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Pulsatile Drug Delivery by Thermoresponsive Polymer Films with Mathematical Model and Evaluation of Thermoresponsive Properties of Block Copolymers

by

Rongbing Yang, M.Sc.

Thesis presented for the Ph.D. degree of the National University of Ireland

School of Chemistry
National University of Ireland Galway
September 2012

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Dr. Fawaz Aldabbagh
Dr. William Carroll

Head of School: Prof. Paul V. Murphy
ABSTRACT

In this thesis, a new controlled thermoresponsive drug delivery system based on UV crosslinkable copolymer films was fabricated and characterised. The characterisation of the drug loaded films has shown an even drug distribution inside of the film which has a smooth surface (Ra = 8.3 nm). The kinetic study has shown that the release of two model drugs can be controlled by the thickness of the films, the initial drug concentration, and the solubility of the drug molecules. This controlled drug delivery system, fabricated from a thermoresponsive polymer, was designed to obtain a pulsatile release profile which is triggered by altering the temperature of the dissolution medium. Two stages of release behaviour were observed: fast release for the swollen state and slow (yet significant and non-negligible) release for the collapsed state.

A mathematical model was developed to evaluate the feasibility of an in vivo implanted drug delivery system. The delivery device consists of a cooling material coated by a drug-loaded thermoresponsive polymer film. Drug release is initiated by remotely dropping the temperature of the cooling material sufficiently, so that the temperature throughout the polymer coating drops below its lower critical solution temperature (LCST), causing the polymer to swell and release the drug. Drug release switches off again when heat conduction from an external fluid medium raises the polymer temperature to above the LCST causing the polymer to collapse. The model was developed based on Ficks law which describes pulsatile release mathematically for the first time. Diffusion coefficients at different temperatures (including temperatures corresponding to both the fully swollen and collapsed states) were estimated by fitting the experimental data with the theoretical release profile given by this model. The effect of temperature on the diffusion coefficient was studied.

The LCST of a series of poly(\(N\)-isopropylacrylamide) (poly(NIPAm)) block copolymers were measured using the cloud point method. This study has found the hydrophobicity of a segment in a copolymer, and the length of the poly(NIPAm) segment affects the LCST. The poly(acrylic acid) (poly(AA))
block supplies the pH sensitivity in the block copolymer. These block copolymers can self assemble into particles which show no cytotoxicity towards 3T3 mouse fibroblast cells.
ACKNOWLEDGMENTS

Many people have helped me through the course of this project. First of all, I want to thank Dr. Fawaz Aldabbagh, Dr. Yury Rochev, and Dr. William Carroll, for giving me the chance to work within this Biomaterials group, for their advice and their guidance. Also, Dr. Natalia Nikolskaya, who taught me patiently and comforted me after failed experiments. Thanks Dr. Alexander V. Gorelov for giving the UV crosslinkable copolymer. Thanks Dr. Padraig O’Connor for supplying me the block copolyemrs. Thanks Dr. Maria Nash who trained me in several techniques and introduced me to research. Thanks Dr. Jennifer Alexander for listening my complain during the years. Thanks Dr. Deirdre Healy for proof reading my whole thesis. Thanks for all other colleges to show me their kindness.

I also give my thanks to Dr. Martin G. Meere and Dr. Tuoi Vo T. N. from School of Mathematics, Statistics & Applied Mathematics, National University of Ireland Galway. Their help and intelligence during the collaboration impressed and inspired. They show me the mystery of the mathematics.

This research has emanated from research work conducted with financial support from Science Foundation Ireland (08/RFP/MTR1201).

Finally, I want to say thank you to my parents who have supported me always.
To my parents, Shiwen Yang and Yanqiu Ma.
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List of abbreviations

3T3 3-day transfer, inoculum $3 \times 10^5$

tBA tert-butylacrylate

A-Hyp-OMe $N$-acryloyl-$trans$-l-proline methyl ester

A-Pro-OMe $N$-acryloyl-l-proline methyl ester

AA acrylic acid

AAm acrylamide

ABzPh acrylamidobenzophenone

AFM Atomic Force Microscopy

Ala l-alanine

AMF alternating magnetic field

BASMC bovine aortic smooth muscle cell

CL $\varepsilon$-caprolactone

CM cooling material

DEA $N,N$-diethylacrylamide

DES drug-eluting stents

DMA $N,N$-dimethylacrylamide

DMAA dimethylarsenic acid

DMAEMA 2-[(dimethylamino)ethyl] methacrylate

DMEM Dulbecco’s Modified Eagle’s Medium

DMS dimethyl sulfide
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<td>dimethyl sulfoxide</td>
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<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
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<td>EE</td>
<td>encapsulation efficiency</td>
</tr>
<tr>
<td>EMA</td>
<td>N-ethylmethacrylamide</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>GPC</td>
<td>gel permeation chromatography</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank’s Buffered Salt Solution</td>
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<tr>
<td>HUVECs</td>
<td>human umbilical vein endothelial cells</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
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<td>LA</td>
<td>lactide</td>
</tr>
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<td>LCST</td>
<td>lower critical solution temperature</td>
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<td>MCE</td>
<td>magnetocaloric effect</td>
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<td>anhydrous methanol</td>
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<td>nPAM</td>
<td>N-n-propylacrylamide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NtBAm</td>
<td>$N$-tert-butylacrylamide</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity</td>
</tr>
<tr>
<td>Pen-Trep</td>
<td>Penicillin-Streptomycin</td>
</tr>
<tr>
<td>poly(EO)M</td>
<td>poly(ethylene oxide) monomethyl ether</td>
</tr>
<tr>
<td>RhB</td>
<td>rhodamine B</td>
</tr>
<tr>
<td>Rms</td>
<td>root mean square</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>scCO$_2$</td>
<td>supercritical CO$_2$</td>
</tr>
<tr>
<td>St</td>
<td>styrene</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
</tbody>
</table>
CHAPTER 1

Introduction

1.1 Background Information

1.1.1 Thermoresponsive polymers

Thermoresponsive polymers are one class of smart polymers. They have a dramatic physical phase transition in response to a change in temperature near a critical value which is defined as the lower critical solution temperature (LCST). Although some of the phase transitions were observed in organic solvents [1], the LCST behaviour is mainly studied in an aqueous environment.

1.1.1.1 Lower critical solution temperature

According to the Goldbook of International Union of Pure and Applied Chemistry (IUPAC), LCST is defined as a critical temperature below which a mixture is miscible. In the case of polymer aqueous solution, the miscibility can be explained as the polymers transit between a hydrophilic state and a hydrophobic state in response to a temperature change.

At a molecular level, this transition is due to hydrogen bonds breaking and forming in response to the change in the temperature. Four types of hydrogen bonds in a polymer-water solution might exist: water-water H-bond, water-polymer H-bond, inter-polymer H-bond\(^1\), and intra-polymer H-bond\(^2\) (Figure 1a). When the temperature is higher than the LCST, some of the water-polymer H-bonds break and more of the inter-polymer H-bonds and intra-polymer H-bonds form instead (Figure 1). Polymer chains collapse and aggregate to result into a cloudy solution at the macro-scale. When the temperature drops below the LCST, the hydrogen bonds between the polymer and water are forming and the polymer chains relax into a coil shape which results in a transparent solution at the macro-scale.

\(^1\)H-bond between hydrophobic and hydrophilic groups in different polymer molecules.
\(^2\)H-bond between hydrophobic and hydrophilic groups in one single polymer molecule.
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Figure 1. The scheme of phase transition of a polymer aqueous solution in macro and micro scale. (a) Polymer aqueous solution is clear when temperature is below its LCST; (b) Polymer solution turns to be cloudy when the temperature is above its LCST.

1.1.1.2 poly(NIPAm) and other thermoresponsive polymers

The LCSTs of a list of thermoresponsive polymers are presented in Table 1. The studies on these polymers have shown that the LCST is dependent on pH, molecular weight and the concentration of the polymer aqueous solution [2–10, 12–26].

Poly(N-isopropylacrylamide) (poly(NIPAm)), structure which is shown below in Figure 2, is one of the most well-studied thermoresponsive polymers. It possesses a hydro-carbon backbone, a strongly hydrophilic amide group, and a hydrophobic isopropyl moiety. The polymeric hydrophilic amide is capable of hydrogen bonding with the aqueous medium (such as water) or another amide group on the same chain or on another chain (Figure 3) [27]. Additionally, when the temperature drops below the LCST, the apolar isopropyl group induces the ordering of surrounding water molecules in a type of hydrophobic hydration. In this state the polymer is completely soluble in water and the polymer chains exist as flexible but extended coils known as Flory coils [2]. As the temperature is raised through the LCST, hydrogen bonds weaken and break. The extrusion of water is followed by polymer precipitation. Intra-polymeric H-bonding
Figure 2. Chemical structures of monomers which can give rise to thermoresponsive polymers.
Table 1. Examples of thermoresponsive polymers.

<table>
<thead>
<tr>
<th>Polymer Name</th>
<th>Molecular Weight</th>
<th>LCST (°C)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(NIPAM)</td>
<td>9,000-8,400,000</td>
<td>30.8-31.7</td>
<td>[2–4]</td>
</tr>
<tr>
<td>poly(DMAEMA)</td>
<td>28,000</td>
<td>53</td>
<td>[5]</td>
</tr>
<tr>
<td>poly(NAPPy)</td>
<td>10,800</td>
<td>52</td>
<td>[6]</td>
</tr>
<tr>
<td>poly(DEA)</td>
<td>1,481-4,780</td>
<td>30.4-41.5</td>
<td>[7]</td>
</tr>
<tr>
<td>poly(nPAM)</td>
<td>1,590,000</td>
<td>22.5</td>
<td>[9]</td>
</tr>
<tr>
<td>poly(A-Pro-OMe)</td>
<td>3,200</td>
<td>20.7</td>
<td>[10]</td>
</tr>
<tr>
<td>poly(A-Hyp-OMe)</td>
<td>11,000</td>
<td>49.5</td>
<td>[11]</td>
</tr>
<tr>
<td>poly(NMPP)</td>
<td>24,400</td>
<td>29.5</td>
<td>[8]</td>
</tr>
<tr>
<td>poly(EMA)</td>
<td>5,400-36,500</td>
<td>58-64</td>
<td>[12]</td>
</tr>
<tr>
<td>poly(NVCL)</td>
<td>330,000-1,500,000</td>
<td>30.8-31.7</td>
<td>[12]</td>
</tr>
</tbody>
</table>

The molecular weight listed in this table were obtained from various techniques, and the details can be obtained from the corresponding references.

The LCST of poly(NIPAM) is dependent on the concentration and the LCST listed here is from its 10 mg/ml aqueous solution.

The detailed information regards the LCST of poly(NIPAm) can be found in the latter section.

The LCST of poly(EMA) is dependent on the concentration and the LCST listed here is from its 10 mg/ml aqueous solution.
facilitates globule aggregation. In this state, hydrophobic forces dominate; the polymer is no longer in solution and thus the solution appears cloudy as the polymer has precipitated.

Figure 3. H-bond formation of poly(NIPAm) and the effect of the salt on it [27].

One of the advantages of poly(NIPAm) in the pharmaceutical applications is the weak dependency of LCST on molecular weight (18 to 8400 KDa) and concentration (0.01 to 1 wt %) [4, 28]. However, pH value does have effects on the LCST of poly(NIPAm) hydrogel [29].

1.1.2 Methods to investigate thermoresponsive polymers in solution

The main property of the thermoresponsive polymer solution is a phase separation when the temperature increases or decreases. As in the previous description, if the phase separation happens when the temperature is increased, an LCST of the polymer system exists. Therefore it is important to have accurate experimental methods to study this phase separation. Three main methods are usually adopted; turbidimetry, light scattering and differential scanning calorimetry. However, other techniques, such as IR spectroscopy which supplies the evidence of two types of water [3]; viscometry which proves the existence of flexible coil [3]; and so on are used.
1.1.2.1 Turbidimetry

Turbidimetry is also known as the cloud point technique. It utilizes the miscibility of the polymer chains and the solvent molecules, usually water, to measure the temperature of phase transition. A polymer solution of known concentration is heated up or cooled down while the strength of the light signal is monitored after it passes through the solution. The LCST is defined as the temperature when the strength of the light signal increases or decreases. However, some studies [30, 31] have found that the temperature obtained when the solution is heated up is not exactly the same as the temperature obtained when the solution is cooled down.

The phase separation of the thermoresponsive polymer and water can be of two types; a) the precipitates are suspended in the water to form a milk like opaque solution; b) the polymers aggregated and settled. The latter would affect the LCST measurement [3].

The methods to determine the LCST from the transmittance curve obtained by the turbidimetry can be various. It can be the temperature corresponding to a 10% reduction [3], or the temperature corresponding to the 50% of the transmittance [32], or the temperature corresponding to the middle value of the transmittance [33].

1.1.2.2 Light scattering

The dynamic light scattering technique is one of the most common methods used to study the size and stability of the collapsed polymer particles when the temperature is above the LCST. In a typical light scattering experiment, a laser beam passes through the medium in which the polymer particles freely swim. By collecting the laser signal at various scattering angle, the size distribution curve of particles can be obtained.

\footnote{It was observed when the LCST of block copolymers were measured.}
1.1.2.3 Differential scanning calorimetry

The differential scanning calorimeter is one of the methods which can be used to obtain the information on the enthalpy of the transition process and the LCST of the polymer in aqueous environment. By using this technology, the sharpness of the phase transition is defined as the half-width of the peak in the transition curve.

1.1.3 Poly(NIPAm) based copolymers

By adding an additive into the polymer, or altering the length of polymer chain, the LCST of the polymer can be tuned. Two types of poly(NIPAm) based copolymers will be reviewed here in relation to their LCST value and applications to drug delivery.

Based on their architecture, the copolymers can be separated into linear and non-linear. In the linear copolymer, the copolymers can be classified into three types: random copolymer, alternating copolymers, block copolymer based on the manner of the presentation of the repeat units in the polymer chain.

In a random copolymer, the monomers are combined in a random pattern; in an alternating copolymer, the monomers repeat in an alternative manner. Block copolymer is derived from two or more monomeric species. If A and B represent two types of units or monomers, then the three types of linear copolymers can be shown as in Figure 4.
Random Copolymer:


Alternating Copolymer:


Block Copolymer:


Figure 4. Architectures of the linear copolymers. A and B represented two different monomers.

1.1.3.1 Poly(NIPAm) based random copolymers

In this thesis, the main thermoresponsive polymer used is poly(NIPAm) which was chosen to build the drug delivery system or block copolymers. Therefore, the poly(NIPAm) random copolymers will be reviewed here.

By adding hydrophobic monomers, the LCST of the copolymers can be decreased from 32°C. Okano et al. [13] copolymerised methacrylic acid stearyl ester with NIPAm and the LCST of the copolymer was decreased to 24.6°C depending on the molecular weight and the molar ratio of the hydrophobic monomers [13]. They also added butyl methacrylate which can decrease the LCST to 25.0°C [34]. 2-hydroxyethyl methacrylate [35] was reported to decrease the LCST to 17.0°C.

By adding hydrophilic monomers, the LCST of the copolymer can be increased. N,N- dimethylacrylamide and acrylamide [35] were copolymerised with NIPAm and the LCST of the copolymers increased (Figure 5). Polyethylene oxide was used to increase the LCST to 34-36°C [36].
By copolymerising the carboxylic acid with NIPAm, the resulting copolymers will be pH sensitive while the LCST was also changed correspondingly. Acrylic acid [37] and methacrylic acid [38] can be used to add a pH sensitivity to the copolymer. While the pH value of the solution is higher than the pKa of acidic monomers, the monomers become hydrophobic and turn back to hydrophilic when the pH is dropped to below pKa.

Some monomers added into the copolymers can introduce the capability of crosslinking. N,N-methylenebisacrylamide [39] and ethylene glycol dimethacrylate [40] can be used as crosslinker agents during the polymerisation process. Acrylamidobenzophenone is a UV-sensitive cross linker [41].

1.1.3.2 Poly(NIPAm) based block copolymers

Block copolymers attract a lot of interest and attention on their possible use in drug delivery applications due to a number of reasons.

First of all, the absorption efficiency of the drug, which has very poor solubility in the physiological environment, can be improved by being delivered by block
copolymer micelles. The drug molecules can be trapped into the core of the block copolymer micelles thus modifying the surface of the drug molecules from hydrophobic to hydrophilic [42]. Therefore, the drug can be absorbed easily by cells. Furthermore, the micelles also give protection to the drug in the core, enhance dispersion, and inhibit aggregation. Also, if the size of the micelles is limited to under 200 nm, the permeability and retention of drugs are enhanced at solid tissue sites while the non-selective uptake by the reticuloendothelial system is reduced [43].

Zhuo et al. [44] in 2009 have carefully reviewed the block copolymer based on poly(NIPAm) on the size of micelles and the drugs incorporated. In table 2, a few of poly(NIPAm) copolymers are list which are not included in this review paper. Although quite a few of poly(NIPAm) copolymers can form micelles or particles, only a small range of drugs were incorporated into these systems and no in vivo study is ever reported to date.

1.1.3.3 Applications of poly(NIPAm) based copolymers

Although the applications of poly(NIPAm) is mainly in the biomedical engineering area, such as tissue engineering and drug delivery, its first application is to be an effective rodent repellent [48] by the Kodak Co.. Later on, its inversible phase transition between a hydrophilic and a hydrophobic strucutre upon heating has turned it into one of the most popular thermoresponsive polymers. Its LCST locates in a rather wide range of 29 - 42 °C, the exact temperature being dependent on a few of parameters, such as molecular weight, microstructure and additives [4, 13, 18, 19, 21, 27, 49]. Before the biological applications were intensively studied, the poly(NIPAm) based gel has been used as the seperation medium. The research group of Cussler has been focus on this area in 1987 [48].

In 1986, Hoffman and his coworks raised the idea to use the thermoresponsive polymer gel structure as the sustained drug delivery system which loading
Table 2. The data on particle size, polydispersity (PDI) and drug encapsulation efficiency (EE) of poly(NIPAm)-based micelles.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Micelle size, PDI in water, temperature</th>
<th>Drug, EE</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(EO)M-b-poly(AA)-b-poly(NIPAm)(^9)</td>
<td>129.9 nm, 0.0822, 37°C</td>
<td>DOX [45]</td>
<td></td>
</tr>
<tr>
<td>poly(NIPAm)-b-poly(Ala)(^10)</td>
<td>280 nm, 20°C</td>
<td>ADR; 9.6 w% [46]</td>
<td></td>
</tr>
<tr>
<td>poly(NIPAm-co-DMA)(<em>{118})-b-poly(LA)(</em>{59})(^11)</td>
<td>170 nm, 0.167, rt</td>
<td>ADR; 3-5 w% [47]</td>
<td></td>
</tr>
<tr>
<td>poly(NIPAm-co-DMA)(<em>{118})-b-poly(CL)(</em>{60})(^12)</td>
<td>87 nm, 0.174, rt</td>
<td>ADR; 3-5 w% [47]</td>
<td></td>
</tr>
</tbody>
</table>

\(^7\) Supplement data for the review paper [44].  
\(^8\) Supplement data for the review paper [44].  
\(^9\) poly(ethylene oxide) monomethyl ether-\textit{block}-poly(acrylic acid)-\textit{block}-poly(NIPAm)  
\(^10\) poly(NIPAm)-\textit{block}-poly(l-alanine)  
\(^11\) poly(NIPAm-co-N,N-dimethylacrylamide)-\textit{block}-poly(lactide)  
\(^12\) poly(NIPAm-co-N,N-dimethylacrylamide)-\textit{block}-poly(\(\varepsilon\)-caprolactone)
the drug in the gel below the LCST and release the drug slowly above the LCST \[50\]. In the following year, Oka \[51\] proposed another thermoresponsive delivery system which the pulsatile release pattern can be obtained by altering the temperature.

1.2 Controlled Thermoresponsive Drug Delivery System

In order to form the polymer based drug delivery system, the free polymer chains would be linked together which results in a polymer network. When the polymer network contains significant amount of water, it is known as a polymer hydrogel.

1.2.1 Diffusion based drug delivery system

Local drug delivery systems are different from the traditional transdermal drug delivery systems and oral drug delivery systems. Compared with these two types of drug delivery methods, the local drug delivery system enables a more targeted strategy and allows a higher concentration of drug to be delivered around the targeted area without systematic toxic side effects being experienced by patients \[52\]. This will also increase the efficiency of the drug and minimize the unspecific in vivo accumulation of the drug molecules which might cause some unexpected damage. However, the bio-compatibility requirements of the drug delivery systems are much higher due to their long-term exposure to the local tissue, and therefore more pre-clinical tests are necessary.

Diffusion is one of the most common mass transport mechanisms for local drug delivery and also one of the most well-studied mechanisms. The polymers which can be used to fabricate the diffusion release system cover a wide range of polymers from hydrophilic to hydrophobic \[53, 54\]. In this section, only the thermoresponsive diffusion delivery system will be focussed on for the purpose of comparison with the system developed in this study.

For the diffusion dominated delivery systems, the water soluble polymers are crosslinked together to maintain the integrity of the system and the drug
molecules can diffuse out of the system through the mesh which is formed when the system swells.

The most common sustained drug delivery system is the hydrogel based system [55, 56]. The drug molecules can be dispersed into the delivery system by a number of techniques, such as immersion or premixing [57, 58]. For some crosslinked techniques, premixing is not an option as crosslinking will damage the activity and validation of drug molecules. The other crosslinking methods allow the drug molecules to be mixed with the polymer before the crosslinking step.

### 1.2.2 Pulsatile drug delivery system

A drug delivery system based on thermoresponsive polymers can utilize the temperature to turn on and off drug release because of the reversible phase transition property of thermoresponsive polymers. When the temperature is above the critical temperature, known as LCST, the polymer molecules are in a hydrophobic state which leads to the collapse of the delivery system. This collapse reduces the mesh size of the delivery system and results in slowing down the elution rate or totally stopping the release. When the temperature drops below LCST, the molecules of polymer become hydrophilic which leads to swelling of the system. The volume of the delivery system increases and drug molecules can diffuse out faster from the bigger mesh space.

