grouping} = C_{M,A} + C_{M,B}$), where $C_{M,A}$ and $C_{M,B}$ are the concentration of A and B in the membrane, and they are dependent (subscript M denotes membrane). Mathematically the above description becomes:

$$dG = -SdT + VdP + (\mu_{M,A}dC_{M,A} + \mu_{M,B}dC_{M,B}) + \sum_{i=3} \mu_i dC_i \quad \text{Equation 65}$$

And

$$\mu_{M,grouping}dC_{M,grouping} = \mu_A dC_A + \mu_B dC_B \quad \text{Equation 66}$$

Where S is entropy.

Hence the combined chemical potential is:

$$\mu_{M,grouping} = \mu_{M,A} \left(\frac{dC_{M,A}}{dC_{grouping}} \right)_{T,P,n_{i>2}} + \mu_{M,B} \left(\frac{dC_{M,B}}{dC_{grouping}} \right)_{T,P,n_{i>2}} \quad \text{Equation 67}$$

And let

$$\alpha = \left(\frac{dC_{M,A}}{dC_{M,grouping}} \right)_{T,P,n_{i>2}}, \text{ and } \beta = \left(\frac{dC_{M,B}}{dC_{M,grouping}} \right)_{T,P,n_{i>2}} \quad \text{Equation 68}$$

At chemical potential equilibrium, it is assumed that the combined chemical potential in the membrane is balanced by the chemical potential of substance A in the donor:

$$\mu_{M,grouping} = \mu_{D,A} \quad \text{Equation 69}$$

Subscript D denotes donor. Substituting the expressions for chemical potential into the above equation:

$$\mu_{M,0} + RT \ln(\gamma_{M,grouping} C_{M,grouping}) = (\mu_{M,0} + RT \ln(\gamma_{M,A} C_{M,A}))\alpha + (\mu_{M,0} + RT \ln(\gamma_{M,B} C_{M,B}))\beta = \mu_{D,0} + RT \ln(\gamma_{D,A} C_{D,A})$$

Equation 70

Where μ_0 is the reference chemical potential, γ is activity coefficient.

Therefore the concentration ratio of substance A in membrane and donor is

$$\frac{C_{M,A}}{C_{D,A}} = \left[\frac{\exp\left(\frac{\mu_{D,o} - \mu_{M,o}}{RT}\right) \gamma_{D,A}}{C_{D,A}^{\alpha-1} \gamma_{M,A}^{\alpha} (\gamma_{M,B} C_{M,B})^{\beta}} \right]^{\frac{1}{\alpha}} \quad \text{for } C_{M,B} > 0 \quad \text{Equation 71}$$

Now consider the partition control principle: Let A stand for the free drugs, and B for the immobilized ones of same type as A. Assuming the donor is an infinite source of A, thus $C_{D,A}$ is constant. The concentration ratio derived in Equation 71 is therefore the partition coefficient of free drugs (K_{eff}):

$$K_{eff} = \left[\frac{\exp\left(\frac{\mu_{D,o} - \mu_{M,o}}{RT}\right) \gamma_{D,free}}{C_{D,free}^{\alpha-1} \gamma_{M,free}^{\alpha} (\gamma_{M,immob} C_{M,immob})^{\beta}} \right]^{\frac{1}{\alpha}} \quad \text{for } C_{M,immob} > 0 \quad \text{Equation 72}$$

Where $\mu_{D,o}$, $\mu_{M,o}$, R , T and $\gamma_{D,free}$ are constants at a given donor condition, and the activity coefficients of the free and the immobilized molecules in the membrane are related through

$$\gamma_{M,immob} = b \gamma_{M,free} \quad \text{Equation 73}$$

This is because the free drugs constantly undergo random movement in the membrane, but when they are immobilized through a spacer they can move in a similar fashion as free drug molecules only within a distance defined by the length of the spacer. Factor b takes this restriction into account. Inserting Equation 73 into Equation 72 gives rise to

$$K_{eff} = \left[\frac{\exp\left(\frac{\mu_{D,o} - \mu_{M,o}}{RT}\right) \gamma_{D,free}}{C_{D,free}^{\alpha-1} \gamma_{M,free} (bC_{M,immob})^\beta} \right]^{\frac{1}{\alpha}} \quad \text{for } C_{M,immob} > 0 \quad \text{Equation 74}$$

In order to test the theory, some minor manipulation of the experimental data is needed. This is because the measured partition coefficient is a combined effect from both steric effect and chemical potential equilibrium. However the equation derived above only considers chemical potential equilibrium. After the manipulation, the fitting result is presented in Figure 46.