Most local drug delivery systems aim to maintain the drug concentration at some appropriate therapeutic level for a specified period of time, and this objective is frequently achieved using sustained release dosage forms. However, for some drugs, an optimum therapeutic effect comes from periodically fluctuating drug concentration [59]. To realise such behaviour, pulsed or pulsatile drug release systems have been developed [60–63]. This type of release system possesses a cycle with two distinct release stages; off/slow release and on/fast release. Usually, the release duration time for the slow release stage is much longer than for the fast stage, and the release rate is much smaller.
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The majority of existing pulsatile release systems can be classified into two categories [59]; time-controlled systems [64–67] and stimuli-induced systems [68–71]. Time-controlled release systems can only release at pre-programmed time points, whereas stimuli-induced pulsatile release systems are more easily manipulated. Stimuli-induced systems have been developed based on thermal, chemical, and electrical stimuli. However, systems based on thermal stimuli are particularly convenient since they can be designed and operated without significantly affecting other critical parameters of the system.

1.2.2.1 Hydrogel based pulsatile drug delivery system

Kashyap et al. [65] investigated a hydrogel system which regulated the release of insulin by using a glucose sensitive chitosan. By changing the glucose concentration, the release of insulin can be controlled. Due to the fact that the concentration of glucose in vivo has pulsatile characteristics, the insulin release via drug delivery also must possess a pulsatile profile (Figure 6).

Figure 6. Pulsatile release of insulin from biosensitive gels [65].
In 1991, Kim et al. [60] prepared a thermoresponsive interpenetrating networks (1-mm thickness and 12-mm diameter) from poly(NIPAm) and poly(tetramethylene ether glycol) (poly(TMEG)) (MW = 2000). The pulsatile release of the model drug, indomethacin, was tested at the temperatures of 30 and 35°C, 25 and 30°C, and 25 and 35°C with the various initial drug concentration of 11.2 to 26.0 wt %. The pulsatile patterns were obtained in 48 hours.

Corrigan et al. [61] obtained a pulsatile release profile of a series model drugs from poly(NIPAm) hydrogel (1.5-mm thickness and 6-mm diameter) at 25 and 37°C in order to compare the release between hydrophobic and hydrophilic drugs, and the effect of molecular weight. They found the release of hydrophobic drug cannot be controlled by the thermoresponsive polymer hydrogel. Comparing with the lower molecular weight (100Da), the larger molecule drugs can be controlled for producing a consistent magnitude of drug pulse.

In previous three examples of the pulsatile release system, 25°C was chosen to be the low temperature. This results to a 12°C gap between the low temperature and the physiological temperature 37°C. With existing medical cooling techniques, there is not an efficient way to locally reduce the temperature to 25°C in vivo. Therefore the application of these systems are quite limited. Peppas and Brazel [72] reported a thermoresponsive and pH-sensitive hydrogel system which can control the release by temperature and pH. They increased the low temperature to 33°C but this temperature only works when the pH value was around 5 to 6.

1.2.2.2 Particle based pulsatile drug delivery system

By utilizing the different temperatures between the lesions and the normal tissue, a thermoresponsive drug delivery system could be designed as a targeting delivery systems. Although there is no study to report that this difference of temperatures is used as a targeting strategy, it has been proven that the heated tumor can accumulate more thermoresponsive micelles compared with other normal tissue [73].
Satarkar et al. [70] presented a thermoresponsive drug delivery system based on pNIPAm. However, within this system, superparamagnetic Fe$_3$O$_4$ particles were incorporated. These particles can be heated up by a high frequency alternating magnetic field (AMF). On the other hand another thermoresponsive delivery system was reviewed here as well, this one utilized the squeezing effect. The squeezing effect is caused by the collapse of the thermoresponsive polymer when it was heated up above its LCST very suddenly. Therefore, pulsatile release profiles can be obtained using AMF as the trigger (Figure 7).

Aminabhavi et al. [62] got the pulsatile release profile of atenolol from microspheres (34 to 76-µm diameter) of gellan gum-poly(NIPAm) at 25 and 37°C. The different swelling ratios at two temperatures had proven that the main release mechanism of atenolol from these micro-spheres was governed by the Fickian diffusion which is very similar to the macro hydrogel systems. Similar results were observed by Ascenzi et al. [49], who reported pulsatile release profile of propranolol and lidocaine free bases from micro-spheres of poly(NIPAm) and poly(acrylic amide) (200-µm diameter).
Figure 7. Schematic showing the effect of ON-OFF cycles of AMF to the magnetic nanocomposites of NIPAm [70].
1.2.3 Advantages and disadvantages of thermoresponsive drug delivery system

By applying the phase transition of thermoresponsive polymers between hydrophilic and hydrophobic states, the drug molecules can be loaded into the drug delivery system after the crosslinking process [57, 58]. The methods to link the polymers into the net-work structure are usually involved with the formation of the physical or chemical bonds [74, 75]. If the drugs are loaded before the crosslinking procedure, there is a high possibility that drug molecules would be linked with the polymers which are composed of the delivery system. This will dramatically drop the total amount of the drug which can be released out. If loading the drug after the crosslinking, the time for the drug diffused into the delivery system can be extremely long.

By utilizing the phase transition of the thermoresponsive delivery system, the drug loading can be done after the crosslinking by simply dropping the temperature to below its LCST. The thermoresponsive delivery system will turn to the swelling state due to their materials being hydrophilic. The volume of the system would be increased and the drug molecules can easily diffuse into the polymer net-works. When the temperature rises back to physiological temperature which is above its LCST, the delivery system will turn to collapsed state and the volume of the delivery system would be shrank which will result in slow release of the drug molecules [50].

However, there are still a few of issues which limited the applications of the thermoresponsive polymers in the drug delivery field. First of all, the most well-known thermoresponsive polymer, poly(NIPAm), have not shown any toxicity to the cells, but its monomer has already been proven to have a negative biological affects. Therefore, a concern regards with the degradation of the non-toxic polymers into toxic segments arises.

Secondly, in order to use the temperature as the trigger to control the release \textit{in vivo}, an effective method to locally drop temperature is required. However, there is no report on this problem to date of this thesis.
In the previous study in our research group, the thermoresponsive polymer as drug delivery system have been considered and characterized to improve the restenosis injury caused the implanted stents. The detail can be found in the papers [57, 76, 77].

1.3 Mathematical Models for Thermoresponsive Drug Delivery System

In this section, a few of cooling techniques will be generally stated here for their potential application in vivo. Some of the mathematical models will be described for comparing with the one developed in this thesis.

1.3.1 Potential cooling techniques in vivo

Thermoresponsive polymers have numerous potential applications in areas such as drug delivery [64–71, 78], gene delivery [79, 80], tissue engineering [81–83], chemical valve technology [84, 85], and catalysis [86]. However, a biomedical device based on a thermoresponsive polymer that enables the pulsatile release of drug in vivo has yet to be developed, principally because of the difficulty of locally decreasing the temperature of human tissue in vivo. Nevertheless, candidate mechanisms for cooling polymers in vivo have been proposed, and some of these are now briefly discussed.

1.3.1.1 Chemical cooling technique

A potential chemically-based cooling mechanism is to add a substance to the polymer’s fluid environment that gives rise to an endothermic chemical process. In an endothermic process, heat is absorbed from the environment as the reactions involved proceed causing a drop in its temperature. The dissolution of ammonium nitrate in water is an example of an endothermic process; when ammonium nitrate is dissolved in water at room temperature at a concentration of 1 M, a drop in fluid temperature of 6 K is observed. However, the choice of substances that could be safely used in vivo is very limited since most
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endothermic reactions involve toxic chemicals [87].

The most typical application of the endothermic chemical reaction as cooling technique is the ice pack which composed of ammonium nitrate and water. It can absorb heat immediately for the emergency cooling in the medical applications [88, 89].

1.3.1.2 Peltier cooling technique

Another candidate cooling mechanism is based on the Peltier effect, which is a thermoelectric phenomenon [90]. The Peltier effect arises at the junction of two dissimilar conductors through which a current is flowing, and refers to the transfer of heat from one of the conductors to the other across the junction. The Peltier effect allows for the transfer of heat at the junction against the temperature gradient, and cooling devices have been manufactured based on the effect [91, 92]. Bi$_2$Te$_3$ and Bi$_2$Se$_3$ [93] are amongst the best performing thermoelectric materials at room temperature, and exhibit a temperature-independent thermoelectric effect. A thermoelectric cooling device based on the Peltier effect has recently been developed by Morizane et al. [94] for the treatment of spinal chord injury in rats, although the cooling component of their device was deployed outside of the body as it was too large to be implanted in vivo. However, extremely thin thermoelectric cooling devices can now be fabricated that are less than 200 $\mu$m thick [95], making feasible the development of devices that may be implanted in vivo.

1.3.1.3 Magnetocaloric cooling technique

The magnetocaloric effect (MCE) provides another possible mechanism for externally lowering the temperature of the cooling material. The MCE refers to the heating or cooling of magnetic materials due to the application of time-varying magnetic fields. The effect, which has been substantially understood for a long time [96, 97], is caused by the change in magnetic entropy of a material due to the application or removal of an external magnetic field. Specifically, the cooling of a magnetic material is caused by an increase in its magnetic en-
entropy when an external magnetic field is removed. The MCE has been a subject of considerable interest since 1997 when Pecharsky and Gschneidner [98] discovered a very large MCE in some Gd$_5$(Si$_x$Ge$_{1-x}$)$_4$ alloys. Many alloys have subsequently been found to possess a colossal MCE [96, 99, 100], although only some of these were found to undergo their maximum temperature change close to room temperature and ambient pressure. Of these, only a very few were found to possess their MCE peak near physiological temperature (37°C). De Campos et al. [101] reported that the MCE peak of Mg$_{1-x}$Fe$_x$As is at 37°C when $x$ is 0.003.

1.3.2 Mathematical models for thermoresponsive drug delivery systems

1.3.2.1 Diffusion models from Fick’s law

There are three parts in a typical drug delivery system: a net-work structure (such as hydrogel), release medium (it can be as simple as water or a complex mixture of proteins and chemicals, such as blood), and drug (it has to be removed from the net-work structure into the environment). The mechanism used by the drug molecules to leave the net-work structure and go into the environmental release medium can vary, but diffusion is one of the most important methods.

In order to model the diffusion of drug molecules, Fick’s equation is used as the starting point. It describes a process by which matter is transported from one location to another as a result of random molecular motions. Fick [102] was the first person to recognize that the equations of heat conduction could be used to describe molecule diffusion.

The Fick’s diffusion equation can be described as follows if the drug is uniformly dispersed throughout the one-dimensional thin slab-shaped matrix:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}(D \frac{\partial C}{\partial x})$$

(1)

where $C$ is the concentration of the drug in solution, $t$ is the time, $D$ is the diffusion coefficient and $x$ is the dimension. This equation can be solved under
some assumptions and conditions.

1.3.2.2 Mathematical models of diffusion release

The most basic presumption of the diffusion equation 1 is to presume the diffusion coefficient constant. Therefore, the equation 1 can be put into:

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

(2)

If the release process is under sink condition and the release through slab edges is neglected, the equation 2 can be solved as:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^\infty \frac{8}{(2n+1)^2 \pi^2} \cdot \exp \left[ -\frac{(2n+1)^2 \pi^2 D}{H^2} t \right]$$

(3)

where $\frac{M_t}{M_\infty}$ is the fraction of release at time t and H is the thickness of the slab.\(^{13}\)

An empirical equation [103] is one of widest used to model the diffusion process:

$$\frac{M_t}{M_\infty} = k t^n$$

(4)

where k is a structural/geometric constant for a particular system and n is designated as release exponent representing the release mechanism.

Alfrey, Gurnee, and Lloyd [102] classified polymer diffusion behaviour into three cases according to the relative rates of diffusion and polymer relaxation:

- Case I, is also called Fickian diffusion, it is where the rate of relaxation is rapid compared to the rate of diffusion;
- Case II, the rate of diffusion is much faster than the rate of relaxation;
- Non-Fickian or anomalous diffusion, in which the rate of diffusion and relaxation is comparable.

\(^{13}\)n is the order of the equation which is used to describe how accurate the calculated result to the true value.
For the slab shape of polymer matrix, if $n = 1$, the release mechanism is Case I and if $n = 0.5$, the release mechanism belongs to Case II.

1.4 Aims

In this thesis, three main projects will be reported and the aims of each of them will be listed in this section.

1.4.1 Fabrication and evaluation of thermoresponsive drug delivery system

- To reproducibly prepare thermoresponsive polymer coatings loaded with model drugs, rhodamine B (RhB) and fluorescein isothiocyanate (FITC).
- To investigate the distribution of rhodamine B within the polymer films.
- To study the kinetics of elution of model drugs from the films and how the kinetics is influenced by the three parameters; thickness of films, solubility of drug in release medium and initial drug concentrations.
- To achieve the pulsatile release profile with various model drugs and try to minimize the difference between low temperature and high temperature ($37^\circ C$).

1.4.2 Mathematical models of controlled drug delivery system and pulsatile release behaviour

- To theoretically design a cooling device which can be incorporated with drug delivery system developed here.
- To model the heating process of films by using the cooling device and study the effect of the polymer films and initial temperature.
- To obtain the diffusion coefficient of model drug release from crosslinked films at various temperatures by applying the diffusion equation from Fick’s law.
• To model the pulsatile release behaviour of model drug from crosslinked films and estimate the time for cold treatment in order to obtain desired dosage of each cycle.

1.4.3 Evaluation of thermoresponsive properties of block copolymers

• To study the factors (hydrophobicity of non-thermoresponsive block, the molecular weight of both blocks, and pH values) that affect the LCST of block copolymers.

• To characterise the surface of dry films made from block copolymers.

• To study the capability of block copolymer to self-assemble into particles.

• To evaluate the biocompatibility of block copolymers on 3T3 mouse fibroblast cells.

List of References


[34] Y. Kaneko, R. Yoshida, K. Sakai, Y. Sakurai, and T. Okano, “Temperature-responsive shrinking kinetics of poly (N-isopropylacrylamide) copolymer gels with hydrophilic and hydrophobic


INTRODUCTION


INTRODUCTION


INTRODUCTION


INTRODUCTION


The methods and techniques will be described in two main sections to correspond to Chapter 4 and 5: thermoresponsive drug delivery system and dual responsive block copolymers.

2.1 Materials

UV crosslinkable polymers of poly(\(N\)-isopropylacrylamide-\(co\)-acrylamidobenzophenone) (poly(NIPAm-\(co\)-ABzPh)) were synthesized by Dr. Alexander V. Gorelov [1]. Block copolymers of poly(\(tert\)-butylacrylate-\(b\)-\(N\)-isopropylacrylamide) (poly(tBA-\(b\)-NIPAm)), poly(acrylic acid-\(b\)-\(N\)-isopropylacrylamide) (poly(AA-\(b\)-NIPAm)), poly(\(N\),\(N\)-dimethylacrylamide-\(b\)-\(N\)-isopropylacrylamide) (poly(DMA-\(b\)-NIPAm)), and poly(styrene-\(b\)-\(N\)-isopropylacrylamide) (poly(St-\(b\)-NIPAm)) were synthesized by Dr. Padrai O’Connor [2]. The chemical structures of the monomers used to make the polymers are presented in Figure 8.

Table 3. Poly(NIPAm) containing block co-polymers used in aqueous cloud point analysis [2].

<table>
<thead>
<tr>
<th>Block copolymers(^{(a)})</th>
<th>(M_n) (GPC)</th>
<th>(M_w/M_n)(^{(b)})</th>
<th>(M_n) (NMR)(^{(c)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(DMA(<em>{58})-b-NIPAm(</em>{82}))</td>
<td>13400</td>
<td>(1.48)</td>
<td>15000</td>
</tr>
<tr>
<td>Poly(DMA(<em>{58})-b-NIPAm(</em>{117}))</td>
<td>21400</td>
<td>(1.55)</td>
<td>18950</td>
</tr>
<tr>
<td>Poly(DMA(<em>{58})-b-NIPAm(</em>{217}))</td>
<td>29250</td>
<td>(2.07)</td>
<td>30250</td>
</tr>
<tr>
<td>Poly(tBA(<em>{62})-b-NIPAm(</em>{81}))</td>
<td>20600</td>
<td>(1.42)</td>
<td>17200</td>
</tr>
<tr>
<td>Poly(tBA(<em>{62})-b-NIPAm(</em>{192}))</td>
<td>30750</td>
<td>(1.33)</td>
<td>29700</td>
</tr>
<tr>
<td>Poly(tBA(<em>{62})-b-NIPAm(</em>{254}))</td>
<td>34550</td>
<td>(1.31)</td>
<td>36750</td>
</tr>
<tr>
<td>Poly(St(<em>{62})-b-NIPAm(</em>{260}))</td>
<td>34200</td>
<td>(1.51)</td>
<td>36500</td>
</tr>
</tbody>
</table>

\(^{(a)}\) \(M_w/M_n\) is calculated from \(M_n\) (GPC) for the first block relative to overall \(M_n\) (NMR) of the block copolymer.

\(^{(b)}\) after purification.

\(^{(c)}\) Calculated from 1H NMR according to [2].

\(N\)-Isopropylacrylamide (NIPAm, 98% TCI) was recrystallized by Dr. Padrai
Figure 8. Chemical structures of the monomers used for synthesis of the polymers in this thesis.

O’Connor\(^1\). poly(\(N\)-isopropylacrylamide) (poly(NIPAm), Aldrich), sodium hydroxide (Aldrich \(\geq 97\%\)), anhydrous methanol (MeOH, Corcoran Chemicals 99.9\%), rhodamine B (RhB, Aldrich \(\sim 95\%\)) and fluorescein isothiocyanate (FITC, Aldrich \(\geq 90\%\)) were all used as received.

Phosphate pH buffer (7.0) was made from 0.1 M sodium hydroxide and 0.1 M potassium dihydrogen phosphate with the volume ratio of 58.1 : 100.

Cell growth cultural medium was composed of 500 ml of Dulbecco’s Modified Eagle’s Medium (DMEM, Aldrich, D6429), 50 ml of fetal bovine serum (FBS, Aldrich, F7524) and 10 ml of Penicillin-Streptomycin (Pen-Trep, Aldrich, P4458). The trypsin (Aldrich, T3924) and Hank’s Buffered Salt Solution (HBSS, Aldrich, H6648) were used for harvesting cells. All other plastic wares were purchased from Aldrich unless stated otherwise.

\(^1\)The recrystallization of commercial NIPAm is to remove the stabilizers which might affect the toxicity results.
2.2 Thermoresponsive Drug Delivery System

2.2.1 Film preparation and drug loading

2.2.1.1 Preparation of crosslinked films

The crosslinkable copolymer, poly(NIPAm-co-ABzPh), was dissolved in dry methanol (2% w/v polymer/methanol). Aliquots (50 and 125 µl respectively) of the solution were applied evenly to the wells of 24-well polystyrene tissue culture plates and polyethylene plastic cover slips (diameter 25 mm). The resulting films (5 µm thick) were allowed to dry at room temperature in a methanol atmosphere overnight. The films were then dried at 40°C for 4 hours under vacuum, and crosslinking initiated by exposure of covered plates to UV light at an intensity of 400 mW/cm² for 20 minutes, followed by inversion of the plates and exposure for a further 20 minutes (Figure 9). During irradiation, the polystyrene served as a long pass filter for UV light [1, 3]. All loading and release experiments were carried out on copolymer films cast on 24-well tissue culture plates. Films used for characterisations were prepared as above on polyethylene plastic cover slips (diameter 25 mm) or other substrates.
Films of various thickness were prepared using the previously described procedure. The volume of the polymer methanol solution ($V_{Ps}$) required was determined by the following equations (equation 5) under a presumption that the density of the dry polymer film ($\rho_{PF}$) is $1 \text{ g/cm}^3$:

$$V_{Ps} = \frac{H_{PF} \times S_{PF} \times \rho_{PF}}{C_{Ps}} \quad (5)$$

where $H_{PF}$ is the desired thickness of dry polymer films, $S_{PF}$ is the coating area of substrates, and $C_{Ps}$ is the concentration of polymer methanol solution.

### 2.2.1.2 Preparation of model drug loaded films

The model drugs (RhB and FITC) were dissolved into anhydrous methanol or millipore water to prepare a 40 mM drug solution. The following equation (equation 6) was used to covert the drug methanol concentration ($C_{Ds}$) from “mM” into “mg/ml”:

$$C_{Ds} (\text{mg/ml}) = M_W \times 40 \text{ mM} \times 10^{-3} \quad (6)$$

The molecular weight $M_W$ of the two model drugs used in this project are listed in Table 4.

<table>
<thead>
<tr>
<th>Model Drugs</th>
<th>Rhodamine B (RhB)</th>
<th>Fluorescein Isothiocyanate (FITC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (g/mol)</td>
<td>479.0</td>
<td>389.4</td>
</tr>
</tbody>
</table>

The drug-methanol solutions were casted on dry crosslinked films to load the model drugs into the film. The samples were placed in a methanol environmental dessicator for over night, after which they were placed in vacuum oven at 40°C for 4 hours. Before characterisation or carrying out a drug release experiment, the drug loaded films (from storage) were dried again at 40°C for 4 hours under vacuum. The volume of drug solution $V_{Ds}$ was determined by
Table 5. AFM parameters for studying of topography of polymer films.

<table>
<thead>
<tr>
<th></th>
<th>Study on drug delivery system</th>
<th>Study on block copolymers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scanning size</strong></td>
<td>25µm × 25µm</td>
<td>500 nm × 500 nm</td>
</tr>
<tr>
<td><strong>Scanning rate</strong></td>
<td>1 Hz</td>
<td>2 Hz</td>
</tr>
<tr>
<td><strong>Number of scanning line</strong></td>
<td>512</td>
<td>512</td>
</tr>
</tbody>
</table>

equation 7 from the surface area of the films to keep the same drug loading concentration.

\[
V_{Ds} = 25 \mu l/cm^2 \times S_P \tag{7}
\]

2.2.2 Physical analysis of polymer films

2.2.2.1 Determination of LCST

In order to estimate the LCST of crosslinked poly(NIPAm-\textit{co}-ABzPh) film, the cloud point measurement of this polymer were carried out using a Cary 100 UV-Vis spectrophotometer at 500 nm, with temperature ramping at 0.1°C/min. Polymer solutions (2 mg/ml) were prepared in Millipore water. The changes of transmittance of each interval of temperature are plotted and analysed by Originlab® to obtain the peak value as LCST and FWHM (full width at half maximum) as the sharpness.

2.2.2.2 Surface characterisation of polymer films

Atomic Force Microscopy (AFM) is used to determinate the roughness and examine the topography of the polymer films. Tapping mode was chosen due to its high sensitivity and limitation on damage of the polymer surface. The parameters used on AFM are listed in Table 5.

The surface topography of four types of films were scanned by AFM: non-crosslinked drug free films, crosslinked drug free films, drug loaded films, and drug loaded films after rinsing. The samples were prepared as previously de-
scribed. After casting and drying the films, the films were scanned before and after exposure of UV light and then, the films were scanned again after loading with the model drugs and further drying. Finally, the drug loaded dry films were scanned again after being rinsed by warm water (40°C) twice and dried.

Roughness measurements were obtained from the topography images. The three most common roughness values were calculated according the following equations.

**RMS** The root mean square roughness is the standard deviation of the Z values within a given area:

\[
RMS = \sqrt{\frac{\sum_{i=1}^{N}(Z_i - Z_{ave})^2}{N}}
\]

where \(N\) is the number of points within a given area, \(Z_i\) is the current Z value and \(Z_{ave}\) is the average Z value within the given area.

**Ra** The mean roughness \((R_a)\) is the arithmetic average of the deviations from the centre plane.

\[
R_a = \frac{\sum_{i=1}^{N}|Z_i - Z_{cp}|}{N}
\]

where \(Z_{cp}\) is the Z value of the centre plane.

**RZ** It is the average difference in height between the five highest peaks and five lowest valleys relative to the centre plane. In cases where five pairs of peaks and valleys do not exist, this value is based on fewer points.

### 2.2.2.3 Drug distribution in films

The samples were prepared by applying an aliquot of copolymer solution (38.5 µl) to glass bottomed culture dishes (P35G-0-14-C, MetTek). The resulting films (5 µm thick) were dried and crosslinked as previously described. Rhodamine B-methanol solution was added to the surface of each film and dried in the methanol atmosphere at room temperature and then dried at 40°C for 4 hours under vacuum. Exposure to light was minimized.
Confocal microscopy was used to study the drug distribution in the film due to the fluorescent property of rhodamine b. By utilizing the high resolution of confocal microscopy, the focus plane in the specimen can be limited to a very thin layer. Therefore, a series of images from different focus planes (z axis) can be obtained at the same location in x-y axis. A 3D image of the specimen is reconstructed by ImageJ®.

The stack of images are obtained by using 40x/1.3 Oil objective lenses and 25 of pinhole so that the resulted resolution of z-axis is around 0.2µm.

2.2.3 Drug release at a constant temperature

Drug release experiments were executed on a new established test system which allows constantly stirring during the drug release process (Figure 10). The drug concentrations were determined by the plate reader with the calibration curves (Figure 11). The details can be found in Chapter 3.

Figure 10. The experimental setup for the drug release experiment at the start.
MATERIALS AND METHODS

2.2.3.1 Effect of film thickness

Films with different thickness (2.5µm, 5µm, and 10µm) were fabricated as previously described and loaded with the same concentration of FITC (25nmol/mg). The drug was released for 10 minutes at two temperatures, 4°C and 40°C. The release rate was calculate and plotted.

2.2.3.2 Effect of drug solubility

FITC has various solubility in the water (< 0.1 mg/ml) [4] and pH=7 buffer (soluble) [5]. Therefore, the effect of solubility of the drug molecules was studied by using the FITC as model drug. In order to minimize the effect of the volume of the release medium, the drug loaded crosslinked films were placed into one big tube with release medium (35 ml). Release medium (0.5 ml) was sampled every time point. The release profile was calculated and plotted.

Figure 11. The calibration curve used for determination of the drug concentration.
2.2.3.3 Effect of initial drug concentration

In theory, the initial drug concentration should not affect the release profile. Two different initial concentrations were used in this experiment: 25 nmol/mg and 250 nmol/mg. The release profile was obtained as previously described.

2.2.3.4 Effect of stirring

In order to study the effect of stirring on the release process, an experiment was set up as described in Chapter 3. The results were calculated and plotted as previously described. The mathematical model was used to fit the release profiles.

2.2.4 Temperature triggered pulsatile release

The effect of thermal cycling on rhodamine B release from UV-crosslinked thermoresponsive polymer films was investigated. The drug loaded samples were first rinsed twice with warm distilled water (40°C) to remove the rhodamine B on the surface of the films. Then the films were soaked into distilled water (1 ml, 37°C) and at pre-designed time intervals, all the dissolution medium was withdrawn and replaced by distilled water (1 ml, low temperature, 4°C, 25°C, 28°C or 30°C). After a pre-calculated time period, all of the dissolution medium was replaced by the same amount of distilled water (37°C). Any residual drug remaining in the samples was then extracted by injecting distilled water at 4°C into the wells (30 minutes). A thermal plate (IC22XT, Torry Pines Scientific) and hotplate combined with magnetic stirrer were used to help maintain the film at the desired temperature. The concentration of the model drug in the sampling solution was determined by a fluorescence plate reader.

2.2.4.1 Pulsatile release with various low temperatures

The results of this experiment were compared with simulations of the mathematical model for values of the diffusion coefficients obtained from the fixed temperature release experiments at 4°C and 40°C, 25°C and 37°C, 28°C and
37°C, 30°C and 37°C. These temperatures were chosen in order to minimize the difference between high temperature and low temperature where pulsatile release profiles are still obtainable.

### 2.2.4.2 Pulsatile release with various model drug

Two model drugs were used to obtain a pulsatile release profile from crosslinked polymer films: poorer solubility model drug, FITC, and good solubility model drug, rhodamine B. With the same initial drug concentration (20 nmol/mg) and same thickness of the film (5 µm), the pulsatile release profile between 4°C and 37°C were obtained.

### 2.2.4.3 Squeeze effect

Thermoresponsive polymer networks possess a phase transition when the temperature is changed around the LCST. This transition is also known as the volume transition due to the dramatic change of their volumes around the LCST. In the case of the thermoresponsive hydrogel based drug delivery system, if this volume transition happens in very short period of time, it might lead to an sudden acceleration release which is not due to the diffusion [6]. This is known as the squeeze effect.

In order to verify if the squeeze effect existed for the thermoresponsive drug delivery system developed here, the release profile was observed when the temperature of the release medium suddenly jumped from the high temperature (37°C) to the low temperature (4°C). After soaking the films in cold water (4°C) for 2 to 3 minutes, 1 ml of the warm water (37°C) was applied on top of the film immediately after removing all the cold water. This portion of the warm water was removed in 30 seconds and another 1 ml of warm water was applied.
2.3 Dual Responsive Block Copolymers

2.3.1 Polymer and micelle preparation

2.3.1.1 Block copolymer preparation

Block copolymers were prepared by Dr. Padraig O’Connor in our lab, and the detailed procedure can be found in the reported publication [2].

2.3.1.2 Polymer particles preparation

Block copolymers were dissolved in double distilled water to reach the concentration of 2 mg/ml. The small particles can be observed under microscopes.

2.3.1.3 Physical analysis of block copolymers and particles

TEM was used to determine the diameter of the particles of block copolymers. The particle-water solution (2 mg/ml) was dropped on top of the TEM grid (Agar, Formvar/Carbon 200 Mesh Cu Grid) and left to dry for 2 hours in the ambient condition.

AFM was used to obtain the topography of block copolymer films and their particles. Block copolymers were dissolved into methanol to prepare 2% w/v solution which were cast on top of cover-slips (diameter 25 mm). Overnight in a methanol environmental dessicator and 4 hours in a vacuum oven (40°C) were adopted to obtain the dry films.

2.3.2 Dual responsiveness of block copolymers

2.3.2.1 LCST determination by cloud point

Aqueous cloud point measurements of block copolymers were carried out using a Cary 100 UV-Vis spectrophotometer at 500 nm, with temperature ramping at 0.1 °C/min. Polymer solutions (0.1% w/v) were prepared in Millipore water. NaOH solutions were used to adjust the pH of samples containing poly(AA)
blocks. Samples were allowed to equilibrate for several hours before analysis. Commercial poly(NIPAM) with $M_n = 56,000$ and $M_w / M_n = 2.70$ was used as reference.

### 2.3.2.2 pH sensitive of block copolymer

The LCST of the different block copolymers was measured in different pH environments. Hydrochloric acid and sodium hydroxide were used to adjust the pH.

3 ml of 2 mg/ml aqueous solution of the block polymers were prepared first. A small volume (less than 0.5 ml $^2$) of the 1 M of HCl and NaOH was used to adjust the pH value.

### 2.3.3 Biocompatibility studies

#### 2.3.3.1 Cell culture

**Subculturing adherent cells** was done under the following procedures. Cells were generally supplied as cryo-preserved vials. Following rapid melting in a 37°C water bath they were transferred to a T75 tissue culture flask (Starstedt). Immediately, 10 ml of medium preheated to 37°C was added, and the flask placed into an incubator (37°C, 5% CO$_2$). The following day, the medium was changed in order to remove remnants of toxic dimethyl sulfoxide (DMSO) (from the freezing process).

When cells formed a monolayer, the flask was rinsed with 5 ml of HBSS. 3ml of 1% trypsin-EDTA (sigma) was added and the flask incubated at 37°C for 5 minutes. Trypsin-EDTA disrupts the attachment the cell makes to the substrate, by complexing with Ca$^{2+}$ ions necessary for cell attachment. However, it is toxic to cells and must be neutralised after 5 minutes by the addition of serum (from medium). Following cell detachment, 3 ml of medium was added and the contents of the flask transferred to a 15 ml centrifuge tube. The cells

---

$^2$The volume of addition was limited to less than 0.5 ml in order to minimize the effect of volume change.
were then centrifuged at 1200-1500 rpm for 5 minutes. The supernatant was discarded and the pellet resuspended in 6 ml of medium. The concentration of cells in the suspension was then counted using a haemocytometer. 1 or 0.5 million cells were transferred to each T75 flask. Flasks were returned to the incubator and re-subcultured after 3 days.

**Cryo-preservation of cells** was necessary to make supplies of frozen cells. Once cells had reached a monolayer, they were trypsinised and spun down to a pellet. The pellet was resuspended in 1 ml of freezing mix (10% DMSO in medium). The cells were transferred to cryo-tubes (Starstedt) and slowly frozen. They were first cooled in ice, then wrapped in tissue paper and placed in the -80°C freezer overnight. Subsequently, they were stored in liquid nitrogen.

**3T3 fibroblast cells** were used to assess the biocompatibility of the block copolymers. Cells were seeded and grown in a 24-well plate first for 24 hours, and then the block copolymers were introduced into the cell culture medium. The cells were continuously grown for another 24 hours before the assays were used.

3T3 mouse fibroblast cells were grown in DMEM (Sigma) supplemented with 1% Pen-strep antibiotic (Sigma) and 10% FBS (Sigma). Cells were grown in an incubator at 37°C and in an atmosphere of 5% CO₂ in air.

Cells were subcultured every 3 days. Cells were pelleted using a centrifugation speed of 1500 rpm.

**2.3.3.2 Cell Morphology**

Bright field microscopy is the simplest of all the optical microscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample. Bright field microscopy is the simplest of a range of techniques used for illumination.
of samples in light microscopes and its simplicity makes it a popular technique. The typical appearance of a bright field microscopy image is a dark sample on a bright background, hence the name.

Pictures of the cells after culturing with block copolymer were taken for 24 hours period. In order to optimize the quality of the images, the medium was removed and replaced by fresh medium before the picture was taken. The thermoplate was used for maintaining the temperature during the whole process.

### 2.3.3.3 Pico Green Assay

The Quant-iT PicoGreen® (Invitrogen) kit was used to determine DNA levels in treated cells. PicoGreen® is a fluorescent stain that is highly selective for solubilised double stranded DNA. It is a very sensitive technology and the DNA is released from the cells using a freeze-thawing procedure.

#### General Procedure:

- Cells of interest were cultured in sterile tissue culture plates for 24 hours after initial seeding (30,000 cells/cm$^2$). They were cultured with the block copolymers for 24 hours.
- Cell growth medium and polymers were removed and the cells were rinsed with HBSS.
- 250 µl of double distilled water was added to each well.
- The cells were then freeze-thawed repeatedly. This was carried out by freezing the cells for 20 minutes at -80°C and subsequent thawing for 30 minutes at room temperature. This was repeated 3 times in order to ensure complete lysis of the cell membranes.
- 100 µl of each sample was transferred into a fresh well of a black 96 well plate.
MATERIALS AND METHODS

- A standard curve was constructed using the 2 µg/ml DNA stock and buffer provided in the kit. Final concentrations of the standards were: 1000 ng/ml, 500 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 10 ng/ml, 5 ng/ml, and 0 ng/ml. 100 µl of each standard was made up in triplicate in the 96 well plate.

- 100 µl of PicoGreen® agent diluted 1:200 in buffer was added to each well.

- Fluorescence was then measured using the plate reader (Victor 3.0) at an excitation wavelength at 480 nm and an emission wavelength at 530 nm.

2.3.3.4 MTT Assay

The MTT assay is a colorimetric technique, first described by Mosmann in 1983 [7], and used for measuring the mitochondrial activity of cells. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphen bromide (MTT) is reduced to purple formazan by mitochondrial reductase, an enzyme active in the mitochondria of living cells. The cell membrane is largely impermeable to formazan resulting in accumulation of purple formazan crystals within the cell. As the reduction only takes place in healthy cells, the amount of formazan product is directly related to the number of viable cells. A detergent (DMS) is used to dissolve the purple crystals and the absorbance of the resultant solution is measured using a spectrophotometer, giving a relative indication of the number of viable cells.

General procedure:

- cells of interest were cultured in sterile 24-well tissue culture plates.

- After 24 hours the tissue culture medium was replaced by block copolymers dissolved in fresh medium. Control cells were only in fresh medium.

- After a further 24 hours of growth 30 µl of 1 mg/ml MTT in HBSS was added to each well and the plate incubated for a further 5 hours.
MATERIALS AND METHODS

• The medium is then removed and 300 µl of DMSO is added per well.
• The formazan crystals adhered to the bottom of the wells were dissolved with gentle swirling.
• 100 µl of the solution was transferred from each well into a clear 96 well plate.
• The absorbance was read at 550 nm using a plate reader (Victor 3.0).

List of References


CHAPTER 3

Fabrication and Evaluation of Thermoresponsive Drug Delivery System

In this chapter, a new thermoresponsive drug delivery system which was fabricated from UV crosslinkable copolymer poly(\(N\)-isopropylacrylamide-\(co\)-acrylamidobenzophenone) (poly(NIPAm-\(co\)-ABzPh)) in the form of thin film will be presented. A list of parameters, film thickness, drug solubility and initial drug concentration, will be studied on how they affect the elution profile of the model drug. This system possesses an ability to control the elution profile into a pulsatile pattern.

3.1 Physical and chemical analysis of polymer films

3.1.1 Surface characterisation of polymer films

3.1.1.1 Surface topography

The topographical images from various stages of film preparation were compared. Figure 13, Figure 14, Figure 15, Figure 16 and Figure 17 show the five surfaces that were examined: before deposition of the polymer film, before and after the crosslinking, after fluorescein isothiocyanate (FITC) loading, and after rinsing by warm water (40°C). All the images were taken after drying due to the limitation of the AFM used.

The patterns shown in Figure 15 are known as the buckling effect. This was caused by the osmotic pressure when the polymer chains swelled in the solvent after they were fixed on a rigid flat surface (Figure 12). A similar phenomenon was observed by other research groups [?]. Therefore, the existence of these patterns proves that the crosslinking was successful.

The disappearance of these patterns (Figure 16) after the rinsing by warm water means the osmotic pressure can be reduced by slowing the evaporation of solvent during the drying process (Figure 12).
Figure 12. The principle of the formation of the buckling effect.

Figure 13. The topographical image and roughness analysis of the 5 µm-thick poly(NIPAm-co-ABzPh) film on the polyethylene coverslip substrate before exposure to UV light in order to facilitate crosslinking.
Figure 14. The topographical image and roughness analysis of the 5 μm-thick poly(NIPAm-co-ABzPh) film on the polyethylene coverslip substrate after crosslinking by UV light.
Figure 15. The topographical image and roughness analysis of the 5 \( \mu \)m-thick poly(NIPAm-co-ABzPh) film on the polyethylene coverslip substrate loaded with the FITC at the initial concentration at 25 nmol/mg.
Figure 16. The topographical image and roughness analysis of the 5 µm-thick poly(NIPAm-co-ABzPh) film on the polyethylene coverslip substrate loaded with the FITC at the initial concentration at 25 nmol/mg and rinsed by warm water (40°C) twice. The sample were dried for two days at 40°C before examined under AFM.
Figure 17. The topographical image and roughness analysis of the substrate (polyethylene coverslip) used to support the polymer films.
The desired loading amount & Rinse off & Total released & Loading efficiency
\(\text{mg}\) & \(\text{mg}\) & \(\text{mg}\) & \(\%\)
---
0.01 & 0.0007 ± 0.00003 & 0.009 ± 0.002 & 92 ± 6

| The desired loading amount & Rinse off & Total released & Loading efficiency |
|-----------------|---------------|-------|--------------|
| \(\text{mg}\) & \(\text{mg}\) & \(\text{mg}\) & \(\%\) |
| 0.01 & 0.0007 ± 0.00003 & 0.009 ± 0.002 & 92 ± 6 |

Table 6. Calculation of drug loading efficiency.

### 3.1.2 Drug distribution in films

Confocal microscopy was used to analyse the distribution of fluorescent model drug rhodamine B (RhB) within the polymer films. Using a process known as optical sectioning, images of various z-axis planes (or slices) within a sample may be combined to give a 3D view of a sample [?].

The aim was to use this z stack technique to analyse the distribution of fluorescent model drug within the polymer films. The distribution of model drug would be observed within each plane of the polymer sample and these planes could then be combined to generate an overall view of drug distribution. The preparation of samples is as described in the previous chapter.

Figure 18 shows a set of z-plane images obtained from a RhB loaded film. The first and last focus position of this scanning was estimated. When the fluorescent signal disappeared on the screen, this position was recorded as the finished position and the signal just before the fluorescence appears is the first focus position. Between these two positions, 38 pictures are taken and thus each picture has a 0.26 \(\mu\text{m}\) gap between it and the previous picture.

By combining all the z-stack pictures, a 3D drug distribution image can be obtained from Zeiss® software. This 3D image is presented in Figure 19. The thickness was measured in the 3D images at six random locations and the result was found to lie in the range 5.0 \(\mu\text{m}\) ± 0.2 \(\mu\text{m}\). It is observed that the distribution of model drug (rhodamine B) is uniformly distributed initially. The drug on the surface was rinsed off by the warm water and the results were present in Table 6.
Figure 18. A series of fluorescent images of Rhodamine B (20 nmol/mg) loaded 5 µm crosslinked poly(NIPAm-co-ABzPh) film obtained in the confocal microscope at different depth of focus plane.
3.1.2.1 Roughness analysis

In Table 7, the roughness of five different surfaces are summarized together. It can be seen that the roughness does not differ strongly after deposition of the polymer film and the roughness drops slightly after crosslinking which can be used to indicate the success of the crosslinking. After loading the FICT into the polymer films, patterns have formed which dramatically increase the roughness. However, after rinsing the surface of the polymer film, these patterns were minimized and as the roughness values show the surface has become smoother.
Due to the limitation of AFM and the definition of the profile roughness, the profile roughness can be changed very substantially by the size of the scanned area [?]. The reason for this is the effect of the distortional plane on the roughness. Therefore, the scanning size of all five surfaces was fixed as 10 × 10 µm².

3.1.3 Determination of lower critical solution temperature

The LCST of poly(NIPAm-co-ABzPh) was measured by using the cloud point method [?]. As shown in Figure 20, the transition temperature of the polymer is around 29°C. However, unlike the sharp transition obtained for commercial poly(NIPAm) (0.5°C), the sharpness of the phase transition for poly(NIPAm-co-ABzPh) is around 2°C. In the Figure 20b is the curve based on the cloud point results in order to make the reading of the LCST and the sharpness of the transition easier. However, there isn’t any literature to use this method to read.

The study of the shape of the transition curve of the poly(NIPAm) are very few. Schild and Tirrel [?] has reported a serie of the poly(NIPAm) with variuos molecular weight and dispersion. However, they can only conclude the dispersion has effects on the shape of the transition curve but they did not give any more detail of this relationship.
Figure 20. Transmittance curve (above) for the uncrosslinked polymer in aqueous solution. The transmittance data was obtained using a UV-Vis spectrophotometer. The polymer used is poly(NIPAm-co-ABzPh) which was employed to construct the thin films. The polymer concentration was 1 mg/ml in double distilled water and the heating ratio was 0.1 °C/min. The changes of transmittance of each interval of temperature are plotted (below) and analyzed to obtain the peak value as LCST and FWHM (full width at half maximum) as the sharpness.
3.2 Drug release at a constant temperature

In vitro drug release kinetic studies were performed, at different temperatures, by soaking the samples in distilled water (2 ml). The drug loaded samples were first rinsed twice with warm distilled water (40°C) to remove the rhodamine B on the surface of the films. At regular time intervals 1 ml of the dissolution medium was withdrawn and analysed by plate reader. The same volume of fresh distilled water was added to replace the volume of the extracted samples. Any residual drug remaining in the samples was then extracted by injecting distilled water at 4°C into the wells (30 minutes). A thermal plate (IC22XT, Torrey Pines Scientific) and hotplate combined with magnetic stirrer (Figure 21) were used to help maintain the film at the desired temperature. All experiments were carried out in triplicate. The release profile was obtained with and without stirring.

![Figure 21. The experimental setup for the drug release experiment at the start.](image)

All of the sampling solution was removed into a black 96-well plate, and then placed in a plate reader to obtain the drug concentration. The resulting data was subsequently analyzed with the aid of Microsoft® office Excel® and the final curves were produced using Originlab®.
Table 8. Value of standard errors of regression with various thickness.