The fitting parameter b is found to be 0.054, $\alpha = 0.595$ and $\beta = 0.405$. Ideally speaking b is unity if there is no restriction in their random motion. A much smaller b found in this particular case suggests that the mobility of the immobilized drug is dramatically limited, which reduces the chemical potential contribution capacity of the immobilized BSA (the chemical potential of more immobilized BSA molecules is equivalent to the chemical potential of a single free BSA). It is expected that b depends on the immobilization agents used, a long and flexible spacer will make the immobilized drug molecules behave more like a free drug molecule, therefore enhance the sensitivity of the control scheme (less amount of drug is needed to be immobilized for a desirable reduction in partition coefficient).

Figure 46 **Partition-coefficient curve** The black dots are the average of effective partition coefficients ($K_{eff} = K / K_{steric}$), calculated from the experimentally measured data (K) of three independent experiments, and K_{steric} is calculated from Appendix C. The error bar is the standard deviation of the measured data. For curve fitting, $\gamma_{M,free}$ is regarded as a constant in the interested concentration range. The curve fitting was performed by a freeware run under Mac OS 10.5: qtiplot.

6.2.3 Molecular simulation study

Thermodynamic study is based on a system of many molecules. It tells how the system as a whole would behave macroscopically under different conditions, but it does not tell much story at molecular level. Molecular simulation enables one to trace macroscopic behavior to molecular events.

The simulation is based on a particular type of statistical mechanics, where drug molecule are randomly inserted into the membrane and the new state (after drug insertion) is subject to a test whether it shall be accepted or rejected under the condition that volume, temperature and chemical potential are held constant (Grand canonical ensemble) [93]. The analogy of this ensemble is that a membrane is placed inside an infinite reservoir of drug molecules where chemical potential of the drug in the reservoir is constant (infinite source condition as we use throughout this thesis).

The drug molecules in the reservoir are allowed to move into the membrane until no more drugs can be inserted at a given pressure. The volume of the membrane (experimental results also suggest that static swelling ratio is not affected by drug immobilization) and the temperature of the system are kept constant. This description fits nicely to the partition control principle proposed in this project, and therefore it is chosen to simulate the partition control principle.

To simulate drug partition behavior in the presence of immobilized drug in the membrane, small molecule analogs are used instead of protein molecules for the sake of efficient computation. In the simulation setup, DEAEMA molecule is chosen to be the constitutional material of membrane, whereas PEGMA is chosen to be a model drug for simulation purposes. All the simulation is done using Materials studio[®].

The simulation volume is a cubic with each edge of 44.32 Å and 100 DEAEMA molecules are placed inside this volume of the membrane (Figure 47). Periodic boundary condition is applied because the simulation volume is a tiny portion of the whole membrane, hence this simulation cubic is supposed to be surrounded by its mirror images in all six surface. This small portion of the membrane is allowed to relax (minimize its energy) for 1,000,000 steps before numerical partition experiment (insertion).

Figure 47 Schematic of the simulation volume of the membrane

Insertion is done by the Sorption module of the Materials studio. 100,000 equilibration steps and 1,000,000 production steps are used in the simulation to find the final state. At 100 kPa, it is found that 65 model drugs (PEGMA) can be inserted into the simulation volume of the membrane.

In order to represent the drug immobilized membrane, a hypothetical molecule is constructed: PEGMA-DEAEMA.

PEGMA-DEAEMA

When PEGMA-DEAEMA and DEAEMA are used together to construct the membrane, PEGMA-DEAEMA represents the immobilized drug. Total number of molecules (PEGMA-DEAEMA + DEAEMA) used to construct the membrane is kept at 100. And

different number of PEGMA-DEAEMA used in the membrane construction corresponds to the different amount of immobilization: when a membrane is constructed by 20 PEGMA-DEAEMA and 80 DEAEMA, it represents the case that 20 PEGMA are immobilized in the DEAEMA matrix.