<table>
<thead>
<tr>
<th>Thickness of the films (µm)</th>
<th>Zero-order Fitting</th>
<th>Fick’s Law Fitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.358</td>
<td>4.675</td>
</tr>
<tr>
<td>2.5</td>
<td>7.945</td>
<td>6.156</td>
</tr>
<tr>
<td>5</td>
<td>6.99</td>
<td>5.935</td>
</tr>
<tr>
<td>10</td>
<td>9.896</td>
<td>2.661</td>
</tr>
</tbody>
</table>

3.2.1 Effect of film thickness

The thickness of the film is one of the most important parameters in the elution process. In order to study the effects of thickness, films were prepared with three different thickness values, 2.5 µm, 5 µm, and 10 µm. The results were plotted and can be seen in Figure 22.

As the results show, the release from the film with thickness of 2.5 µm, 5 µm, and 10 µm are relatively the same, which does not follow Fick’s law as expected. The release results were fitted into the zero-order model and the Fick’s law diffusion model and the standard errors of regression (S) are listed in Table 3.2.1. This suggests other release mechanisms might be involved in the elution process. One possible reason is the crosslink ratio on the surface of the film with the various thickness value are the same which means the size of the mesh of these films are very similar. More studies are needed to verify this inference. However, when the films are as thin as 1 µm, the release mechanism is zero-order release. This might be because the films are so thin that the crosslinked network lost the control of the diffusion process. When the thickness of the films is a thick as 10 µm, the main release mechanism is the Fick’s law diffusion (minimum S value). When the thickness in the range of 1 to 10 µm, the release of the model drug are governed by both two mechanisms.
Figure 22. Effect of thickness of polymer films. FITC was loaded into films with three different thickness, 2.5 µm, 5 µm, and 10 µm. The initial drug concentration was 25 nmol/mg and the release profiles were obtained in water medium at both 4°C and 40°C.
3.2.2 Effect of drug solubility

The solubility of the drug molecule in the release medium can affect the release profile. This is verified by using FITC as model drug and two types of release medium were chosen: water where FITC has poor solubility and pH = 7 buffer where FITC has better solubility.

It is easy to conclude that with better solubility, the release is faster in the buffer solution. Figure 23 shows that the solubility does not only affect the release rate, it also affects the total amount of releasable drug. With higher solubility, the release can reach nearly 100%, but for the poorly soluble drugs, the amount of releasable drug in the same period is even less than 50% of loaded drug.

There are a few possible reasons for these results. The limited volume of release medium will restrain the total amount of drug which can be released. However, due to the fixed volume of total release medium, this effect was minimized in this experiment. Another possible reason is that the poorly soluble drug molecules tend to aggregate together to form particles. These particles might accumulate on the surface of the film to prevent the further drug release. In this experiment, this effect was reduced by mixing the release medium. The third possible reason is due to the slow self diffusion rate of poorly soluble drugs. Another possible reason would be the reaction between amide group of poly(NIPAm) and FITC. However, the possibility of this reaction are unclear and there is no reported on this issue.
Figure 23. Effect of solubility of drug. FITC was loaded into 5 µm thick crosslinked polymer film at initial drug concentration of 25 nmol/mg. The release profiles were obtained in water medium and water buffer (pH = 7) at two temperatures 4°C and 40°C.
3.2.3 Effect of initial drug concentration

The original amount of drug in the film at the start of the released process is another critical parameter. Two different initial drug concentrations were adopted in this experiment; 1% (25 nmol/mg) and 10% (250 nmol/mg). The results are plotted in Figure 24.

As the results show the effect of initial drug concentration is the same for both temperatures (4°C and 40°C). Although the Fick’s law has indicated that the initial concentration of the drug molecules in the film will not affect the cumulative release, the results show that a larger initial concentration will slow down the elution process. Figure 25 shows that the fractional release profiles are similar when the initial concentration is from 10 nmol/mg to 100 nmol/mg. However, when the initial concentration is small as 5 nmol/mg, the release becomes faster. One of the possible reasons is because the diffusion coefficient is not independent on the concentration [?]. However, the results has shown in the range of 10 to 100 nmol/mg, the diffusion coefficient can be treated as the constant parameter.
Figure 24. Effect of initial drug concentration. FITC was loaded into 5 μm thick crosslinked polymer films at two different initial drug concentration, 1% (25 nmol/mg) and 10% (250 nmol/mg). The release profiles were obtained in water medium at two temperatures 4°C and 40°C.
Figure 25. Effect of initial drug concentration. Rhodamine B was loaded into 5 µm thick crosslinked polymer films at five different initial drug concentration, 5 nmol/mg, 10 nmol/mg, 20 nmol/mg, 50 nmol/mg, and 100 nmol/mg. The release profiles were obtained in water medium at 37°C.
3.3 Pulsatile Release

3.3.1 Pulsatile release with various low temperatures

The model drug, RhB, was loaded into UV crosslinked 5 µm-thick films with the initial drug concentration at 20 nmol/mg. After twice rinsing by warm water (37°C), the elution profiles were obtained based on the procedure described in Chapter 2.

Figure 26 shows that the pulsatile release pattern can be obtained by applying 4°C as the low temperature. There are a total of 6 cycles of temperature changing. Similar dosages of the model drug was released in each cycle except the first one. In the first cycle, the release at low temperature is extremely fast and this might result in a burst effect. The reason for this boost of releasing might be due to the fact that the difference of the water content in the gel between high temperature (40°C) and low temperature (4°C) is too large. When the temperature drops, large amount of water diffuses into polymer network. The drug molecules dissolved by this water and release at a relative fast rate.

In order to minimize the burst effect caused by difference between temperatures, other low temperatures were adopted, including 25°C, 28°C, 30°C and 32°C, while the high temperature was chosen as physiological temperature 37°C.

Figures 27-29 have shown that the pulsatile release can be obtained when low temperature located at 25°C, 28°C and 30°C are used, while Figure 30 shows that the pulsatile release cannot be realized if the low temperature was at 32°C. Therefore, the minimum temperature difference is 7°C in order to control the drug release in pulsatile patterns successfully.

By comparing with LCST measurement of the copolymers, the 32°C is outside the range of the LCST of poly(NIPAm-co-ABzPh). Therefore, the pulsatile release profile cannot be obtained.
Figure 26. (a) Pulsatile release profile between 4 °C and 40 °C. The cumulative release of rhodamine B (20 nmol/mg) from 5 µm thick UV-crosslinked polymer films in distilled water at 4 °C and 40 °C were sampled at selected time points. (b) Release rate profile corresponding to the cumulative release profile in (a).
Figure 27. Pulsatile release profile between 25°C and 37°C. The cumulative release of RhB (20 nmol/mg) from 5 µm thick UV-crosslinked polymer films in distilled water at 25°C and 37°C were sampled at selected time points.
Figure 28. Pulsatile release profile between 28°C and 37°C. The cumulative release of RhB (20 nmol/mg) from 5 µm thick UV-crosslinked polymer films in distilled water at 28°C and 37°C were sampled at selected time points.
Figure 29. Pulsatile release profile between 30°C and 37°C. The cumulative release of RhB (20 nmol/mg) from 5 µm thick UV-crosslinked polymer films in distilled water at 30°C and 37°C were sampled at selected time points.
Figure 30. Pulsatile release profile between 32°C and 37°C. The cumulative release of RhB (20 nmol/mg) from 5 µm thick UV-crosslinked polymer films in distilled water at 32°C and 37°C were sampled at selected time points.
3.3.2 Pulsatile release with various model drugs

The other model drug, FITC, was loaded into the 5 µm thick UV crosslinked films at the initial drug concentration 20 nmol/mg and its pulsatile release profile was obtained at temperatures 4°C and 40°C (Figure 31).

A total of 6 cycles were obtained and around 10% of total drug were released in each cycle. Compared with the release profile of RhB, the release of FITC is more controlled. This might be because that the solubility of FITC limits the amount of drug which can be released from the each cycle.

3.3.3 Squeeze effect

When the temperature was jumped from 4°C to 40°C, the swollen films will collapse suddenly. This will lead to a squeeze effect where the drug molecules are pushed out due to mechanical force. In order to observe this effect, sampling time points were designed after the low temperature release medium was replaced by 1 ml of hot water (40°C), the hot water will be replaced by another 1 ml of fresh hot water in 30 seconds. The release profile is presented in Figure 32a while the release rate is presented in Figure 32b. It is obvious that the more rapid release continued for another 30 seconds after the temperature jumped back from 4°C to 40°C. This might be because the swollen films take longer time to collapse or the drug was squeezed out when the film shrank. The same leaking phenomenon was also observed by Satarkar et al. [?] and by Chang et al. ( [?]). However, neither of them justified adequately whether the continued leaking is caused by squeezing or by slow shrinking.
Figure 31. Pulsatile release profile between 4°C and 40°C. The cumulative release of FITC (20 nmol/mg) from 5 µm thick UV-crosslinked polymer films in distilled water at 4°C and 40°C were sampled at selected time points.
Figure 32. (a) Pulsatile release profile between 4 °C and 40 °C. The cumulative release of FITC (20 nmol/mg) from 5 µm thick UV-crosslinked polymer films in distilled water at 4 °C and 40 °C were sampled at selected time points to show the continued release after the temperature raised. (b) Release rate profile corresponding to the cumulative release profile in (a)
3.4 Conclusions

In this chapter, a new controlled drug delivery system was fabricated and characterised. The effects of three parameters on the release kinetics were studied: the thickness of the films, the solubility of the drug (FITC), and the initial concentration of the drug. By altering the temperature, the release profile can be tuned into pulsatile patterns.

The results have shown that the films have buckling patterns after loading the drugs and that these patterns can be removed after rinsing by warm water (40°C). The film thickness does not affect the release profile as contrary to what Fick’s law predicted, when the thickness was less than 2.5 nm. This means that another physical mechanism might be involved in the release process, such as surface adsorption. The release of the model drug from this system has been proven that it can be regulated by varying the temperature. Therefore, the pulsatile release profiles of two model drugs were obtained at low and high temperatures. The study has shown that the difference between high and low temperatures can be limited as 7°C due to the LCST of the polymer is in the range of 29.0 ± 2.0 °C.
CHAPTER 4
Mathematical Models of Controlled Drug Delivery System and Pulsatile Release Behaviour

Mathematical modelling for controlled drug delivery systems can be very helpful to evaluate the possibility of a theoretical design of the delivery system, to speed up the development of the delivery system, to better understand mechanisms involving the drug delivery process, and to predict quantitatively the resulting drug release profile under various conditions.

In this chapter, a mathematical model of a cooling device will be set up for a thermoresponsive controlled drug delivery system. The parameters of the cooling materials (CMs) will be discussed based on the cooling time. The thermoresponsive controlled drug delivery system presented in the previous chapter will be modelled here and its pulsatile release behaviour will be described mathematically. The temperature dependent diffusion coefficient of the model drug will be estimated and compared with the lower critical solution temperature (LCST) of the polymer aqueous solution.

4.1 Theoretical design of cooling device for controlled drug delivery system

Although a huge number of the thermoresponsive drug delivery systems were developed in the last decades, there is not a device which can put these systems in action. The major issue for this problem would be that there isn’t a proper method to regulate the temperature in vivo. In this part, a cooling device will be designed theoretically and its heating process will be modelled in order to compare the various materials of cooling device for their cooling capability. It is presumed the time for the device to cool down to the beginning cooling temperature is time zero. Based on the study shown in the previous chapter [1], the reasonable cooling time of this device would be in the range of 1-30 minutes.
4.1.1 Model set up

Although there are at least three possible cooling techniques (see section 1.3.1) which can be used to regulate the temperature in human body, no study has verified it. The following mathematical model is aimed to evaluate the requirements for a cooling device to reduce the temperature \textit{in vivo} in order to turn on the thermoresponsive drug delivery system.

4.1.1.1 Physical model

In this model, the cooling device is simplified into a slab of CMs. A thermoresponsive polymer film based drug delivery system is attached on the CMs. In order to turn on the drug delivery system, the CMs have to have an initial temperature which is low enough to cool the polymer film down below its LCST. The whole system\(^1\) is placed into a water environment where an initial temperature is set to 37$^\circ$C. Considering there are two types of heat source in human body; the metabolism of surrounding tissue and the nearby blood vessels, a heat source (constantly at 37$^\circ$C) is also included at a variable distance.

4.1.1.2 Modelling assumptions

The basic assumptions made in formulating the mathematical model are now listed.

(i) Heat conduction in the CM, polymer and water is governed by Fourier’s law of conduction, and the temperature and heat flux are taken to be continuous at the CM-polymer and polymer and water interfaces.

(ii) The thermal conductivity and diffusivity of the CM, polymer and water are taken to be constant, and the presence of the drug and the change of the temperature does not affect these parameters.

(iii) The phase transition for the polymer is assumed to be perfectly sharp, so

\(^1\)For convenience, the system would represent the cooling device and the adjacent polymer film instead of the drug delivery system as in the text.
Based on these three assumptions, a mathematical model will be set up to describe a heat-up process. The process of the CM to cool down is dependent on the cooling technology which we do not include in this model. The thickness of the polymer film will be dependent on the temperature due to the different swelling ratio in the range of the LCST. However, in the case of the thin film, this difference is not critical for a final conclusion. The detailed discussion can be found in later sections in this chapter.

4.1.1.3 List of denotations

There are a few of denotations that will be used later in this chapter:

- $H_M$ – Half thickness of the cooling device;
- $H_W$ – Distance between heat source to the surface of the polymer;
- $H_P$ – Thickness of polymer coating on the cooling device;
- $t$ – Time;
$T_i^M$ – Initial temperature of the cooling device;
$T_M$ – Temperature of the cooling device;
$T_P$ – Temperature of the polymer coating;
$T_i^W$ – Initial temperature of the water;
$T_W$ – Temperature of the water;
$T_L$ – LCST of the polymer;
$k_M$ – Thermal diffusivity of the cooling device;
$k_P$ – Thermal diffusivity of the polymer coating;
$k_W$ – Thermal diffusivity of water;
$K_M$ – Thermal conductivity of the cooling device;
$K_P$ – Thermal conductivity of the polymer coating;
$K_W$ – Thermal conductivity of water;
$\rho_c$ – Volumetric density;
$\rho_M c_M$ – Volumetric heat capacity of the cooling device;
$\rho_P c_P$ – Volumetric heat capacity of the polymer coating;
$\rho_W c_W$ – Volumetric heat capacity of water;
$t_L$ – Time for keeping the temperature on the interface of polymer coating and water below the LCST;
$I_M$ – Thermal inertias of the cooling device
$I_W$ – Thermal inertias of water

### 4.1.1.4 Geometry and model equations

For simplicity, the cooling device is taken to be composed of an infinite slab of homogeneous material of thickness $2H_M$ that occupies $-2H_M < x < 0, -\infty < y, z < \infty$. At $x = -2H_M$ and $x = 0$, the CM is taken to be in thermal contact with polymer films of thickness $H_P$. At $x = -H_M - H_P$ and $x = H_P$, the polymer films are in turn taken to be in thermal contact with water that occupies $-\infty < x < -2H_M - H_P$ and $H_P < x < \infty$. Water is chosen as the fluid medium here since it is the dominant component of biological fluids. The system is clearly symmetric about the centreline of the CM at $x = -H_M$, and so a symmetry condition is imposed at $x = -H_M$, and the semi-infinite domain $-H_M < x < \infty$ need only be considered; see Fig. 34.
Figure 34. A schematic representation of a slice of the CM, polymer, and water at time $t = 0$ in the semi-infinite domain $-H_M < x < \infty$. The complete system lies along the infinite domain $-\infty < x < \infty$, but is symmetric about the centreline of the CM at $x = -H_M$. The temperatures in the CM, polymer and water at location $x$ and time $t$ are denoted by $T_M(x, t)$, $T_P(x, t)$ and $T_W(x, t)$, respectively. The initial temperature of the entire system is taken to be at the constant temperature of the water, $T_W^i$, say. Since the behaviour of the device in the human body is being modelled, $T_W^i$ is taken to be $37^\circ C$ in the numerical calculations of this paper. The LCST of the thermoresponsive polymer is denoted by $T_L$, and it is supposed that $T_L < T_W^i$ so that the polymer is initially in its collapsed state. At time $t = 0$, it is assumed that the temperature throughout the CM is instantaneously lowered to $T_M^i < T_L$. The purpose of the analysis of this paper is to investigate the subsequent temperature evolution for $t > 0$ in the adjoining polymer, and in particular, the possibility of the polymer temperature dropping to below its LCST throughout its thickness so that it can swell and release drug. The subsequent reheating of the polymer above its LCST due to heat conduction from the adjacent fluid medium will also be modelled.
In view of the discussion immediately above and the modelling assumptions of the previous section, the temperature in the CM $T_M(x,t)$ satisfies:

$$\frac{\partial T_M}{\partial t} = k_M \frac{\partial^2 T_M}{\partial x^2} \quad \text{for} \quad -H_M < x < 0, \quad t > 0,$$

$$\frac{\partial T_M}{\partial x} = 0 \quad \text{on} \quad x = -H_M, \quad t \geq 0,$$

$$T_M = T^i_M \quad \text{for} \quad -H_M < x < 0, \quad t = 0,$$

(8)

where $k_M$ is the constant thermal diffusivity of the material. The boundary condition (8)$_2$ is the symmetry condition that allows the spatial domain to be halved. The temperature in the polymer $T_P(x,t)$ satisfies:

$$\frac{\partial T_P}{\partial t} = k_P \frac{\partial^2 T_P}{\partial x^2} \quad \text{for} \quad 0 < x < H_P, \quad t > 0,$$

$$T_P = T^i_P \quad \text{for} \quad 0 < x < H_P, \quad t = 0,$$

(9)

where $k_P$ is the constant thermal diffusivity of the polymer, and the temperature in the water medium $T_W(x,t)$ satisfies:

$$\frac{\partial T_W}{\partial t} = k_W \frac{\partial^2 T_W}{\partial x^2} \quad \text{for} \quad H_P < x < \infty, \quad t = 0,$$

$$T_W = T^i_W \quad \text{for} \quad H_P < x < \infty, \quad t = 0,$$

$$T_W \rightarrow T^i_W \quad \text{as} \quad x \rightarrow \infty, \quad t \geq 0,$$

(10)

where $k_W$ is the constant thermal diffusivity of the water. Imposing continuity in temperature and heat flux at the CM-polymer and the polymer-water interfaces gives:

$$T_M = T_P, \quad -K_M \frac{\partial T_M}{\partial x} = -K_P \frac{\partial T_P}{\partial x} \quad \text{on} \quad x = 0, \quad t \geq 0,$$

$$T_P = T_W, \quad -K_P \frac{\partial T_P}{\partial x} = -K_W \frac{\partial T_W}{\partial x} \quad \text{on} \quad x = H_P, \quad t \geq 0,$$

(11)

where $K_M, K_P, K_W$ give the constant thermal conductivities of the CM, polymer, and water, respectively. The thermal diffusivities are related to the thermal conductivities via:

$$k_M = \frac{K_M}{\rho_M c_M}, \quad k_P = \frac{K_P}{\rho_P c_P}, \quad k_W = \frac{K_W}{\rho_W c_W},$$

(12)

where, using an obvious notation, the $\rho$’s give the densities and the $c$’s the specific heat capacities of the three media. The ratio of the conductivity to the
thermal diffusivity, \( K/k = \rho c \), is referred to as the volumetric heat capacity of a material.

The mathematical model is now complete and consists of equations (8)-(11).

4.1.2 Discussions of model

4.1.2.1 Non-dimensionalisation and non-dimensional parameters

The independent non-dimensional parameters that may be varied to tune the system are identified by non-dimensionalising the governing equations (8)-(11).

Introducing the dimensionless variables:

\[
\bar{x} = \frac{x}{H_M}, \quad \bar{t} = \frac{t}{(H_M^2/k_M)}, \quad \bar{T}_M = \frac{T_M}{T_W}, \quad \bar{T}_P = \frac{T_P}{T_W}, \quad \bar{T}_W = \frac{T_W}{T_W},
\]

the following dimensionless equations are obtained:

**Cooling Material**

\[
\frac{\partial \bar{T}_M}{\partial \bar{t}} = \frac{\partial^2 \bar{T}_M}{\partial \bar{x}^2} \quad \text{for} \quad -1 < \bar{x} < 0, \quad \bar{t} > 0,
\]

\[
\frac{\partial \bar{T}_M}{\partial \bar{x}} = 0 \quad \text{on} \quad \bar{x} = -1, \quad \bar{t} \geq 0,
\]

\[
\bar{T}_M = \bar{T}_{M/W} \quad \text{for} \quad -1 < \bar{x} < 0, \quad \bar{t} = 0,
\]

(13)

**Cooling material/polymer interface**

\[
\bar{T}_M = \bar{T}_P, \quad -\frac{\partial \bar{T}_M}{\partial \bar{x}} = -K_{P/M} \frac{\partial \bar{T}_P}{\partial \bar{x}} \quad \text{on} \quad \bar{x} = 0, \quad \bar{t} \geq 0;
\]

(14)

**Polymer**

\[
\frac{\partial \bar{T}_P}{\partial \bar{t}} = k_{P/M} \frac{\partial^2 \bar{T}_P}{\partial \bar{x}^2} \quad \text{for} \quad 0 < \bar{x} < \varepsilon, \quad \bar{t} > 0,
\]

\[
\bar{T}_P = 1 \quad \text{for} \quad 0 < \bar{x} < \varepsilon, \quad \bar{t} > 0,
\]

(15)

**Polymer/water interface**

\[
\bar{T}_P = \bar{T}_W, \quad -\frac{\partial \bar{T}_P}{\partial \bar{x}} = -K_{W/P} \frac{\partial \bar{T}_W}{\partial \bar{x}} \quad \text{on} \quad \bar{x} = \varepsilon, \quad \bar{t} \geq 0,
\]

(16)
MATHEMATICAL MODELS OF CONTROLLED DDS

Water

\[
\frac{\partial T_W}{\partial t} = k_{W/M} \frac{\partial^2 T_W}{\partial x^2} \quad \text{for } \varepsilon < x < \infty, \quad t = 0,
\]

\[T_W = 1 \quad \text{for } \varepsilon < x < \infty, \quad t = 0,\]

\[T_W \to 1 \quad \text{as } x \to \infty, \quad t \geq 0,
\]

(17)

where the over bars have been dropped for convenience, and where:

\[
T_{M/W}^i = \frac{T_M^i}{T_W^i}, \quad \varepsilon = \frac{H_p}{H_M}, \quad K_{P/M} = \frac{K_P}{K_M}, \quad K_{W/P} = \frac{K_W}{K_P},
\]

\[k_{P/M} = \frac{k_P}{K_M} = \frac{\rho_M c_M}{\rho_P c_P}, \quad k_{W/M} = \frac{k_W}{K_M} = \frac{\rho_W c_W}{\rho_M c_M},
\]

(18)

are the non-dimensional parameters arising.