It can be seen that different number of drug molecules can be inserted/loaded into the membrane with different amount of immobilization (Figure 48). As more drug molecules are immobilized in the membrane, less free drug from the reservoir can be inserted into the membrane, which is consistent to the experimental results and thermodynamic models.

Figure 48 Loading capability of membranes with different amount of immobilization of PEGMA

To translate the loading data into partition coefficient, it is assumed that the partition coefficient of the drug without immobilization is unity, the partition coefficients with different amount of immobilization can be deduced (Figure 49). Although the trend is

different at very concentrated regime compared to experimental data and thermodynamic simulation, molecular simulation confirms that partition coefficient is reduced with drug immobilization due to chemical potential contribution. The difference in the trend between molecular simulation and thermodynamic modeling is probably due to the ignorance of the mobility restriction in the molecular simulation.

Figure 49 Molecular simulation of partition coefficient as a function of different immobilization of PEGMA

6.3 Enhancement of switching effectiveness of pulsatile drug delivery systems by partition control principle

For pulsatile drug delivery systems, one of the biggest concerns is loss of controllability. Controllability refers to the ability that a delivery system switches on and off their release. The issue arises because of two competing factors: on one hand, hydrogel based pulsatile delivery systems hardly switch themselves off completely, which means that there are still some drugs being released during the off state, only at

a slower rate; on the other hand, plasma drug concentration has to be within a range for the drug to be effective (Figure 10). As long as the plasma concentration is out of that range and lower, it does not exert therapy hence can be considered as being switched off. Since hydrogel based pulsatile drug delivery system never switches off completely, when the difference between on and off release rates is too small, the switching is not effective from the perspective of a patient.

It can be seen that the difference in release rate between on and off states can be increased with drug immobilization (Figure 30); hence the release system is more effective. The underlying reason of increased switching effectiveness can be explained by the concentration of immobilized drug in the membrane. For the same amount of immobilization (same mass), its concentration in the membrane during off state is higher than on state because gel absorbs less water at off state. Since the concentration of immobilized drug in the off state is higher, the reduction in the partition coefficient during off state is more significant. However the reduction in the release rate during on state is less prominent due to lower concentration of the immobilized drug. Therefore the difference in the release rate between on and off states is enlarged with drug immobilization.

But the switching effectiveness does not keep increasing as more drug is immobilized (Figure 30). This is because as more and more drug is immobilized, the reduction in partition coefficient becomes less sensitive to concentration (Figure 46) as both shown experimentally and predicted by the thermodynamic model. Since the improvement of switching effectiveness is due to the concentration difference of immobilized drug during on and off state, at low immobilization levels, a given change in concentration of immobilized drug could result in a bigger change in partition coefficient, which

causes an improvement in switching effectiveness. Whereas at higher immobilization levels, the change in partition coefficient with a given change in concentration is smaller (less steep of curve, hence less sensitive Figure 46), therefore the improvement of switching effectiveness is gradually lost.

The implication to drug delivery system design is that lower immobilization levels are preferred for pulsatile drug delivery systems for the sake of improved switching effectiveness.

6.4 Alteration of release kinetics of sustained drug delivery system by partition control principle

The requirement for sustained drug delivery system differs from pulsatile drug delivery system. For sustained drug delivery systems, two requirements need to be satisfied: prolonged release time and linear release rate. A prolonged release time alone is not necessarily good for chronic disease if its release rate is volatile. Even the device could release drug for a very long time, it does no good if the drug concentration is out of the therapeutic window. A linear release profile is preferred because it corresponds to a constant release rate (first derivative of the release profile is release rate). A prolonged and constant rate drug delivery device will be able to maintain the plasma concentration within the therapeutic window for a longer period of time.

In Figure 50, the two hypothetical systems release their drug for the same period of time; however their concentration profiles are different. Since a drug is only effective if its concentration is within its therapeutic window, the effective acting durations of these two systems are different even though their total release time is the same.

Therefore both release kinetics and the total release time are important in sustained drug delivery systems.