It is noteworthy from (18) that for a given polymer and CM, there are two parameters that may be independently varied to tune the system, namely the temperature ratio, \(T_{M/W}^i\), and the geometrical parameter, \(\varepsilon\). The remaining four quantities appearing in equation (18) are material parameters, and are fixed for a given choice of polymer and CM. If both the CM and the polymer are allowed to change, then in principle all six parameters in equation (18) may be independently varied.

The problem defined by equation (13)-(17) is linear and analytical progress is possible using, for example, the method of Laplace transforms [2]. However, the algebra arising is quite heavy and it is more convenient to solve the full problem numerically. Fortunately, a convenient analytical solution may be written down at leading order in the thin polymer film limit (which is probably the most important case from the point of view of applications), and this is considered now.

4.1.2.2 The thin polymer film limit

The asymptotic limit \(\varepsilon = H_p/H_M \to 0\) is considered, which corresponds to the thickness of the polymer being small compared to the thickness of the CM. The remaining parameters are taken to be \(O(1)\), and as \(\varepsilon \to 0\) the following expansions are posed for \(t = O(1)\):

\[T_M \sim T_{M0}(x, t) \quad \text{in } -1 < x < 0, \quad T_W \sim T_{W0}(x, t) \quad \text{in } x > 0.
\]
It is easily shown that $T_{M_0}$ satisfies (13), $T_{W_0}$ satisfies (17) with $\varepsilon = 0$, and:

$$T_{M_0} = T_{W_0}, \quad -\frac{\partial T_{M_0}}{\partial x} = -K_{W/M} \frac{\partial T_{W_0}}{\partial x} \quad \text{on} \quad x = 0, \quad t \geq 0,$$

where $K_{W/M} = K_{P/M} K_{W/P} = K_W/K_M$. This leading order problem can be readily solved using Laplace transforms (similar problems are considered in [2], for example) to obtain:

$$T_{M_0} = T_{W_0} - \frac{1}{2} (T_{M/W}^i - 1)(1 - \alpha) \sum_{n=0}^{\infty} \alpha^n \left( \text{erfc} \left( \frac{2n - x}{2\sqrt{t}} \right) + \text{erfc} \left( \frac{2(n + 1) + x}{2\sqrt{t}} \right) \right),$$

$$T_{W_0} = 1 + \frac{1}{2} (T_{M/W}^i - 1)(1 + \alpha) \sum_{n=0}^{\infty} \alpha^n \left( \text{erfc} \left( \frac{2n + x \sqrt{K_{M/W}}}{2\sqrt{t}} \right) - \text{erfc} \left( \frac{2(n + 1) + x \sqrt{K_{M/W}}}{2\sqrt{t}} \right) \right),$$

where:

$$\alpha = \frac{\sqrt{k_{W/M} - K_{W/M}}}{\sqrt{k_{W/M} + K_{W/M}}} = \frac{\sqrt{K_M \rho_M c_M} - \sqrt{K_W \rho_W c_W}}{\sqrt{K_M \rho_M c_M} + \sqrt{K_W \rho_W c_W}},$$

so that $|\alpha| < 1$. The leading order temperature in the polymer film, $T_{P_0}$ (say), is then given by:

$$T_{P_0}(t) = T_{M_0}(0, t) = T_{M/W}^i - \frac{1}{2} (T_{M/W}^i - 1)(1 - \alpha) \sum_{n=0}^{\infty} \alpha^n \left( \text{erfc} \left( \frac{n}{\sqrt{k_{M/W} t}} \right) + \text{erfc} \left( \frac{n + 1}{\sqrt{k_{M/W} t}} \right) \right),$$

or, in dimensional terms:

$$T_{P_0}(t) = T_M - \frac{1}{2} (T_M - T_W)(1 - \alpha) \sum_{n=0}^{\infty} \alpha^n \left( \text{erfc} \left( \frac{H_M n}{\sqrt{k_M t}} \right) + \text{erfc} \left( \frac{H_M (n + 1)}{\sqrt{k_M t}} \right) \right).$$

In previous section, a non-dimensionalisation of the governing equations (8)-(11) is presented. The asymptotic limit $\varepsilon = H_p/H_M \to 0$ is considered, with the other independent dimensionless parameters arising being taken to be $O(1)$ as above discussions. This limit is of practical relevance since it corresponds to the case of a thin polymer film coating. It is found that $T_p \sim T_{P_0}(t)$ as $\varepsilon \to 0$, where:

$$T_{P_0}(t) = T_M - \frac{1}{2} (T_M - T_W)(1 - \alpha) \sum_{n=0}^{\infty} \alpha^n \left( \text{erfc} \left( \frac{H_M n}{\sqrt{k_M t}} \right) + \text{erfc} \left( \frac{H_M (n + 1)}{\sqrt{k_M t}} \right) \right),$$

with:

$$\alpha = \frac{\sqrt{K_M \rho_M c_M} - \sqrt{K_W \rho_W c_W}}{\sqrt{K_M \rho_M c_M} + \sqrt{K_W \rho_W c_W}} = \frac{I_M - I_W}{I_M + I_W},$$
where:

\[ I_M = \sqrt{K_M \rho_M c_M}, \quad I_W = \sqrt{K_W \rho_W c_W}, \]

give the thermal inertias of the cooling device and water, respectively. It is clear from (21) that \(|\alpha| < 1\). It is noteworthy that at leading order, the temperature in the polymer does not depend on position, and is also independent of the conductivity, thermal diffusivity and thickness of the polymer. Hence, for a thin polymer film, the temperature in the polymer will frequently be dominated by the properties of the CM and the water medium, as would be expected.

An elementary calculation shows that \(T_{p_0}\) is an increasing function of time for \(|\alpha| < 1\) and \(T_{M}^{i} < T_{W}^{i}\) so that the polymer has its minimum temperature at leading order immediately after the temperature in the CM has been lowered. From equation (20), this minimum temperature is given by:

\[ T_{p_0}(t) \to \frac{1}{2} \left((1 + \alpha)T_{M}^{i} + (1 - \alpha)T_{W}^{i}\right) = \frac{I_M T_M^{i} + I_W T_W^{i}}{I_M + I_W} \quad \text{as} \quad t \to 0^+, \]

which is a weighted average of the initial temperatures of the CM and the water, with the weights being given by the thermal inertias of the two media. As time progresses, the leading order temperature of the polymer increases toward the initial water temperature, \(T_{W}^{i}\). Hence, in the thin film limit, a necessary condition for the polymer to fully swell is that:

\[ \frac{I_M T_M^{i} + I_W T_W^{i}}{I_M + I_W} < T_L, \quad \text{or}, \quad T_M^{i} < T_L - \frac{I_W}{I_M}(T_W^{i} - T_L). \quad (22) \]

The second inequality in (22) is of considerable practical value since it provides an upper bound for the temperature the CM must be dropped to for drug release to be initiated.

If equation (22) is satisfied, it is of interest to estimate the time \(t = t_L\) by which the polymer has heated back up to its LCST, as \(t_L\) then provides an estimate for the duration the polymer releases drug. If \(t_L \sim t_{L_0}\) as \(\varepsilon \to 0\), then \(T_{p_0}(t_{L_0}) = T_L\), and using equation (20):

\[ \sum_{n=0}^{\infty} \alpha^n \left( \text{erfc} \left( \frac{H_M n}{\sqrt{K_M t_{L_0}}} \right) + \text{erfc} \left( \frac{H_M (n + 1)}{\sqrt{K_M t_{L_0}}} \right) \right) = \frac{2}{1 - \alpha} \frac{T_L - T_M^{i}}{T_W^{i} - T_M^{i}}. \quad (23) \]
Elementary calculations show that equation (23) has a unique solution $0 < t_{L0} < \infty$ provided $|\alpha| < 1$, $T_M^i < T_L < T_W^i$, and equation (22) is satisfied. It also follows from equation (23) that $t_{L0}$ has the general structure:

$$t_{L0} = \frac{H_M^2}{k_M} F \left( \frac{T_L - T_M^i}{T_W^i - T_M^i}, \frac{I_M - I_W}{I_M + I_W} \right),$$

(24)

for some function $F$, or:

$$t_{L0} = t_M F(\theta, \alpha),$$

(25)

where:

$$t_M = \frac{H_M^2}{k_M}, \quad \theta = \frac{T_L - T_M^i}{T_W^i - T_M^i}. \quad (26)$$

Note that $0 < \theta < 1$ for $T_M^i < T_L < T_W^i$ and that equation (22) corresponds to $\theta > (1 - \alpha)/2$. In Table 9, values are displayed for $F(\theta, \alpha)$ in the range $(1 - \alpha)/2 < \theta < 1$, $|\alpha| < 1$, which were found by numerically solving equation (23) using MAPLE.

The formula (24) is instructive because it implies that in the thin film limit, the duration the polymer is held below its LCST is proportional to the square of the thickness of the CM. Since $F(\theta, \alpha) > 0$ for $(1 - \alpha)/2 < \theta < 1$, $|\alpha| < 1$, it is in principle possible to adjust the thickness of the CM so as to maintain the polymer in the swollen state for a desired time interval for any parameter pair $\theta, \alpha$ with $(1 - \alpha)/2 < \theta < 1$, $|\alpha| < 1$. Furthermore, since the dependence on the thickness of the CM is quadratic, it is an effective parameter to adjust to tune the system. It is also noteworthy in equation (24) that the three temperature parameters $T_M^i, T_L, T_W^i$ appear only in a single divided difference ratio. However, it remains to show that the system is realisable for realistic parameter values, and this issue is now addressed.
<table>
<thead>
<tr>
<th>$\theta$</th>
<th>$\alpha = 0$</th>
<th>$\alpha = 0.2$</th>
<th>$\alpha = 0.4$</th>
<th>$\alpha = 0.6$</th>
<th>$\alpha = 0.67$</th>
<th>$\alpha = 0.8$</th>
<th>$\alpha = 0.82$</th>
<th>$\alpha = 0.83$</th>
<th>$\alpha = 0.9$</th>
<th>$\alpha = 0.92$</th>
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<tr>
<td>$0.05$</td>
<td>0.01</td>
<td>0.57</td>
<td>0.72</td>
<td>3.05</td>
<td>5.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0.10$</td>
<td>1.57</td>
<td>2.10</td>
<td>2.37</td>
<td>7.98</td>
<td>12.89</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$0.15$</td>
<td>0.85</td>
<td>3.38</td>
<td>4.33</td>
<td>4.94</td>
<td>15.90</td>
<td>25.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0.20$</td>
<td>0.98</td>
<td>1.76</td>
<td>6.06</td>
<td>7.70</td>
<td>8.76</td>
<td>27.70</td>
<td>44.32</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>$0.25$</td>
<td>1.79</td>
<td>3.00</td>
<td>9.89</td>
<td>12.53</td>
<td>14.23</td>
<td>44.66</td>
<td>71.35</td>
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<td>4.73</td>
<td>15.27</td>
<td>19.31</td>
<td>21.91</td>
<td>68.52</td>
<td>109.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0.35$</td>
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<td>4.41</td>
<td>7.13</td>
<td>22.77</td>
<td>28.77</td>
<td>32.62</td>
<td>101.81</td>
<td>162.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0.40$</td>
<td>1.25</td>
<td>3.18</td>
<td>9.44</td>
<td>15.1</td>
<td>47.89</td>
<td>60.46</td>
<td>68.53</td>
<td>213.51</td>
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<td>33.22</td>
<td>41.94</td>
<td>47.55</td>
<td>148.24</td>
<td>236.56</td>
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<td>$0.50$</td>
<td>1.86</td>
<td>4.13</td>
<td>9.87</td>
<td>28.80</td>
<td>145.39</td>
<td>183.48</td>
<td>207.95</td>
<td>647.60</td>
<td>1033.23</td>
<td></td>
</tr>
<tr>
<td>$0.55$</td>
<td>2.82</td>
<td>6.20</td>
<td>14.80</td>
<td>43.21</td>
<td>69.08</td>
<td>218.17</td>
<td>275.32</td>
<td>312.04</td>
<td>971.80</td>
<td>1550.48</td>
</tr>
<tr>
<td>$0.60$</td>
<td>4.40</td>
<td>9.66</td>
<td>23.11</td>
<td>67.53</td>
<td>107.98</td>
<td>341.11</td>
<td>430.48</td>
<td>487.91</td>
<td>1519.58</td>
<td>2424.49</td>
</tr>
<tr>
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<td>7.27</td>
<td>16.07</td>
<td>38.55</td>
<td>112.83</td>
<td>180.46</td>
<td>570.27</td>
<td>719.70</td>
<td>815.72</td>
<td>2540.75</td>
<td>4053.94</td>
</tr>
<tr>
<td>$0.70$</td>
<td>13.47</td>
<td>29.96</td>
<td>72.11</td>
<td>211.39</td>
<td>388.19</td>
<td>1069.06</td>
<td>1349.25</td>
<td>1529.29</td>
<td>4763.69</td>
<td>7602.26</td>
</tr>
<tr>
<td>$0.75$</td>
<td>31.16</td>
<td>69.73</td>
<td>168.28</td>
<td>493.96</td>
<td>790.45</td>
<td>2499.43</td>
<td>3154.59</td>
<td>3575.56</td>
<td>11139.17</td>
<td>17790.80</td>
</tr>
<tr>
<td>$0.80$</td>
<td>126.66</td>
<td>284.57</td>
<td>688.12</td>
<td>2021.60</td>
<td>3235.60</td>
<td>10233.04</td>
<td>12925.79</td>
<td>14639.28</td>
<td>45625.83</td>
<td>73126.11</td>
</tr>
</tbody>
</table>
4.1.2.3 Cooling materials

The feasibility of a particular system may be evaluated using the formula (25) since $t_{L0}$ estimates the time the polymer will remain in the swollen state. In (25), it is seen that $t_{L0}$ depends on the three parameters $t_M$, $\theta$ and $\alpha$. The parameter $t_M$ depends on the properties of the CM only, $\theta$ depends on the three temperature scales that arise in the system (one each for the CM, polymer and water), and $\alpha$ depends on the ratio of the thermal inertias of the CM and water. It is noteworthy that in the thin film limit, the properties of the polymer only enter at leading order via its LCST in the parameter $\theta$. However, it is clear that the properties of the CM are critical to the behaviour of the system.

In this section, two CMs are evaluated, water and copper. Water is chosen because it may be used as the medium for a cooling system based on endothermic chemical reactions. Copper has a very high thermal and electrical conductivity, and has been chosen to represent metallic materials; thermoelectric and magnetocaloric cooling technologies typically involve metals. Parameter values for both water and copper are displayed in Table 10.

<table>
<thead>
<tr>
<th>Material</th>
<th>$c_\rho$ (J m$^{-3}$K$^{-1}$)</th>
<th>$K$ (J m$^{-1}$K$^{-1}$s$^{-1}$)</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>$4.2 \times 10^6$</td>
<td>0.6</td>
<td>0.00</td>
</tr>
<tr>
<td>Copper</td>
<td>$3.5 \times 10^6$</td>
<td>372.0</td>
<td>0.92</td>
</tr>
</tbody>
</table>

In Fig. 35 (a), the leading order polymer temperature, $T_{p0}(t)$, has been plotted as a function of time, with water being the CM. In the plot, $T_M^i = 32^\circ$C, $T_W^i = 37^\circ$C (physiological temperature), and various thicknesses for the CM are used, ranging from 1 mm to 5 mm. The strong dependency of the behaviour on CM thickness is evident in these plots. If, for example, the LCST of the polymer is $35.5^\circ$C, then it is clear from the figure that a drug release time of some minutes may be achieved for a device thickness of some millimetres if a temperature drop of the order of $5^\circ$C can be induced in the CM. A drug release duration of a couple minutes for a single dose can be appropriate for many systems of practical interest; see, for example, [1].
In Fig. 35 (b), plots for the time (to leading order) it takes the polymer to reheat to its LCST, $t_{L0}$, are given as a function of $T_M^i$, the temperature to which the CM is initially dropped. In these plots, parameter values for both copper and water being the CM are used, and $T_L = 35.5^\circ C$, $T_W^i = 37^\circ C$. It is evident from this figure that the system is realisable for reasonable parameter values for both materials.

### 4.1.2.4 Thicker polymers

The case where the polymer thickness is not small compared to that of the CM is now very briefly considered. For this case, the full set of equations (8)-(11) are integrated numerically. However, it should be emphasised again here that some of the modelling assumptions may not now be valid; see Section 4.1.1.2.

In Figure 36, numerical solutions are plotted for the temperature of the polymer at its interface with the water medium as functions of time, that is, $T_P(H_P, t)$ versus $t$. The model predicts that the polymer will remain swollen while $T_P(H_P, t) < T_L$ since $T_P(x, t)$ has its maximum value at $x = H_P$. In the figure, parameter values typical of polymeric materials have been used for the polymer. The CM is water in Figure 36 (a), and copper in Figure 36 (b). The model predicts, as would be expected, that thicker polymers are more difficult to cool thoroughly, although the results also indicate that the system can produce reasonable results for ratios $H_P/H_M$ as large as $O(1/10)$.

### 4.1.2.5 Effects of the convective heat transfer

Except the diffusion discussed above as the main heat transfer mechanism, the convection is the other main mechanism would affect the cooling time of the cooling device described here. The free convective heat transfer coefficient of water is in the range of 20 - 100 W/m²K. Therefore, for a small cooling device with the surface area of 4 cm² and the cooled temperature of 5 K, the power of the cooling device has to be higher than 0.04 - 0.2 W. The time for the cooling device (3mm thickness) heating up back to $37^\circ C$ would be around 175 - 210 minutes for copper and 35 - 42 minutes for water as cooling materials.
Therefore, unless the forced convective heat transfer involved, the main heat transfer mechanism would be the heat diffusion.

4.1.3 Incorporating a heat source in the modelling

A limitation of the modelling presented here is that no attempt has been made to incorporate the possible presence of heat sources or sinks. In the human body, tissues generate heat via metabolic processes, and blood flowing in or nearby to tissue can exchange thermal energy with it. The subject of bioheat transfer has been extensively studied in the past, and numerous mathematical models have been proposed to describe it [4–8].
Figure 35. (a) The leading order temperature in the polymer, $T_{Po}(t)$, with water being the CM. Here $T_M^i = 32^\circ C$, $T_W^i = 37^\circ C$ and various CM thicknesses have been used. (b) Plots of $t_{L0}$ as a function of $T_M^i$ for $T_W^i = 37^\circ C$, $T_k = 35.5^\circ C$ and various CM thicknesses. Parameter values with both copper and water being the CM have been used.
Figure 36. Plots of the temperature at the polymer-water interface as a function of time for various polymer thicknesses. The cooling material is water in (a), and copper in (b). The parameter values used here are \( k_p = 5 \times 10^{-8} \text{m}^2 \text{s}^{-1} \), \( K_p = 0.12 \text{J m}^{-1} \text{K}^{-1} \text{s}^{-1} \), \( T_{iM} = 32^\circ \text{C} \), \( T_{iw} = 37^\circ \text{C} \), and \( H_M = 4 \text{ mm} \).

The model presented here can be justified for systems where heat conduction due to the initial temperature difference between the CM and the water makes the dominant contribution to heat transfer in the polymer as it reheats to its LCST. Fourier’s law implies that heat transfer via conduction at a given
location is proportional to the temperature gradient at that location. In our system, the temperature gradients in and near a thin polymer are large for times $t \ll \frac{H^2}{k_{M}}$ and $t = O(\frac{H^2}{k_{M}})$ compared to these gradients for times $t \gg \frac{H^2}{k_{M}}$ (with the dimensionless parameters other than $H_{p}/H_{M}$ being $O(1)$), so that heat conduction in the polymer is strong in this sense for $t = O(\frac{H^2}{k_{M}})$ or smaller. However, it should be emphasised that the model presented here cannot be used to estimate the time taken for a polymer to reheat to 37°C in the human body. This is because the model predicts that $T_{P} \rightarrow 37°C$ as $t \rightarrow \infty$, and since heat conduction becomes weaker as time progresses, other factors that can contribute to heat transfer, such as the presence of blood vessels or metabolism, can become significant, or even dominant, as time goes on.

A simple illustrative calculation is now presented that models the presence of a constant heat source at 37°C that is at a distance $H_{W}$ from the polymer. This is achieved by replacing the boundary condition $T_{W} \rightarrow 37°C$ as $x \rightarrow +\infty$ by:

$$T_{W} = 37°C \text{ on } x = H_{p} + H_{W}. \quad (27)$$

This condition could serve as a crude model for the presence of a major artery at $x = H_{p} + H_{W}$, for example; more realistic models for heat transfer in the body can be found in the literature cited above. Numerical solutions corresponding to the boundary condition (27) are presented in Fig. 37, and the results are broadly as expected. It is seen that for $H_{W} \leq 6$ mm, the profiles for the first few minutes are close to the $H_{W} \rightarrow \infty$ curve, but that the curves with $H_{W} = 1, 6, 12$ mm recover to close to 37°C much more quickly than the $H_{W} \rightarrow \infty$ curve. This suggests that for some geometries and materials, heat conduction due to the initial temperature mismatch between the CM and the water can make the major contribution to heat transfer in the polymer in the first few minutes. In Table 11, numerical estimates for the time taken for the polymer to reheat to its LCST and to 36.9°C are displayed for various separations between the polymer and the constant heat source, $H_{W}$. 