Figure 50 Concentration profile of two different systems with the same total release time but different concentration profile

It has been demonstrated experimentally that the order of release can be altered by drug immobilization and a near zero-order release profile can be achieved by a suitable amount of immobilization (Table 7).

In order to understand why order of release can be controlled by drug immobilization, it is important to understand the release profile of diffusion controlled monolith system ($n=0.5$). In diffusion controlled monolith system, release rate gradually reduces till zero. The reduction in release rate is not because of changes in diffusivity but because the concentration of the drug in the matrix is reducing as more and more drugs are released. In another words, if the drug concentration in the matrix reduces slowly, the release profile would be more linear. This effect has been reported in [33], in which partition coefficient plays a critical role in affecting the overall drug release profile. The contribution of the current work is to provide a flexible means to control the partition coefficient, which in turn tailor the drug release profile.

In the microsphere embedded matrix system studied in this project, the drug available for release is provided by the drug loaded microspheres. The concentration in the microsphere and the matrix is related by the partition coefficient. With drug being immobilized in the matrix, release rate is smaller and therefore the variation in the concentration is smaller, hence a more linear profile is maintained.

As more and more drugs are immobilized, the order of release is greater than unity. Because the fitting of the profile is done only for the first 40% of the release, the meaning of the higher orders is that the release rate during early stage of the drug release is very small (silent period). Compared to the usual cases of burst release, the systems with higher immobilization levels are better because it does not only get rid of the burst release problem but also it gives travel allowance for the system to reach the targeted site before drug is released. The drug immobilized microsphere embedded system could achieve such effect because the amount of drug that could travel from the microsphere to the outer surface of the system is very small in the early stage of the release (controlled by a small partition coefficient).

It has been demonstrated in this work that the proposed control method should be applied to different drug delivery system configurations as long as a partition phase is present. It is therefore predicted that the proposed method could be widely used in oral, injection (nano/microsphere based) and implantable drug delivery devices, including drug containing medical implants such as stents. However the major technological difficulty is to remove those drugs that are failed to be immobilized during the process to avoid any detrimental effect.

Chapter 7 Conclusions

Drug release rate can be controlled by a drug immobilization method due to chemical potential contribution. Bovine Serum Albumin (BSA) has been used as a model drug to demonstrate the partition control principle by immobilizing the model drug in a hydrogel membrane. The effect of drug immobilization on hydrogel swelling behavior and drug release properties are summarized as follows:

1. Dynamic swelling of a pH sensitive hydrogel is dominated by network relaxation with drug immobilization compared to diffusion dominance for hydrogels without drug immobilization. The extra force to drive network relaxation is contributed by the charges carried by the immobilized drug and osmotic pressure. At equilibrium, the final swelling ratio at different pH values is not affected by drug immobilization.
2. Drug immobilization does not affect the diffusivity of the free drug in the hydrogel membrane, but the partition coefficient of free drug from the donor to the membrane is reduced with more drugs being immobilized. The reduction in the partition coefficient of free drug is due to the chemical potential contribution of the immobilized ones. The relationship between the concentration of immobilized drug and the partition coefficient of the free drug is:

$$K_{eff} = \left[\frac{\exp(\frac{\mu_{D,0} - \mu_{M,0}}{RT}) \gamma_{D,free}}{C_{D,free}^{\alpha-1} \gamma_{M,free} (bC_{M,immob})^\beta} \right]^{\frac{1}{\alpha}} \text{ for } C_{M,immob} > 0$$

where K_{eff} is the partition coefficient of free drugs; $\mu_{D,0}$ and $\mu_{M,0}$ are the

reference chemical potentials in the donor and membrane respectively; $\gamma_{D,free}$ and $\gamma_{M,free}$ are the activity coefficients of free drugs in the donor and membrane respectively; $C_{D,free}$ is the free drug concentration in the donor and $C_{M,immob}$ is the concentration of immobilized drug in the membrane; R is the gas constant and T is the temperature; α , β and b are fitting parameters. Quantitative control of drug release rate is possible based on the above thermodynamic model. The cell viability is not negatively affected by BSA immobilization.