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Figure 37. Numerical solutions for the temperature at the polymer-water interface as a function of time, and for various separations $H_w$ between the polymer and a constant heat source held at $37^\circ C$. The polymer is of thickness $H_p = 150 \mu m$, and the CM is water of thickness $H_m = 3 mm$. The thermal diffusivity and conductivity chosen for the polymer are $k_p = 1 \times 10^{-7} m^2 s^{-1}$ and $K_p = 0.15 J m^{-1}K^{-1}s^{-1}$, respectively. The initial temperature of cooling device and water are $T_{iM} = 32^\circ C$ and $T_{iW} = 37^\circ C$, respectively.

4.2 Mathematical models for pulsatile drug delivery system

In this section, a pulsatile drug delivery system based on the thermoresponsive polymer film will be modelled. This system was characterized in a previous chapter (Chapter 3) and can be attached to the cooling device described in a previous section (Section 4.1).
Table 11. Estimated times taken for the polymer to heat back to its LCST, $T_L = 35.5\,^\circ C (t_L)$, and to 36.9$^\circ C (t_{36.9^\circ C})$, with water being CM. The times are estimated for various separations $H_w$ between the polymer and the heat source. The thickness of the CM and the polymer here are $2H_M = 6\, mm$ and $H_P = 150\, \mu m$, respectively. The initial temperature of the CM is $T_{i M} = 32^\circ C$, and of the water is $T_{i W} = 37^\circ C$.

<table>
<thead>
<tr>
<th>$H_w$ (mm)</th>
<th>$t_L$ (min)</th>
<th>$t_{36.9^\circ C}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>2.85</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>$\infty$</td>
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<td>840.0</td>
</tr>
</tbody>
</table>

4.2.1 Non-pulsatile release behaviour

Before the system is used to obtained the pulsatile release profile of model drug, the kinetic release mechanism studied in previous chapter will be modelled for obtaining the diffusion coefficients of model drug in the films.

4.2.1.1 Mathematical model for diffusion

The motion of the drug molecules through the film is assumed to be governed by Fick’s law and we denote by $c(x, t)$ the concentration of drug at penetration $x$ and time $t$ in the film.

If $H(t)$, $D(t)$ denote the thickness of the film and the drug diffusivity at time $t$, respectively, then under the assumptions stated above, the concentration $c(x, t)$ of drug in the film is governed by:

$$\frac{\partial c}{\partial t} = D(t) \frac{\partial^2 C}{\partial x^2}$$

Equations 28 can be solved subject to the following conditions.

(i) The film is initially uniformly loaded with drug, so we take $c = c_0$ at $t = 0$ in $0 < x < H_c$ where $c_0$ is constant.
(ii) The bottom of the film (Fig. 21), \( x = 0 \) is attached to a plastic cover slip substrate, which is taken to be impermeable to the drug, and so we impose \(-D \frac{\partial c}{\partial x} = 0\) on \( x = 0 \).

(iii) Perfect sink conditions are assumed for the drug at the top surface of the film which is in contact with the eluting medium, and so we set \( x = 0 \) on \( x = H(t) \).

The solution is:

\[
\frac{M(t)}{M(\infty)} = 1 - \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} e^{\exp \left( -\frac{(2n-1)^2 \pi^2 Dt}{4H^2} \right)}
\]  

(29)

All the one temperature release profiles were fitted with this equation to test if Fick’s diffusion is the dominant release mechanism.

4.2.1.2 Fixed temperature release

The fraction of total drug released from the system as a function of time was measured for various temperatures, ranging from 4°C to 40°C. It is emphasised that the temperature is kept fixed for each release experiment; the pulsatile release behaviour in which the temperature is varied in the experiment is discussed separately below. It is clear from this data that the rate at which drug releases from the system decreases significantly as the temperature is increased through the range 27°C to 34°C. However, the release rate is less sensitive to changes in temperature for ranges below 27°C or above 34°C.

In Figure 38, a selection of the experimental release profiles is displayed, together with theoretical curves obtained from the mathematical model used to fit to the experimental data. The selection includes examples where the film is fully collapsed, fully swollen, and in an intermediate state between these two extremes. For each of these release experiments, the temperature is held fixed so that the appropriate theoretical release profile is given by equation (29) above. This expression was fitted to the experimental data using the method of least squares, with \( D/H^2 \) being the unknown parameter to be estimated.
Once the estimate for $D/H^2$ has been obtained, $D$ follows immediately if $H$ is known. The nonlinear equation for $D/H^2$ arising from this fitting procedure was solved numerically using the mathematical package MAPLE®\textsuperscript{®}, and the estimates for $D/H^2$ obtained from the fitting procedure are given in Figures 39. In Figure 38, it is observed that in all cases the fit is good between the experimental and theoretical curves.

The diffusion coefficient of the model drug cannot be estimated because the thickness of the films is unknown in the range of the LCST. However, when the film is fully collapsed, the thickness of the film would be very near the thickness of the dry sample ($5.0 \pm 2.0 \mu m$). Therefore, it can be calculated that the diffusion coefficient of the rhodamine B is in the order of $10^{-12} \text{ cm}^2/\text{s}$ comparing with the free diffusion coefficient of in the order of $10^{-5} \text{ cm}^2/\text{s}$ [9].
Figure 38. Influence of temperature and stirring on the release of rhodamine B (20 nmol/film) from 5 µm thick UV-crosslinked polymer films (16 mm in diameter) in distilled water for 120 minutes. (a) Release profile with stirring. (b) Release profile without stirring. The continuous line curves correspond to solutions (29) of the mathematical model which have been fitted to the experimental data.
Figure 39. Transmittance curve for the uncrosslinked polymer in aqueous solution together with $D/H^2$ values for the crosslinked film. The $D/H^2$ values (■) were obtained by fitting a theoretical curve to the experimental data (without stirring) and the transmittance (continuous line) was obtained using the UV-Vis spectrophotometer of p(NIPAm-co-ABzPh) used to construct the thin films. The polymer concentration was 1 mg/ml in double distilled water and the heating ratio was 0.1°C/min.

By comparing the release profile of rhodamine B at 4°C, 25°C, 32°C and 37°C with and without stirring, it can be seen that the release profiles were very similar when the temperature is below LCST (4°C and 25°C). The release was slightly slower without stirring when the temperature is above the LCST (37°C). At 32°C, it is clear from Figure 38 that stirring has dramatically increased the drug release rate. However 32°C is close to the polymer’s LCST, and the composition of the polymer is uncertain in this regime. We could not obtain reproducible experimental results close to the LCST, and the model described here is not appropriate for this regime.

4.2.1.3 Lifetime of system

In order to acquire the lifetime of this system, long-term release profiles of rhodamine B were obtained at 4°C and 37°C. The system was kept at 4°C and
37°C for 2 and 48 hours respectively. The resulting release profiles are presented in Figure 40. It is noted in Figure 40 (b) that there is some discrepancy between the theoretical and experimental data. This can be accounted for by noting that a non-negligible fraction of the drug has been retained by the collapsed polymer even after 40 hours of release; a better fit can be obtained if the data is re-scaled so that the last data point of Figure 40 (b) corresponds to 100% release. This suggests that for the collapsed polymer, a small fraction of the drug releases extremely slowly, if at all. However, we have not attempted to incorporate this effect in the current modelling.

In one hour, 98.6 ± 0.4% of rhodamine B was released at 4°C and 91.1 ± 1.7% of model drug was released in 42 hours at 37°C.

4.2.1.4 Temperature dependence of the drug diffusivity across the LCST

In Figure 39, transmittance is plotted versus temperature for the heating process of the copolymer poly(NIPAm-co-ABzPh) aqueous solution, and these data shows that the transition temperature lies in the range 28°C to 30°C. The scaled diffusivities \( D/H^2 \) were estimated at different temperatures by fitting the experimental data with the theoretical release profile given by equation (29), and the results are displayed in Figure 39. This data reveals that the scaled diffusivity \( D/H^2 \) decreases by approximately one order of magnitude as the temperature is increased through the LCST, which conforms to our intuitive expectations since this temperature change corresponds to the transition from the swollen to the shrunken state for the film. The volume phase transition of the films used in this study was in the range of 22°C to 30°C. This transition temperature range is 6°C wider than that for the uncrosslinked polymer in aqueous solution. It should be noted, however, that the LCST for the polymer in aqueous solution was found to be 3°C higher than that for the crosslinked film [10, 11].
Figure 40. Lifetime of the system. (a) Release profile of rhodamine B at 4°C over 2 hours. (b) Release profile of rhodamine B at 37°C over 48 hours.

4.2.2 Pulsatile release behaviour

In this section, the pulsatile release behaviour of model drugs will be modelled and this model used to help design of the time scale of low temperatures regulated to obtain a controlled pulsatile release profile.
4.2.2.1 Mathematical model for pulsatile release

A one-dimensional model is formulated for drug diffusion in the film, and the release behaviour is considered for the case in which the temperature of the film is quickly and repeatedly switched between a value above the LCST and a value below the LCST. When the film is held at a temperature above the LCST, it is in a condensed state, and we denote by $H_c$, $D_c$ the constant thickness and diffusivity of the condensed film, respectively. If the film is held at a temperature below the LCST, it is in a swollen state, and we denote by $H_s$, $D_s$ the constant thickness and diffusivity of the swollen film, respectively. The lateral dimensions of the film are fixed because they are constrained by the wall of the containing well, and swelling/collapsing can only occur in the $x$ direction. We shall find, as would be expected, that $D_c << D_s$, so that alternating the temperature between values above and below the LCST results in a release profile with an on/off pulsatile character.

The time the polymer was left in either the fully swollen or fully collapsed state was typically of the order of minutes. However, in the experimental work for this paper, the time it took for the thin polymer film to either fully swell or fully collapse was considerably shorter than a minute (see section 3.3.3). Hence, in the current model, we assume that the swelling and collapsing processes occur instantaneously. This assumption simplifies the problem considerably since incorporating the detail of the swelling or collapsing behaviour in the model would require the tracking of moving boundaries [12]. This would lead to a much more challenging mathematical problem, from which an analytical expression for the release profile could not in general be obtained.

We suppose that at time $t = 0$ the film is at the temperature above the LCST and is fully collapsed. At time $t = t_1$ the temperature of the film is taken to instantaneously switch to the value below the LCST and the film is fully swollen; at time $t = t_2$ the film instantaneously reverts to the collapsed state, and so on (collapsed swollen collapsed ...).

Then the thickness of the film and the drug diffusivity at time $t$ can be ex-
pressed:

\[
D(t) = \begin{cases} 
D_c, & 0 \leq t < t_1 \\
D_s, & t_1 \leq t < t_2 \\
D_c, & t_2 \leq t < t_3 \\
\vdots 
\end{cases}
\]

and

\[
H(t) = \begin{cases} 
H_c, & 0 \leq t < t_1 \\
H_s, & t_1 \leq t < t_2 \\
H_c, & t_2 \leq t < t_3 \\
\vdots 
\end{cases}
\]

The model of 28 and 30 is readily solved subject to the conditions list above (i)-(iii) by separating variables (Crank, 1975) and the amount of drug released per unit area from the film by time \(t\), \(M(t) = H_c c_0 - \int_0^t c(x, t) dx\) is easily calculated. The fraction of drug released from the film by time \(t\) can be found to be:

\[
\frac{M(t)}{M(\infty)} = \begin{cases} 
1 - \sum_{n=1}^{\infty} \frac{2}{\lambda_n} \exp \left( -\lambda_n \frac{D_c t}{H_c^2} \right), & 0 \leq t < t_1 \\
1 - \sum_{n=1}^{\infty} \frac{2}{\lambda_n} \exp \left( -\lambda_n \left( \frac{D_c t_1}{H_c^2} + \frac{D_s (t - t_1)}{H_s^2} \right) \right), & t_1 \leq t < t_2 \\
1 - \sum_{n=1}^{\infty} \frac{2}{\lambda_n} \exp \left( -\lambda_n \left( \frac{D_c t_1}{H_c^2} + \frac{D_s (t_2 - t_1)}{H_s^2} + \frac{D_c (t - t_2)}{H_c^2} \right) \right), & t_2 \leq t < t_3 
\end{cases}
\]

where \(\lambda_n = \frac{(2n - 1)^2 \pi^2}{4}\). Equation 31 gives an analytical expression for \(M(t)\), the total drug released from the pulsatile system at time \(t\). The development of such an expression is of significant practical value in both the design and operation of a pulsatile release device. In the design stage, it can be used to predict the thickness, surface area, and initial drug load to be used so that the resulting device can deliver doses of appropriate strength over an appropriate time-scale. In the operation stage, it can be used to devise a detailed schedule for when the device should be switched on and off so as to deliver the required doses at the desired time intervals.

To see how equation 31 might be used in practice, a hypothetical example is considered. Suppose it is required to deliver \(n\) equal doses of a drug, with each dose delivering an amount of drug \(d\). It is further supposed that there is a fixed time interval \(T\) between the deliveries of each dose. In this example, it shall be assumed that some mechanism has been devised that quickly switches the device on and off and that the parameters \(D_c/H_c^2\) and \(D_s/H_s^2\) are known; a procedure for estimating these parameters from experimental data has been given in this paper.

The total drug loaded onto the device, \(M(\infty)\), which of course must exceed \(nd\)
here, can be controlled by varying either the volume of the device or the initial
drug concentration. It is supposed that the device is deployed in the off state
at time \( t = 0 \) and that it is first switched on at \( t = t_1 \). To determine the time
\( t = t_2 \) at which the device should be switched off, we solve the equation:

\[
M(t_2) - M(t_1) = d
\]  

(32)

for \( t_2 \) where \( M(t) \) is given by equation 31; notice that this ensures that the
required dose \( d \) is delivered over the time interval \( (t_1, t_2) \). Although equation
32 gives rise to a rather complicated expression for \( t_2 \) that cannot be solved
analytically, \( t_2 \) can be readily estimated numerically with the aid of mathematical
packages such as MATLAB\textsuperscript{\textregistered} or MAPLE\textsuperscript{\textregistered}. The device is switched on for
the second time at \( t = t_2 + T \), and then switched off again at \( t = t_3 \) where \( t_3 \)
is determined by numerically solving: \( M(t_3) - M(t_2 + T) = d \).

Proceeding in this way, it is clear how a schedule may be devised for switching
the device on and off so as to deliver the required doses at the desired intervals.

The appropriate release formula for an isothermal system which does not swell
or collapse is given by equation 29. This simpler form is very familiar and
has been used on numerous occasions previously to describe diffusion from a
planar sheet \([13, 14]\). We now obtain an estimate for the time it takes for a
release device with a single constant diffusivity to empty its drug load; more
precisely, we estimate the time it takes for there to be only a small fraction
\( r \ll 1 \) of the initial drug load left remaining in the device. This time is
such that \( Dt/H_2 \gg 1 \) and from equation 29 the following approximation is
obtained:

\[
\frac{M(t)}{M(\infty)} \approx 1 - \frac{8}{\pi^2} \exp\left(-\frac{\pi^2 Dt}{4H^2}\right)
\]

for \( Dt/H^2 \gg 1 \). This expression is used to solve for \( t_r \), in
\( M(t_r)/M(\infty) = 1 - r \) to obtain:

\[
 t_r \approx \frac{4H^2}{\pi^2 D} \ln\left(\frac{\pi^2 r}{8}\right)
\]  

(33)

as the estimate for the time at which there is a fraction \( r \ll 1 \) of the initial
drug left in the device. Equation 33 can be used to estimate the effective life-
span of the pulsatile device. Clearly the device will have its minimum effective
life-span, \( t_{min} \), if it is continually left on, so that \( t_{min} = \frac{4H_s^2}{\pi^2 D_s} \ln\left(\frac{8}{\pi^2 r}\right) \). The
device will have its maximum effective life-span, $t_{max}$, if it is continually left off, and $t_{max} = \frac{4H^2}{\pi^2 D_c} \ln \left( \frac{8}{\pi^2 r} \right)$. The effective life-span of the pulsatile device then lies in the range $[t_{min}, t_{max}]$, and these useful quantities are readily calculated; for the system described in this paper, $t_{min}$ is approximately 1 hour and $t_{max}$ is approximately 42 hours for 95% of drug released ($r = 5\%$).

In equation 33, it is seen that there is a strong quadratic dependency on the thickness of the film, but that the dependence on the diffusivity is weaker, being inversely proportional to only the first power. The diffusivities $D_c$ and $D_s$ are determined largely by the nature of the material being used, although there is some flexibility in material choice and preparation. The thickness of the film, however, is a quantity that can be readily varied in practice, and given that the release time-scales have a strong dependence on film thickness, it is probably the most effective parameter to vary when designing a release device.

4.2.2.2 Experimental results fitted with model

In Figure 41, experimental drug release data is displayed for a system in which the temperature is repeatedly switched between $40^\circ$C (above the LCST, slow release) and $4^\circ$C (below the LCST, fast release). The resulting release profile has a clear on/off pulsatile character as would be expected. In the experiment, the system was held in the collapsed state for a fixed three minutes in each cycle, but the time it was left in the swelling state was increased with increasing cycle number so as to achieve an approximately uniform dose for each cycle. However, for the first cycle, a larger dose was delivered. In Figure 41, a theoretical curve based on equation (31) is also displayed which has been fitted to the experimental data using the method of least squares, with $D_c/H^2_c$, $D_s/H^2_s$ being the unknown parameters to be estimated. The results of the analysis reveal that $D_c/H^2_c$ is one order of magnitude smaller than $D_s/H^2_s$, which is consistent with the results of the fixed temperature experiments. The fit is again quite good indicating that our model, despite the simplifying assumptions made in its development, is adequate for capturing the essential features of the release behaviour. In this case, a total of six pulses were obtained. It should be noted
that it is very difficult to realize a temperature transition of 36°C (from 4°C to 40°C) \textit{in vivo}. However, we shall show that the system is capable of successfully exhibiting pulsatile release for temperature transitions as modest as 7°C (from 30°C to 37°C), which is clearly a much more realistic scenario from the point of view of potential applications.

Having established that the model adequately fits the experimental data, we used it as an aid in the design of two further pulsatile release experiments. Specifically, we used the model to estimate when the temperature of the polymer should be changed in the experiments so as to ensure that there would be five cycles, each of duration of 20 minutes approximately, and that between 10% and 20% of the total drug would be released in each cycle. The results of the experiments are displayed in Figure 42, and we see that these objectives were broadly achieved, except for the first temperature drop where less than 10% of the drug was released. It is also noteworthy that there is a much smaller jump in the temperature for these experiments, such as 12°C for the pulsatile release of 25°C and 37°C, 9°C for the pulsatile release of 28°C and 37°C, and only 7°C for the pulsatile release 30°C and 37°C.
Figure 41. (a) Pulsatile release profile between 4°C and 40°C. The cumulative release of rhodamine B (20 nmol/film) from 5 µm thick UV-crosslinked polymer films (16 mm of diameter) in distilled water at 4°C and 40°C were sampled at selected time points. The data points have been fitted to the mathematical model using equation (31). (b) Release rate profile corresponding to the cumulative release profile in (a)
Figure 42. Pulsatile release profiles for the UV-crosslinked polymer films (see Figure 41 for details) where the temperature is varied between (a) 25°C and 37°C, (b) 28°C and 37°C, and, (c) 30°C and 37°C

4.3 Conclusions

A mathematical model was developed to evaluate the feasibility of an in vivo implanted drug delivery system. The delivery device consists of a cooling material coated by a drug-loaded thermoresponsive polymer film. Drug release is initiated by dropping the temperature of the cooling material sufficiently, so that the temperature throughout the polymer coating dropped below its lower critical solution temperature (LCST), causing the polymer to swell and release the drug. Drug release switches off again when heat conduction from an external fluid medium raises the polymer temperature to above the LCST causing the polymer to collapse. Candidate cooling mechanisms based on endothermic chemical reactions, the Peltier effect, and the magnetocaloric effect were discussed.

Due to the thin polymer film limit, the model must account for an upper boundary limit which is the temperature that the cooling material must be dropped to in order for drug release to be initiated. Significantly, the model
predicts that the duration time, for which a thin polymer will continue to release drug in a single cycle, is proportional to the square of the thickness of the cooling material. It was found that the system may be realised for realistic parameter values and materials. In particular, if water is chosen as the cooling medium, it is observed that a device with a thickness of a few millimetres may release the drug for a few minutes over a single cycle if a temperature drop of approximately 5°C may be induced in the cooling material. The model is also used to assess the behaviour of thicker polymers.

A model was developed based on Fick’s law which describes pulsatile release mathematically for the first time. Diffusion coefficients at different temperatures (including temperatures corresponding to both the fully swollen and collapsed states) were estimated by fitting the experimental data with the theoretical release profile given by this model. The effect of temperature on the diffusion coefficient was studied and it was found that in a range of the lower critical solution temperature (LCST), the diffusion coefficient increased with decreasing temperature. The model predicts that the effective lifetime of the system lies in the approximate range of 1 to 42 hours (95% of drug released), depending on how long the system was kept at low temperature (below the LCST). Therefore this system can be used to obtain a controllable pulsatile release profile for small molecule drugs thereby enabling optimum therapeutic effects.

This model was used to estimate when the temperature of the polymer should be changed in the experiments so as to ensure that there would be five cycles, each of duration of 20 minutes approximately, and that between 10% and 20% of the total drug would be released in each cycle. The experimental results confirmed these predictions.

List of References


CHAPTER 5

Evaluation of Thermoresponsive Properties of Block Copolymers

Block copolymers, which possess two physicochemical properties in one polymer chain, attract a lot of attention in the area of drug delivery. In this chapter, a list of poly(NIPAm) block copolymers (poly(tert-butylacrylate)-b-poly(N-isopropylacrylamide), poly(tBA-b-NIPAm); poly(acrylic acid)-b-poly(N-isopropylacrylamide), poly(AA-b-NIPAm); poly(N,N-dimethylacrylamide-b-N-isopropylacrylamide), poly(DMA-b-NIPAm); poly(styrene-b-N-isopropylacrylamide), poly(St-b-NIPAm)) will be assessed for future applications in e.g. fabricating a thermoresponsive controlled drug delivery system.

In the section on physical characterisation, the lower critical solution temperature (LCST) will be measured and a few parameters (chemical properties of non-thermoresponsive block, molecular weight, and pH value) will be studied to determine their effect on the LCST of the block copolymer. The block copolymers’ capability of self-assembling into the formation of particles were investigated later.