3. For pulsatile drug delivery systems made of pH sensitive hydrogel, its controllability (change in drug release rate upon switching) is enhanced with drug immobilization because with the same amount of immobilization, the concentration of the immobilized drug is different in the swollen and dry states, hence the chemical potential contribution in the swollen state is less than the dry state, therefore the reduction in the partition coefficient is more significant at dry state than the swollen state. The overall effect is an increase in the difference of release rate between swollen and dry states.
4. For sustained drug delivery system, a microsphere embedded hydrogel system demonstrates that the release kinetics can be altered by the drug immobilization method. A linear release profile with prolonged release duration (from hours to half a month) can be realized by drug immobilization in the hydrogel matrix. The system with drug immobilization shows no burst release in the early stage of the experiment.
5. Conventional thermodynamics ignores dependence between different

components in a system, which cannot describe some situations such as the partition behavior of free drug in the presence of immobilized ones. The dependence shall be considered when the amount of one component in a system affects the amount of the other in the partial differentiation operation by chain rule.

List of Publications

Journal papers

- 1, L. Shang, S. Zhang, H. Du, S. S. Venkatraman, A novel approach for the control of drug release rate through hydrogel membrane: I. Effect of drug immobilization on drug release rate by copolymerization method. *European Journal of Pharmaceutics and Biopharmaceutics* 68 (2008) 715–723
- 2, L. Shang, S. Zhang, H. Du, S. S. Venkatraman, A novel approach for the control of drug release rate through hydrogel membrane: II. Thermodynamic modeling of the partition control scheme. *Journal of Membrane Science*. 321 (2008) 331-336
- 3, L. Shang, S. Zhang, S. S. Venkatraman, H. Du and Meenubharathi Natarajan, Partition control Scheme in hydrogel coated BioMEMS drug delivery device for release rate controllability. *Nanoscience and Nanotechnology letters*. 2 (2010) 11-15
- 4, L. Shang, S. Zhang, S. S. Venkatraman, H. Du, Sustained drug delivery of microsphere embedded drug immobilized film. *Nanoscience and Nanotechnology letters*. 2 (2010) 16-20

Book Chapters

- 1, Lei Shang, Sam Zhang, Subbu S Venkatraman and Hejun Du, Physiological insights of pharmacokinetics and host response for drug delivery system design, *Biomaterials and Engineering* (ISBN 0-87849-480-4) Edited by W. Ahmed, N. Ali, A. Öchsner, Published by Trans Tech Publications, 419-472 (chapter 11), 2007
- 2, Lei Shang, Sam Zhang, Subbu S Venkatraman and Hejun Du, Application of surface coating technology in the release profile control of drug delivery systems. *CRC Handbook of Biological and Biomedical Coatings* Edited by Sam Zhang, to be published by CRC Press. In Press (2010)

Appendix

Appendix A: Prediction of unloading time

%both boudary kept at constant concentration, used for loading and
%unloading

```
clear all
D=10^-7;
l=0.042; % membrane thickness 0.42mm
C_donor=5;%donor concentration
j=30; % 5 space intervals
space_step=l/j;
n=10000;% 10 time interval
t=12*3600;% total time for simulation
time_step=t/n;% delta t
f(1:n+1,2:j)=5; % intial condition, 0 concentration inside membrane,
column 1 and j+1 are boundaries
f(:,j+1)=0;% acceptor boudary condition
f(:,1)=0;%donor side boundary condition
a=D*time_step/(space_step)^2
for time=2:1:n+1
    for jj=2:1:j
        f(time,jj)=f(time-1,jj)+a*(f(time-1,jj+1)-2*f(time-
1,jj)+f(time,jj-1));
    end
end
```

```
x=0:space_step:1;
subplot(2,1,1)
hold on
plot(x,f(time,:))
x=0:space_step:1;
subplot(2,1,2)
hold on
plot(x/l,f(time,:)/C_donor)
end
hold off
```