In the section on biocompatibility studies, 3T3 mouse fibroblast cells exposed to two block copolymers (poly(tBA-b-NIPAm) and poly(AA-b-NIPAm)) were compared to the cells exposed to commercial poly(N-isopropylacrylamide) (poly(NIPAm)) and its monomers.

5.1 Physical characterisation of block copolymers and their particles

In the first section of this chapter, the LCST of a series of AB type poly(NIPAm) block copolymers will be measured by cloud point techniques. The effects of a list of parameters (the length and the hydrophobicity of non-thermoresponsive block, the pH value of solution) on LCST will be studied. Due to the different solubilities of the two blocks in poly(tBA-b-NIPAm) which
forms micelles, its particles were characterised by transmission electron microscopy (TEM) and atomic force microscopy (AFM).

5.1.1 LCST of block copolymers

In this section, the LCST was measured by cloud point technique in aqueous solution. All of the block copolymers were synthesized using the same initiating system and polymerisation protocols and thus they have the same chemical end-group. Therefore, it can be assumed that the influence of end-group on their LCST is negligible.

LCST determination involved the preparation of purified and dried block copolymer samples (1 mg/ml) in de-ionized water. 0.1 M phosphate pH buffers were used to dissolve block copolymer poly(AA-b-NIPAm) instead of de-ionized water because its LCST is sensitive to pH. Upon heating at a ramping speed of 0.1 °C/min, the solutions became turbid above the LCST, which corresponds to 50% transmittance on the heating curves obtained from the UV-Vis spectrophotometer with temperature controller. Commercially available high MW polydisperse poly(NIPAM) (LCST = 31.7 °C) was used as a reference.

The LCST of following block copolymers will be measured and their molecular weights are listed in Table 12.

Table 12. Poly(NIPAm) containing block co-polymers used in aqueous cloud point analysis [1].

<table>
<thead>
<tr>
<th>Block copolymers(^{(a)})</th>
<th>(M_n) (GPC)</th>
<th>(M_w/M_n)(^{(b)})</th>
<th>(M_n) (NMR)(^{(c)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(DMA(<em>{58})-b-NIPAm(</em>{82}))</td>
<td>13400</td>
<td>1.48</td>
<td>15000</td>
</tr>
<tr>
<td>Poly(DMA(<em>{58})-b-NIPAm(</em>{117}))</td>
<td>21400</td>
<td>1.55</td>
<td>18950</td>
</tr>
<tr>
<td>Poly(DMA(<em>{58})-b-NIPAm(</em>{217}))</td>
<td>29250</td>
<td>2.07</td>
<td>30250</td>
</tr>
<tr>
<td>Poly(tBA(<em>{62})-b-NIPAm(</em>{81}))</td>
<td>20600</td>
<td>1.42</td>
<td>17200</td>
</tr>
<tr>
<td>Poly(tBA(<em>{62})-b-NIPAm(</em>{192}))</td>
<td>30750</td>
<td>1.33</td>
<td>29700</td>
</tr>
<tr>
<td>Poly(tBA(<em>{62})-b-NIPAm(</em>{254}))</td>
<td>34550</td>
<td>1.31</td>
<td>36750</td>
</tr>
<tr>
<td>Poly(St(<em>{62})-b-NIPAm(</em>{266}))</td>
<td>34200</td>
<td>1.51</td>
<td>36500</td>
</tr>
</tbody>
</table>

\(^{(a)}\) \(M_w/M_n\) is calculated from \(M_n\) (GPC) for the first block relative to overall \(M_n\) (NMR) of the block copolymer.

\(^{(b)}\) after purification.

\(^{(c)}\) Calculated from 1H NMR according to [1].
5.1.1.1 Effects of non-thermoresponsive block

In order to study the effect of the non-thermoresponsive block on the LCST of the block copolymer, four block copolymers were synthesized: poly(DMA\textsubscript{58-b-NIPAm\textsubscript{217}}), poly(tBA\textsubscript{62-b-NIPAm\textsubscript{192}}), poly(AA\textsubscript{62-b-NIPAm\textsubscript{192}}) and poly(St\textsubscript{62-b-NIPAm\textsubscript{266}}). Figure 43 shows the block copolymer solutions containing a hydrophobic part, i.e. poly(St) and poly(tBA), became turbid at a lower temperature than those block copolymers containing a hydrophilic parts, i.e. poly(AA) and poly(DMA) parts.

This observation is same as in other studies [2]. One of the possible reasons might be that the introduction of hydrophobic segments in polymers will depress the formation of the hydrogen bonds between polymer chains and water molecules and encourage the hydrogen bonds between polymer chains [3].
Figure 43. Light transmittance at 500 nm wavelength as a function of temperature of 1 mg/ml aqueous solutions at pH 7 of commercial poly(NIPAm) (∗), poly(DMA58-b-NIPAm217) (●), poly(tBA62-b-NIPAm192) (■), poly(AA62-b-NIPAm192) (▲), and poly(St62-b-NIPAm266) (♦).
5.1.1.2 Effects of molecular weight

Figures 44 and 45 respectively show the effects of molecular weight on LCST of poly(NIPAm) block copolymers containing the poly(DMA) and poly(AA). As a greater number of NIPAM monomeric units are incorporated in each case, the copolymers reach the cloud point at temperatures closer to that of commercial poly(NIPAM). Small differences in polydispersity have a negligible effect on cloud points, as indicated upon examination of Table 12. Strong decreases in the phase transition temperature with increasing MW of poly(NIPAM) have been reported by Stover and co-workers for narrow dispersity homopolymers prepared using room temperature ATRP [4].

One possible reason that longer NIPAm chains have a lower LCST is that the effect of the non-thermoresponsive block is weakened due to the reduce it molar ratio in the block copolymer chains. Another possible reason is that with shorter chains of poly(NIPAm), the polymer has less of chance to form H-bonds between the polymer chains.
Figure 44. Light transmittance at 500 nm wavelength as a function of temperature of 1 mg/ml aqueous solution at pH 7 of commercial poly(NIPAm) (continuous line), poly(DMA_{58}-b-NIPAm_{82}) (dotted line), poly(DMA_{58}-b-NIPAm_{117}) (dashed line) and poly(DMA_{58}-b-NIPAm_{217}) (long dashed line).
Figure 45. Light transmittance at 500 nm wavelength as a function of temperature of 1 mg/ml aqueous solution at pH 7 of commercial poly(NIPAm) (continuous line), poly(AA_{62}-b-NIPAm_{81}) (dotted line), poly(AA_{62}-b-NIPAm_{192}) (dashed line) and poly(AA_{62}-b-NIPAm_{254}) (long dashed line).
5.1.1.3 Effect of pH value on poly(AA-b-NIPAm)

Poly(AA-b-NIPAm) is a well-studied dual responsive copolymer [5–8], and it is now observed that the cloud point is lowered significantly at pH 4 close to the pKa (4.5 - 5.0) of the poly(AA) part, where it is most hydrophobic (Figure 46). This is in agreement with the findings of Kulkarni et al [9] with the dependence of cloud point measurements on pH being small once the pKa is exceeded.

However, when the pH value increase from 7 to 10, the LCST of poly(AA-b-NIPAm) decreased slightly (less than 0.5 °C) and this can be observed in other poly(AA-b-NIPAm) block copolymers synthesized in our group. The reason for this decrease is unclear.
Figure 46. Light transmittance at 500 nm wavelength as a function of temperature of 1 mg/ml aqueous solution of poly(AA$_{62}$-b-NIPAm$_{254}$), at pH = 4 (dashed line), pH = 7 (continuous line) and pH = 10 (dotted line).
5.1.2 Topography of particles by TEM

Block copolymer of poly(tBA-b-NIPAm) with a monomer ratio of tBA$_{42}$ to NIPAM$_{41}$ was dissolved in double distilled water. The concentration used was 2 mg/ml which is above the critical micelle concentration in [10]. This solution was then deposited on the transmission electron microscopy (TEM) grid at room temperature (18°C) and examined by TEM after drying for 2 hours.

As Figure 47 shows, the block copolymer self-assembled into the form of particles at the room temperature (18°C) which is below its LCST (28°C-30°C). Although all of these particles have a wide range of diameters from a few of micrometers to less than 100 nanometers (Figure 48 and Figure 49), most of the particles have diameters in the range of 1 µm to 2 µm. The dispersion of these particles in terms of diameter can be improved by dialysis method [11].
Figure 47. Particles of the block copolymer poly(tBA$_{42}$-$b$-NIPAm$_{41}$) in 2 mg/ml aqueous solution at room temperature ($18^\circ$C) with various diameters under TEM.
Figure 48. Particles of the block copolymer poly(tBA_{42}-b-NIPAm_{41}) in 2 mg/ml aqueous solution in micro-meter scale at room temperature (18°C) under TEM.
Figure 49. A particle of the block copolymer poly(tBA_{42}-b-NIPAm_{41}) in 2 mg/ml aqueous solution in nano-meter scale at room temperature (18°C) under TEM.
5.1.3 Topography of films by AFM

Methanol block copolymer solution (20 mg/ml) was prepared by dissolving 10 mg of poly(tBA-b-NIPAm) in 0.5 ml of dry methanol. This solution was then cast on top of a glass cover slip and dried in a dessicator with a methanol environment overnight and followed by incubation in a vacuum oven (40°C) for a further 4 hours. The dried film was then scanned by AFM with the following settings scanning rate at 2Hz and scanning size as 500 nm × 500 nm.

As Figure 50 shows, the surface of the block copolymer film has patterns that are not present on the homopolymer films. These patterns confirm that the two blocks of the copolymer have two different physical properties, hydrophobic and hydrophilic. During the drying process, the block copolymers have phase separated in the small scale and thus the patterns seen in Figure 50 on the surface of the dried films [12, 13].

In order to determine if the block copolymer will form particles, a few drops of cool water (room temperature, 18°C) were scattered on top of the dried block copolymer films. The samples were dried in an ambient environment for 24 hours before being examined by AFM.

Figure 51 and Figure 52 show that particles have formed on the areas after the treatment. The size of the particles is around 150 nm. However, the topography of these particles has shown the same aggregation trend as the results from the TEM.
Figure 50. AFM image of the block copolymer film (5 µm thick). Methanol solution of poly(tBA_{42}-b-NIPAm_{41}) (10kDa) was casted on a glass cover slip and dried in a methanol environment dessicator and a vacuum oven (40°C).
Figure 51. AFM 2D image of a few of particles on the block copolymer film. A few drops of cool water (room temperature, 18°C) were scattered on top of the dried block copolymer films of the poly(tBA_{42}-b-NIPAm_{41}). The sample was dried under atmospheric conditions before being scanned by AFM.
Figure 52. AFM 3D image of a few of particles on the block copolymer film. A few drops of cool water (room temperature, 18°C) were scattered on top of the dried block copolymer films. The sample was dried under atmospheric conditions before being scanned by AFM.
5.2 Biocompatibility studies

In the second section of this chapter, two block copolymers poly(AA-\textit{b}-NIPAm) and poly(tBA-\textit{b}-NIPAm) were tested to determine their biocompatibility using 3T3 mouse fibroblast cells. Cell morphology, cell proliferation and cytotoxicity of these block copolymers will be reported here. These results will be compared with the results of the homopolymer poly(NIPAm) and its monomers.

5.2.1 Morphology of cells with particles of block copolymers

Cell morphology was studied using three polymers, poly(AA_{42}-\textit{b}-NIPAm_{41}), poly(tBA_{42}-\textit{b}-NIPAm_{41}) and commercial poly(NIPAm). These polymers were used to create 5\mu{}m thick films in small Petri dishes (35 mm diameter). After sterilization by UV light, the films were dissolved in 1 ml of hot (37\degree{}C) or cold (4\degree{}C) cell culture medium.

3T3 mouse fibroblast cells were seeded on the tissue culture plastic surface at an initial cell concentration of 30,000/cm$^2$ in 24-well plate and allowed to grow for 24 hours before any treatment. The cells were then grown for a further 24 hours after changing the old cell culture medium and replacing it with 0.5 ml of the polymer-medium solution in each well. Images were then taken using the bright field microscope (10× and 20×).

As Figure 53 shows, after a 48 hours incubation period, the 3T3 cells formed a confluent cell sheets and the cells were totally spread out. Commercial poly(NIPAm) has been proven as a non-toxic polymer [14] and Figure 54 has shown the topography of the 3T3 cells are very similar as the ones in Figure 53 (controlled) which confirmed this. The block copolymer of poly(tBA-\textit{b}-NIPAm) formed particles during the incubation period and Figure 55 has shown that this causes no change in the morphology of the 3T3 cells. The block copolymer of poly(AA-\textit{b}-NIPAm) does not form particles (or size of particles are too small to be seen under microscope) (Figure 56) in the cell medium, and this might be because the pAA part of the block copolymer was neutralized by the chemicals in the cell medium and this prevent the polymer molecule aggregating as
Figure 53. Bright field images of 3T3 cells on tissue culture plastic (TCP) without polymers. Cells were seeded on TCP with an initial density of 30,000 /cm² and grown for 48 hours before pictures were taken using the microscope.
Figure 54. Bright field images of 3T3 cells on tissue culture plastic with commercial poly(NIPAm). Cells were seeded on TCP with an initial density of 30,000 /cm$^2$ and grown for 24 hours without any treatment. Cells were then grown for a further 24 hours with the addition of homopolymer poly(NIPAm) dissolved from its corresponding films by hot (37 °C) or cold (4°C) cell growth medium before the pictures were taken.
Figure 55. Bright field images of 3T3 cells on tissue culture plastic with block copolymer of poly(tBA_{42}-b-NIPAm_{41}). Cells were seeded on TCP with an initial density of 30,000 /cm² and grown for 24 hours without any treatment. Cells were then grown for a further 24 hours with the addition of poly(tBA_{42}-b-NIPAm_{41}) dissolved from its corresponding films by hot (37 °C) or cold (4°C) cell growth medium before the pictures were taken.
Figure 56. Bright field images of 3T3 cells on tissue culture plastic with block copolymer of poly($\text{AA}_{42-b}\text{-NIPAm}_{41}$). Cells were seeded on TCP with an initial density of 30,000 /cm$^2$ and grown for 24 hours without any treatment. Cells were then grown for a further 24 hours with the addition of poly($\text{AA}_{42-b}\text{-NIPAm}_{41}$) dissolved from its corresponding films by hot ($37\,^\circ\text{C}$) or cold ($4\,^\circ\text{C}$) cell growth medium before the pictures were taken.
5.2.2 Cytotoxicity assessment of block copolymers

Two assays were used to assess the toxicity of the synthesized block copolymers. As seen in Figure 57, cells numbers did not change after the exposure to poly(NIPAm), poly(tBA_{42}-b-NIPAm_{41}) and poly(AA_{42}-b-NIPAm_{41}) compared to cells grown on tissue culture plastic surfaces. Figure 58 shows that all three polymers are not toxic towards 3T3 cells in the concentration range from 0.1 mg/ml to 10 mg/ml.

NIPAm monomers were used as the negative control in this experiment and as shown in both Figure 57 and Figure 58, monomers of NIPAm have negative effects on cell proliferation and viability when the concentration is higher than 5 mg/ml; this is in agreement with the results in paper [15].
Figure 57. Results of PicoGreen® assay for the number of cells with and without polymers and monomers. Cells were seeded on TCP with an initial density of 30,000 /cm² and grown for 24 hours without any treatment. Cells were then grown for a further 24 hours with the addition of poly(NIPAm), poly(tBA₄₂-b-NIPAm₄₁), poly(AA₄₂-b-NIPAm₄₁), and NIPAm monomers at various concentrations (10 mg/ml, 5 mg/ml, 1 mg/ml and 0.5 mg/ml) before the PicoGreen® assay was carried out.
Figure 58. Results of MTT assay for cytotoxicity of polymers (commercial poly(NIPAm), poly(tBA_{42-b}-NIPAm_{41}), and poly(AA_{42-b}-NIPAm_{41})) and NIPAm monomer. Cells were seeded on TCP with an initial density of 30,000 /cm² and grown for 24 hours without any treatment. Cells were then grown for a further 24 hours with the addition of poly(NIPAm), poly(tBA_{42-b}-NIPAm_{41}), poly(AA_{42-b}-NIPAm_{41}), and NIPAm monomers at various concentrations (10 mg/ml, 5 mg/ml, 1 mg/ml and 0.5 mg/ml) before the MTT assay was carried out.
5.3 Conclusions

In this chapter, a list of the AB type poly(NIPAm) block copolymers were measured for their LCST dependent on the molecular weight and the hydrophobicity of non-thermoreponsive block. It was found that the longer segment of poly(NIPAm) has lower LCST. The hydrophobic block will decrease the LCST of copolymer while the hydrophilic block increase it.

The block copolymer poly(AA-b-NIPAm), which possesses both the thermoresponosive property and pH sensitivity was examined. When the pH value is below the pKa of AA (4.5), poly(AA) block is hydrophobic leads to a decrease in the LCST. When the pH value is above the pKa, poly(AA) becomes hydrophilic leads to an increase in the LCST.

Due to the amphiphilic properties of the block copolymers, they can form particles which were characterised by TEM and AFM. It has shown that the range of the diameter covers from 20 nm to 3 µm. These particles were proven to be non-toxic for the 3T3 mouse fibroblast cells.

List of References


In this thesis, three projects with regard to thermoresponsive drug delivery system were completed. A new thermoresponsive drug delivery system was based on UV crosslinkable copolymer films which were fabricated and characterised. Pulsatile release profiles of two small molecule model drugs (FITC and RhB) were successfully obtained by controlling the temperature of the dissolution medium. In order to apply this drug delivery system to real applications, a conceptual drug delivery system with the capability of dropping temperature in vivo was proposed and a mathematical model was used to assess its possibility and feasibility of controlling a thermoresponsive drug delivery system based on polymer thin films. A series of thermoresponsive block copolymers, which possess the ability to self-assemble into particles, were characterised and evaluated as the preliminary study of their application on delivery large molecules drugs.

The drug loaded thermoresponsive polymer thin films were prepared by the solvent casting technique and the drying process was optimized to achieve a smooth surface. The drug distribution inside of the thin film was examined by using 3D-confocal microscopy. The results of the RhB loaded films confirmed an even distribution of the drug and a well-defined thickness of the thin film.

The effects of three parameters (thickness of films, solubility of drug in release medium, and initial drug concentration) on the kinetics of elution of the model drugs were studied. When the thickness is as thin as 1 µm, the release mechanism is zero-order and when the thickness becomes thicker than 10 µm, the release curve possesses the same shape as a Fick’s law diffusion curve. For the thickness in the range of 1 - 10 µm, the release is governed by both mechanisms. The possible reason for this phenomenon suggested the effects from other release mechanisms, such as the physical adsorption of drug molecules on the surface of the film slowing down the release. The kinetic release results
verified that drugs with better solubility diffuse faster than ones with poorer solubility. This shows the diffusion of the drug molecules inside of the films is dependent on the diffusion of the drugs in the release medium. The fractional release is not dependent on the initial drug concentration as is expected from Fick’s equation, this happens when the initial concentration is greater than 5 nmol/mg. This indicated that effects of the initial concentration of the drug is not as strong as others parameters.

The pulsatile release profiles of two model drugs, which were triggered by altering the temperature of the dissolution medium, were obtained successfully. Two stages of release behaviour were found: fast release for the swollen state and slow (yet significant and non-negligible) release for the collapsed state. Six cycles of pulsatile release between 4°C and 40°C were obtained. The dosage of drug (RhB) released in these cycles could be controlled to deliver approximately equal doses by altering the release time in the swollen state. However, for the first cycle, the swollen release rate was found to be larger than subsequent release rates, and the release time could not be made short enough to prevent a larger dose than desired being delivered.

A mathematical model was developed to evaluate the feasibility of an in vivo implanted drug delivery system. The delivery device consists of a cooling material coated by a drug-loaded thermoresponsive polymer film. Drug release is initiated by dropping the temperature of the cooling material sufficiently, so that the temperature throughout the polymer coating dropped below its lower critical solution temperature (LCST), causing the polymer to swell and release the drug. Drug release switches off again when heat conduction from an external fluid medium raises the polymer temperature to above the LCST causing the polymer to collapse. Candidate cooling mechanisms based on endothermic chemical reactions, the Peltier effect, and the magnetocaloric effect were discussed.

Due to the thin polymer film limit, the model must account for an upper boundary limit which is the temperature that the cooling material must be dropped to in order for drug release to be initiated. Significantly, the model
predicts that the duration time, for which a thin polymer will continue to release drug in a single cycle, is proportional to the square of the thickness of the cooling material. It was found that the system may be realised for realistic parameter values and materials. In particular, if water is chosen as the cooling medium, it is observed that a device with a thickness of a few millimetres may release the drug for a few minutes over a single cycle if a temperature drop of approximately 5°C may be induced in the cooling material. The model is also used to assess the behaviour of thicker polymers.

A model was developed based on Fick’s law which describes pulsatile release mathematically for the first time. Diffusion coefficients at different temperatures (including temperatures corresponding to both the fully swollen and collapsed states) were estimated by fitting the experimental data with the theoretical release profile given by this model. The effect of temperature on the diffusion coefficient was studied and it was found that in a range of the lower critical solution temperature (LCST), the diffusion coefficient increased with decreasing temperature. The model predicts that the effective lifetime of the system lies in the approximate range of 1 to 42 hours (95% of drug released), depending on how long the system was kept at low temperature (below the LCST). Therefore this system can be used to obtain a controllable pulsatile release profile for small molecule drugs thereby enabling optimum therapeutic effects.

This model was used to estimate when the temperature of the polymer should be changed in the experiments so as to ensure that there would be five cycles, each of duration of 20 minutes approximately, and that between 10% and 20% of the total drug would be released in each cycle. The experimental results confirmed these predictions.

The LCST of a series of poly(N-isopropylacrylamide) (poly(NIPAm)) block copolymers was measured by cloud point method. This study has found that hydrophobic segments in copolymers decrease the LCST and that hydrophilic segments increase the LCST. The length of the poly(NIPAm) segment also affects the LCST, longer poly(NIPAm) chains have lower LCST values. The
poly(acrylic acid) (poly(AA)) segment provides the pH sensitivity in the block copolymer. When the pH value of the solution is higher than its pKa (4.5), the poly(AA) segment become hydrophilic and increases the LCST of block copolymer. When the pH value of the solution is lower than 4.5, the segment poly(AA) become hydrophobic and decreases the LCST of the block copolymer.