Appendix B: Matlab code for partition coefficient calculated from hydrogel partition model

```
%*****
%*****
%partition coefficient modeling by 'Phase equilibria for aqueous
protein/polyelectrolyte gel systems'
%'partitioning of proteins between aqueous solution and a weakly-
ionizable polyelectrolyte hydrogel'
%the method is to divide partition contribution into two parts, one
is by
%steric, size exclusion, and the other is by electrostatic interaction
%between ions and polymer, but ignore all other interactions.
%this program calculates partition coefficient under influence of
steric
%and electrostatic interaction (protein-polymer) with BSA
%immobilization.
%*****
%*****
%partition contribution from steric exclusion
%*****
%*****
%parameters initiation
BSA_mass=20^-3/90;% gram mass of BSA per hydrogel piece
BSA_mol=BSA_mass/69000;
volume_fraction_polymer=0.1;
volume_fraction_polymer_syn=0.3;
volume=0.3;%ml of the hydrogel per piece at partition experiment
volume_syn=0.1
molar_volume_protein=128000*10^(-30)*6.03*10^(23); %m^3/mole
%pore_radi_polymer, defined by mesh size
%in order to calculate mesh size, we need number of repeating units
between
%crosslinks and volume swelling ratio, which is the inverse of
%volume_fraction_polymer
Q=1/volume_fraction_polymer;
%number of monomer per chain is defined as molar ratio of
%monomer/crosslinking
DEAEM=0.00954/90;
PEGMA=0.00823/90;% 90 pieces synthesized from the amount of monomer
used monomer per hydrogel per piece
TEGDMA=0.000182/90;
molar_monomer=DEAEM+PEGMA;
%PEGMA:DEAME ratio in a chain is assumed to be the same as added
number_monomer_chain=molar_monomer/TEGDMA;
number_repeating_units=number_monomer_chain/(1+1); % because
PEGMA:DEAME=1:1
solute_radi=31.3;%amstron, BSA radius, from 'the osmotic pressure of
concentrated protein solutions'
carbon_bond=1.54; % carbon-carbon bond length, amstron
rigidity_factor=14.4;%for PEGMA:DEMEA hydrogel
%*****
%*****
%calculation of mesh size
%*****
%*****
```

```

end_to_end_sqr=number_repeating_units*carbon_bond^2*rigidity_factor;
mesh_size=Q^(1/3)*sqrt(end_to_end_sqr)
%*****
%calculation of partition coefficient from steric exclusion
%*****
partition_steric=(1-volume_fraction_polymer)*(1-
solute_radi/mesh_size)^2
%pore_raius is taken as the same as the mesh size

%*****
% partition coefficient from electrostatic interaction
%*****
%fraction of charged monomer
pK=7;% read from swelling v.s. pH curve, where swelling ratio changes
pH=7.4;% fraction of charged monomer at this pH.
fraction_charged_monomer=1/(1+10^abs(pH-pK));
concentration_charged_monomer=fraction_charged_monomer*(DEAEM-
20.4*BSA_mol)/(volume*(1-volume_fraction_polymer))%concentration of
charges, DEAEM(1+)+BSA(20.4-)
%calculation of activity coefficient of protein-ion pair;
ion_concentration=0.1;% ion concentration in the membrane is assumed
to be 0.1M;
pI=4.9;
constant=7.14/2.52;% 2.52 is the length of unit charge of poly-N-
isoprolacrylamide, but for DEAEM+PEGMA, it should be different
if pH>pI
    %polymer can only be positively charged, however, at high pH,
    protein
    %is negatively charged 7.4, so protein is counter ion

activity_coefficient_counter=(0.7*(concentration_charged_monomer/ion_
concentration)/(constant*20.4)+1)/((concentration_charged_monomer/ion_
concentration)+1);
    %here, 20.4 is the total number of charges at pH=7.4

activity_coefficient_coion=(0.7*(concentration_charged_monomer/ion_co
ncentration)/(constant*1)+1)/(0.53*(concentration_charged_monomer/ion_
concentration)/(constant*1)+1);
else

activity_coefficient_counter=(0.7*(concentration_charged_monomer/ion_
concentration)/(constant*1)+1)/((concentration_charged_monomer/ion_co
ncentration)+1);

activity_coefficient_coion=(0.7*(concentration_charged_monomer/ion_co
ncentration)/(constant*20.4)+1)/(0.53*(concentration_charged_monomer/
ion_concentration)/(constant*20.4)+1);
end
activity_coefficient=sqrt(activity_coefficient_counter*activity_coeff
icient_coion);
%*****
%partition contribution from osmotic pressure
%*****
concentration_crosslinking_syn=TEGDMA/(volume_syn*10^-6);%mol/m^3
osmotic=concentration_crosslinking_syn*molar_volume_protein*(volume_f
raction_polymer/((volume_fraction_polymer_syn)^(1/3)))

```

```
%*****
%*****
%calculation of partition from electrostatic
%*****
lnK=-log(activity_coefficient)+osmotic;
partition_elec=exp(lnK)
%*****
%total partition coefficient
%*****
partiton=partition_steric*partition_elec
```

Appendix C: Matlab code for UNIQUAC three-component system

```

%1:water
%2:solute
%3:polymer
clear all
format long
DEAEM=0.00954/90
PEGMA=0.00823/90;
% 90 pieces synthesized from the amount of monomer used monomer
per %hydrogel per piece
TEGDMA=0.000182/90;
molar_monomer=DEAEM+PEGMA;
polymer_mole_fraction=molar_monomer/(molar_monomer+0.1/18);
% polymer mole fraction assuming solute partition does not
affect %polymer fraction.
T=273.23+25;
r=[0.92 2483 25 2483];
% r for water and for protein and polymer polymer taken as PEG1000
as %in 'on extension of UNIQUAC-NRF model to study' and 'a new
modified %Wilson equation for the calculation of
q=[1.4 1873 31.59 1873];
%protein is taken as chymotrypsin 'a modified extended UNIQUAC
model %for proteins' 'correlation and prediction of thermal
properties'
g=[-8524.99 -8739.09 2049.1 -8739.09; -8739.09 -8892.08 1000 -8892.08;
8516.19 -1000 -2000 -1000;-8739.09 -8892.08 -1000 -8892.08];
%water-water is zero 'correlation and prediction of
mineral %solubilities'
%interaction parameter with polymer is unknown yet, r and q taken
as %PEG value
q_pri=[1.4 1873 31.59 1873];
N=3;
for solute=10^-5:10^-4:0.001
%solute=0.512*1.3*10^-6
%polymer=polymer_mole_fraction_beforeImmo;
polymer=polymer_mole_fraction;

solvent=1-polymer-solute;
x=[solvent solute polymer];
phi_denominator=0;
theta_denominator=0;
theta_pri_denominator=0;
for i=1:1:N
    phi_denominator=r(i)*x(i)+phi_denominator;
    theta_denominator=q(i)*x(i)+theta_denominator;
    theta_pri_denominator=q_pri(i)*x(i)+theta_pri_denominator;
    l(i)=5*(r(i)-q(i))-(r(i)-1);
end
for j=1:1:N
    phi(j)=r(j)*x(j)/phi_denominator;
    theta(j)=q(j)*x(j)/theta_denominator;
    theta_pri(j)=q_pri(j)*x(j)/theta_pri_denominator;
end
for p=1:1:N

```

```

        for k=1:1:N
            tor(p,k)=exp(-(g(p,k)-g(k,k))/T);
        end
    end
    xtimestl=0;
    for h=1:1:N
        xtimestl=x(h)*l(h)+xtimestl;
    end
    for m=1:1:N
        thetapritimestor=0;
        lastterm=0;
        for n=1:1:N
            thetapritimestor=theta_pri(n)*tor(n,m)+thetapritimestor;
            theta_pri_summation=0;
            for o=1:1:N

theta_pri_summation=theta_pri(o)*tor(o,n)+theta_pri_summation;
            end
            lastterm=(theta_pri(n)*tor(m,n)/theta_pri_summation)+lastterm;
        end
        phiphi=phi(m);
        logactivity(m)=log(phi(m)/x(m))+5*q(m)*log(theta(m)/phi(m))+l(m)-
(phi(m)/x(m))*xtimestl-q_pri(m)*log(thetapritimestor)+q_pri(m)-
q_pri(m)*lastterm;
    end
    soluteactivity=exp(logactivity(1))
    solventactivity=exp(logactivity(2));
    hold on
    plot(solute,soluteactivity,'--s',
'MarkerEdgeColor','k','MarkerFaceColor','g','MarkerSize',3)
%plot(solute,solventactivity,'--
s', %'MarkerEdgeColor','k','MarkerFaceColor','r','MarkerSize',3)
end

```

Appendix D: Matlab code for UNIQUAC four-component system

```

%1:water
%2:solute
%3:polymer
%4:immobilized protein
clear all
format long
BSA_mass=1/90% gram mass of BSA per hydrogel piece
BSA_mol=BSA_mass/69000
DEAEM=0.00954/90
PEGMA=0.00823/90;
% 90 pieces synthesized from the amount of monomer used monomer
per %hydrogel per piece
TEGDMA=0.000182/90;
molar_monomer=DEAEM+PEGMA;
polymer_mole_fraction=molar_monomer/(molar_monomer+0.1/18)
% polymer mole fraction before immobilization
immobilized_protein_fraction=BSA_mol/((BSA_mol+molar_monomer+0.1/18))
T=273.23+25;
r=[0.92 2483 25 2483];
% r for water and for protein and polymer polymer taken as PEG1000
as %in 'on extension of UNIQUAC-NRF model to study' and 'a new
modified %Wilson equation for the calculation of
q=[1.4 1873 31.59 1873];
%protein is taken as chymotrypsin 'a modified extended UNIQUAC
model %for proteins' 'correlation and prediction of thermal
properties'
g=[-8524.99 -8739.09 2049.1 -8739.09; -8739.09 -8892.08 -1000 -
8892.08; 8516.19 -1000 -2000 -1000;-8739.09 -8892.08 -1000 -8892.08];
%water-water is zero 'correlation and prediction of
mineral %solubilities'
%interaction parameter with polymer is unknown yet, r and q taken as
PEG value
q_pri=[1.4 1873 31.59 1873];
N=4;
for solute=10^-5:10^-4:0.001
%polymer=polymer_mole_fraction_beforeImmo;
polymer=polymer_mole_fraction;
immobilized_protein=immobilized_protein_fraction;
solvent=1-polymer-solute-immobilized_protein;
x=[solvent solute polymer immobilized_protein];
phi_denominator=0;
theta_denominator=0;
theta_pri_denominator=0;
for i=1:1:N
phi_denominator=r(i)*x(i)+phi_denominator;
theta_denominator=q(i)*x(i)+theta_denominator;
theta_pri_denominator=q_pri(i)*x(i)+theta_pri_denominator;
l(i)=5*(r(i)-q(i))-(r(i)-1);
end
for j=1:1:N
phi(j)=r(j)*x(j)/phi_denominator;
theta(j)=q(j)*x(j)/theta_denominator;
theta_pri(j)=q_pri(j)*x(j)/theta_pri_denominator;

```

```

end
for p=1:1:N
    for k=1:1:N
        tor(p,k)=exp(-(g(p,k)-g(k,k))/T);
    end
end
xtimesl=0;
for h=1:1:N
    xtimesl=x(h)*l(h)+xtimesl;
end
for m=1:1:N
    thetapritimestor=0;
    lastterm=0;
    for n=1:1:N
        thetapritimestor=theta_pri(n)*tor(n,m)+thetapritimestor;
        theta_pri_summation=0;
        for o=1:1:N

theta_pri_summation=theta_pri(o)*tor(o,n)+theta_pri_summation;
        end
        lastterm=(theta_pri(n)*tor(m,n)/theta_pri_summation)+lastterm;
    end
    logactivity(m)=log(phi(m)/x(m))+5*q(m)*log(theta(m)/phi(m))+l(m)-
(phi(m)/x(m))*xtimesl-q_pri(m)*log(thetapritimestor)+q_pri(m)-
q_pri(m)*lastterm;
end
soluteactivity=exp(logactivity(1));
solventactivity=exp(logactivity(2));
hold on
plot(solute,soluteactivity,'--s',
'MarkerEdgeColor','k','MarkerFaceColor','b','MarkerSize',3)
%plot(solute,solventactivity,'--
s', %'MarkerEdgeColor','k','MarkerFaceColor','r','MarkerSize',3)
end

```

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