Particles were found on the surface of the block copolymer films after the water treatment which suggested their ability of their self-assembling. These block copolymers can also self-assemble into particles in water solution and these particles demonstrate no cytotoxicity towards 3T3 mouse fibroblast cells when the concentration is lower than 10 mg/ml.
CHAPTER 7

Future Works

Chapter 3 has reported on a new controlled drug delivery system based on poly(NIPAm-co-ABzPh) films. It has been proven that this system can control the release profile of model drugs, RhB and FITC, in pulsatile patterns by altering the temperature. As a continuation of this research, perhaps this system can be used to control the dosage of some real drugs, such as an anti-restenotic drug, Vinblastine, or an anti-cancer drug, mitomycin C. A previous study [1, 2] on the effect of Vinblastine has shown that the working range of this drug in vitro would be in the range of 1 nM to 0.1 nM, or the toxic effect would be strong enough to cause irreparable damage. The pulsatile release profile can limit the drug concentration to the appropriate range in order to optimize the effect of the treatment while limiting its cytotoxicity.

The limitation on the size of molecules which can be carried by this system (a controlled drug delivery system based on poly(NIPAm-co-ABzPh) films) can be improved by carefully decreasing the crosslinking ratio while still maintaining the integrity of the films. In this way, this system is able to deliver larger drug molecules such as small proteins or small segments of gene.

In previous publications from our group, quantum dots [3, 4] have been shown to possess a strong and stable fluorescent property for both short- and long-term use. However, high toxicity is one of the limitations on the applications of quantum dots. Therefore, the thermoresponsive drug delivery system based on thin polymer film could be used to carry quantum dots around to cells and only release small doses when fluorescent characterisation is needed.

The block coopolymers characterised in this thesis possesses the ability to self-assemble into particles which can be used to deliver hydrophobic drugs or big molecule drugs. Some anti-cancer drugs have very limited solubility in vivo environment, and these block copolymers can be used to encapsulate them and deliver them into cells. The challenge of this work would be overcoming the
limitation on the size of particles, as the particles would be limited to the range of 100 to 200 nm in order to optimize the transfection rate.

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APPENDIX

Paper Published
A mathematical model for pulsatile release: Controlled release of rhodamine B from UV-crosslinked thermoresponsive thin films

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ABSTRACT

A controlled drug delivery system fabricated from a thermoresponsive polymer was designed to obtain a pulsatile release profile which was triggered by altering the temperature of the dissolution medium. Two stages of release behaviour were found: fast release for a swollen state and slow (yet significant and non-negligible) release for a collapsed state. Six cycles of pulsatile release between 4 °C and 40 °C were obtained. The dosage of drug (rhodamine B) released in these cycles could be controlled to deliver approximately equal doses by altering the release time in the swollen state. However, for the first cycle, the swollen release rate was found to be large, and the release time could not be made short enough to prevent a larger dose than desired being delivered. A model was developed based on Fick's law which describes pulsatile release mathematically for the first time, and diffusion coefficients at different temperatures (including temperatures corresponding to both the fully swollen and collapsed states) were estimated by fitting the experimental data with the theoretical release profile given by this model. The effect of temperature on the diffusion coefficient was studied and it was found that in the range of the lower critical solution temperature (LCST), the diffusion coefficient increased with decreasing temperature. The model predicts that the effective lifetime of the system lies in the approximate range of 1–42 h (95% of drug released), depending on how long the system was kept at low temperature (below the LCST). Therefore this system can be used to obtain a controllable pulsatile release profile for small molecule drugs thereby enabling optimum therapeutic effects.

1. Introduction

Most local drug delivery systems aim to maintain the drug concentration at some appropriate therapeutic level for a specified period of time, and this objective is frequently achieved using sustained release dosage forms. However, for some drugs, an optimum therapeutic effect comes from a periodically fluctuating drug concentration (Kikuchi and Okano, 2002). To realise such behaviour, pulsed or pulsatile drug release systems have been developed (Bae et al., 1991; Coughlan et al., 2004; Ishino et al., 1992; Lowman and Peppas, 1999; Mundargi et al., 2010; Siegel and Pitt, 1995; Vertommen et al., 2008). This type of release system possesses a cycle with two distinct release stages: off/slow release and on/fast release. Usually, the release duration time for the slow release stage is much longer than that for the fast stage, and the release rate is much smaller.

The majority of existing pulsatile release systems can be classified into two categories (Kikuchi and Okano, 2002); time-controlled systems (Intra et al., 2008; Kashyap et al., 2007; Liu et al., 2007; Makino et al., 2000) and stimuli-induced systems (Li and D’Emanuele, 2001; Mohammad and Dashevsky, 2006; Satarkar and Hilt, 2008; Schellekens et al., 2008). Time-controlled release systems can only release at pre-programmed time points, whereas stimuli-induced pulsatile release systems are more easily manipulated. Stimuli-induced systems have been developed based on thermal, chemical, and electrical stimuli. However, systems based on thermal stimuli are particularly convenient since they can be designed and operated without significantly affecting other critical parameters of the system.

Thermoresponsive polymers undergo dramatic changes in conformation in response to a small change in temperature. They
possess a lower critical solution temperature (LCST) in aqueous solution, below which they are hydrophilic and absorb water to become swollen, and above which they are hydrophobic and expel water to become dense and dry. The thermoresponsive polymer, poly(N-isopropylacrylamide) (pNIPAm) has been extensively studied. Its LCST (Heskins and Guillet, 1968) is near physiological temperature (37°C), and the highly temperature-sensitive transition between the hydrophobic and hydrophilic states (also known as the volume phase transition) is independent of other factors, such as pH (Pei et al., 2004). This polymer has been used in the construction of many pulsatile drug release systems that are triggered by altering the temperature, such as hydrogel matrices (Caykara et al., 2006; Coughlan et al., 2004), microspheres (Fundueanu et al., 2009b; Mundargi et al., 2010; Wei et al., 2009), membranes (Li and D’Emanuele, 2001), porous systems (Fundueanu et al., 2009a; Vertoommen et al., 2008) and thin films (Doorty et al., 2003; Kavanagh et al., 2005). The release time for a single cycle for these various delivery systems ranged from approximately 20 min (Fundueanu et al., 2009b) to 15 h (Coughlan et al., 2004). In the current study, the release profiles indicate that the behaviour is diffusion dominated, although some behaviour characteristic of zero-order release was also observed (Fundueanu et al., 2009b). The parameters that can be used to control drug release from the system include the initial drug loading concentration, the geometrical dimensions of the system (such as thickness and surface area), and the durations the device is left switched on/off; the governing mathematical model described here incorporates all of these quantities.

A few models have previously been developed to describe the release behaviour from swelling delivery systems (Brazel and Peppas, 2000; Crank, 1975; Fujita, 1961; Grassi and Grassi, 2005; Kikuchi and Okano, 2002; Lee, 1985; Siegel and Pitt, 1995; Siepmann et al., 1998; Siepmann and Siepmann, 2008), and diffusion from thin films has been well studied (Cook and Chen, 1995; McCaig et al., 2000; Sanches Silva and Cruz, 2007; Wang et al., 2007). However, in this paper, the first model to incorporate release from a system that alternates between a swollen hydrophilic state and a film-like hydrophobic state is described.

In this work, a fabrication procedure is described for thin hydrogel films loaded with rhodamine B, and the drug release behaviour from these films is analyzed. The objective of the study is to develop a new controllable drug delivery system based on thin UV-crosslinked thermoresponsive films, which can be characterized and tuned with the aid of a mathematical model.

2. Modelling pulsatile release

A one-dimensional model is formulated for drug diffusion in the film, and the release behaviour is considered for the case in which the temperature of the film is quickly and repeatedly switched between a value above the LCST and a value below the LCST. The motion of the drug molecules through the film is assumed to be governed by Fick’s law and we denote by \( c(x, t) \) the concentration of drug at penetration \( x \) and time \( t \) in the film. When the film is held at a temperature above the LCST, it is in a condensed state, and we denote by \( H_c, D_c \) the constant thickness and diffusivity of the condensed film, respectively. If the film is held at a temperature below the LCST, it is in a swollen state, and we denote by \( H_s, D_s \) the constant thickness and diffusivity of the swollen film, respectively. The lateral dimensions of the film are fixed because they are constrained by the wall of the containing well, and swelling/collapsing can only occur in the \( x \) direction. We shall find, as would be expected, that \( D_s \approx D_c \), so that alternating the temperature between values above and below the LCST results in a release profile with an on/off pulsatile character.

The time the polymer was left in either the fully swollen or fully collapsed state was typically of the order of minutes. However, in the experimental work for this paper, the time it took for the thin polymer film to fully swell or fully collapse was considerably shorter than a minute. Hence, in the current model, we assume that the swelling and collapsing processes occur instantaneously. This assumption simplifies the problem considerably since incorporating the detail of the swelling or collapsing behaviour in the model would require the tracking of moving boundaries (Siepmann and Siepmann, 2008). This would lead to a much more challenging mathematical problem, from which an analytical expression for the release profile could not be in general obtained.

We suppose that at time \( t=0 \) the film is at the temperature above the LCST and is fully collapsed. At time \( t=t_1 \), the temperature of the film is taken to instantaneously switch to the value below the LCST and the film is fully swollen; at time \( t=t_2 \) the film instantaneously reverts to the collapsed state, and so on (collapsed → swollen → collapsed → ...).

If \( H(t), D(t) \) denote the thickness of the film and the drug diffusivity at time \( t \), respectively, then under the assumptions stated above, the concentration \( c(x, t) \) of drug in the film is governed by:

\[
\frac{\partial c}{\partial t} = D(x) \frac{\partial^2 c}{\partial x^2} \quad \text{for} \quad 0 < x < H(t),
\]

where

\[
D(t) = \begin{cases} 
D_c, & 0 \leq t < t_1, \\
D_l, & t_1 \leq t < t_2, \\
D_s, & t_2 \leq t < t_1, \\
& \vdots 
\end{cases}
\]

and

\[
H(t) = \begin{cases} 
H_c, & 0 \leq t < t_1, \\
H_l, & t_1 \leq t < t_2, \\
H_s, & t_2 \leq t < t_1, \\
& \vdots 
\end{cases}
\]

Eqs. (1) and (2) are solved subject to the following conditions.

(i) The film is initially uniformly loaded with drug, so we take \( c = c_0 \) at \( t = 0 \) in \( 0 < x < H_c \), where \( c_0 \) is constant.

(ii) The bottom of the film (see Fig. 1, \( x = 0 \)), is attached to a plastic cover slip substrate, which is taken to be impermeable to the drug, and so we impose \( -D(x) \frac{\partial c}{\partial x} \bigg|_{x=0} = 0 \).

(iii) Perfect sink conditions are assumed for the drug at the top surface of the film which is in contact with the eluting medium, and so we set \( c = 0 \) on \( x = H(t) \).

The model (1) and (2) is readily solved subject to (i)-(iii) by separating variables (Crank, 1975) and the amount of drug released per unit area from the film by time \( t \), \( M(t) = H_c c_0 - \int_0^t c(x, t) \, dx \).
is easily calculated. The fraction of drug released from the film by time \( t \) is found to be:

\[
\frac{M(t)}{M(\infty)} = \begin{cases} 
1 - \sum_{n=1}^{\infty} \frac{2}{n^2} \exp \left( -\frac{t}{n^2 D_H^*} \right), & 0 \leq t < t_1, \\
1 - \sum_{n=1}^{\infty} \frac{2}{n^2} \exp \left( -\frac{t_1}{n^2 D_H^*} \frac{D_{1-H}^*}{D_{1-H}^*} \right), & t_1 \leq t < t_2, \\
1 - \sum_{n=1}^{\infty} \frac{2}{n^2} \exp \left( -\frac{t_2 - t_1}{n^2 D_H^*} \frac{D_{1-H}^*}{D_{1-H}^*} \right), & t_2 \leq t < t_3, \\
\vdots 
\end{cases}
\]  

(3)

where \( \lambda_n = ((2n - 1)^2 \pi^2)/4 \). For the case in which the temperature in the film is held fixed for all time (non-pulsatile), the appropriate result can be obtained by simply setting \( D_{1-H}^* = D_{1-H}^* = D^*/H^2 \) (constant) in Eq. (3) to obtain:

\[
\frac{M(t)}{M(\infty)} = 1 - \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n - 1)^2} \exp \left( -\frac{(2n - 1)^2 \pi^2 D}{4H^2} \right) \text{ for all } t > 0. 
\]  

(4)

Eq. (3) gives an analytical expression for \( M(t) \), the total drug released from the pulsatile system at time \( t \). The development of such an expression is significant in practical value in both the design and operation of a pulsatile release device. In the design stage, it can be used to predict the thickness, surface area, and initial drug load to be used so that the resulting device can deliver doses of appropriate strength over an appropriate time-scale. In the operation stage, it can be used to devise a detailed schedule for when the device should be switched on and off so as to deliver the required doses at the desired time intervals.

To see how Eq. (3) might be used in practice, a hypothetical example is considered. Suppose it is required to deliver \( n \) equal doses of a drug, with each dose delivering an amount of drug \( d \). It is further supposed that there is a fixed time interval \( T \) between the deliveries of each dose. In this example, it shall be assumed that some mechanism has been devised that quickly switches the device on and off and that the parameters \( D_{1-H}^* \) and \( D_{1-H}^* \) are known; a procedure for estimating these parameters from experimental data has been given in this paper.

The total drug loaded onto the device, \( M(\infty) \), which of course must exceed \( nd \) here, can be controlled by varying either the volume of the device or the initial drug concentration. It is supposed that the device is deployed in the off state at time \( t = 0 \) and that it is first switched on at \( t = t_1 \). To determine the time \( t = t_2 \) at which the device should be switched off, we solve the equation:

\[
M(t_2) - M(t_1) = d
\]  

(5)

for \( t_2 \) where \( M(t) \) is given by Eq. (3); notice that this ensures that the required dose \( d \) is delivered over the time interval \( [t_1, t_2) \). Although Eq. (5) gives rise to a rather complicated expression for \( t_2 \) that cannot be solved analytically, \( t_2 \) can be readily estimated numerically with the aid of mathematical packages such as MATLAB® or MAPLE®. The device is switched on for the second time at \( t = t_1 + T \), and then switched off again at \( t = t_3 \) where \( t_3 \) is determined by numerically solving: \( M(t_3) - M(t_2 + T) = d \).

Proceeding in this way, it is clear how a schedule may be devised for switching the device on and off so as to deliver the required doses at the desired intervals.

The appropriate release formula for an isothermal system which does not swell or collapse is given by Eq. (4). This simpler form is very familiar and has been used on numerous occasions previously to describe diffusion from a planar sheet (Crank, 1975). We now obtain an estimate for the time it takes for a release device with a single constant diffusivity to empty its drug load; more precisely, we estimate the time it takes for there to be only a small fraction \( r < 1 \) of the initial drug load left remaining in the device. This time is such that \( DT/H^2 > 1 \) and from Eq. (4) the following approximation is obtained:

\[
\frac{M(t)}{M(\infty)} = 1 - \frac{8}{\pi^2} \exp \left( -\frac{\pi^2 DT}{4H^2} \right) \text{ for } DT/H^2 > 1.
\]  

This expression is used to solve for \( t_r \), in \( M(t_r)/M(\infty) = 1 - r \) to obtain:

\[
t_r \approx \frac{4H^2}{\pi^2} \ln \left( \frac{8}{\pi^2} \right)
\]  

(6)

as the estimate for the time at which there is a fraction \( r < 1 \) of the initial drug left in the device. Eq. (6) can be used to estimate the effective life-span of the pulsatile device. Clearly the device will have its minimum effective life-span, \( t_{\text{min}} \), if it is continually left on, so that \( t_{\text{min}} = ((4H^2)/(\pi^2 DT)) \ln(8/(\pi^2 r)) \). The device will have its maximum effective life-span, \( t_{\text{max}} \), if it is continually left off, and \( t_{\text{max}} = ((4H^2)/(\pi^2 DT^2)) \ln(8/(\pi^2 r^2)) \). The effective life-span of the pulsatile device then lies in the range \([t_{\text{min}}, t_{\text{max}}]\), and these useful quantities are readily calculated; for the system described in this paper, \( t_{\text{max}} \) is approximately 1 h and \( t_{\text{min}} \) is approximately 42 h for 95% of drug released \( (r = 0.05) \).

In Eq. (6), it is seen that there is a strong quadratic dependency on the thickness of the film, but that the dependence on the diffusivity is weaker, being inversely proportional to only the first power. The diffusivities \( D_1 \) and \( D_2 \) are determined largely by the nature of the material being used, although there is some flexibility in material choice and preparation. The thickness of the film, however, is a quantity that can be readily varied in practice, and given that the release time-scales have a strong dependence on film thickness, it is probably the most effective parameter to vary when designing a release device.

3. Materials and methods

3.1. Materials

N-Isopropylacrylamide (NIPAm, Aldrich) was re-crystallised from hexane and acetone and dried at room temperature under vacuum. Acrylamidobenzophenone was prepared from acryloyl chloride and 4-aminobenzophenone by a standard amidation reaction, with triethylamine as the acid scavenger. 2,2'-Azobisobutyronitrile (AIBN, Phase Separation Ltd.) was re-crystallised from methanol. Benzene was dried under sodium-wire and distilled before use. All other solvents were reagent grade and purified by conventional methods before use. Rhodamine B was purchased from Aldrich and used as received.

3.2. Preparation of crosslinkable nNIPAm

N-Isopropylacrylamide (NIPAm) and the UV sensitive crosslinker, acrylamidobenzophenone (ABzPh) (molar ratio was 98.8 mol%: 1.2 mol%, 5 g of total monomers) were copolymerised by radical polymerisation using AIBN (0.7 mol%) as an initiator in benzene (30 ml) under argon to form poly(NIPAm-co-ABzPh). After polymerisation at 60 °C for 24 h, the mixture was precipitated in n-hexane. Precipitation was repeated three times using acetone as a solvent and n-hexane as a non-solvent. The polymer was dried at 45 °C in a vacuum oven, and after three precipitations the yield was 70%.
3.3. Lower critical solution temperature LCST of p(NIPAm-co-ABAPh)

The LCST was determined by the cloud point measurement obtained on a Cary 100 UV-VIS spectrophotometer equipped with a temperature controller, and twelve-position sample holder. Polymer aqueous solution (3.5 ml of 1 mg/ml) in a cuvette was heated at a rate of 0.1 °C/min whilst obtaining the absorbance at 500 nm wavelength. The solution temperature was determined by the internal temperature probe with a resolution of 0.5 °C and an accuracy of ±0.1 °C, were monitored.

3.4. Preparation of films

For experiments, crosslinkable copolymer solution was prepared in dry methanol (2% (w/v) polymer/methanol). Aliquots (50 μl and 125 μl respectively) of the solution were applied evenly to the wells of 24-well polystyrene tissue culture plates and polyethylene plastic cover slips (diameter 25 mm). The resulting films (5 μm thick) were allowed to dry at room temperature in a methanol atmosphere overnight. The films were then dried at 40 °C for 4 h under vacuum, and crosslinking initiated by exposure of covered plates to UV light at an intensity of 400 mW/cm² for 20 min, followed by inversion of the plates and exposure for a further 20 min. During irradiation, the polystyrene served as a long pass filter for UV light. All loading and release experiments were carried out on copolymer films cast on 24-well tissue culture plates. Films used for characterizations were prepared as above on polyethylene plastic cover slips (diameter 25 mm) unless stated otherwise.

3.5. Drug distribution in the film

An aliquot of copolymer solution (38.5 μl) was applied to glass bottom culture dishes (P35G-0-14-C, MetTek). The resulting films (5 μm thick) were dried and crosslinked as previously described. Rhodamine B-methanol solution was added to the surface of each film and dried in the methanol atmosphere at room temperature and then dried at 40 °C for 4 h under vacuum. Exposure to light was minimised. Images were recorded at room temperature using a Leica TCS SL confocal system and z-stack technique, which is described elsewhere (Kavanagh et al., 2005) in order to confirm the thickness and observe the rhodamine B distribution in the film.

3.6. Drug loading

Rhodamine B (50 μl, 0.4 mM) solution in dehydrated methanol was cast on top of crosslinked dry films of thickness (5 μm), which were deposited in a 24-well plate. The samples were dried in ethanol atmosphere at room temperature and then dried at 40 °C for 4 h under vacuum. Exposure to light was minimised. In order to obtain the loading efficiency, the solution used for rinsing off the rhodamine B on the surface of films before the release experiment and the solutions used for extracting the remaining rhodamine B after the release were collected and the rhodamine B concentration was determined by plate reader (FLx800, Bio-tek, Ex: 548 nm/Em: 590 nm). The drug loading efficiency was calculated by one minus the ratio of the number of moles of rhodamine B rinsed to the number of moles of total rhodamine B coated (composed of rinsed, controlled released and extracted).

3.7. In vitro drug release kinetics

In vitro drug release kinetic studies were performed, at different temperatures, by soaking the samples in distilled water (2 ml). The drug loaded samples were first rinsed twice with warm distilled water (40 °C) to remove the rhodamine B on the surface of the films. At regular time intervals 1 ml of the dissolution medium was withdrawn and analysed by plate reader. The same volume of fresh distilled water was added to replace the volume of the extracted samples. Any residual drug remaining in the samples was then extracted by injecting distilled water at 4 °C into the wells (30 min). A thermal plate (IC22XT, Torry Pines Scientific) and hot-plate combined with magnetic stirrer (Fig. 1) were used to help maintain the film at the desired temperature. All experiments were carried out in triplicate. The release profile was obtained with and without stirring.

All of the sampling solution was removed into black 96-well plate, and then placed in a plate reader to obtain the drug concentration. The resulting data was subsequently analyzed with the aid of Microsoft® Excel® and the final curves were produced using OriginLab®.

3.8. Pulsatile release

The effect of thermal cycling on rhodamine B release from UV-crosslinked thermo-responsive polymer films was investigated. The drug loaded samples were first rinsed twice with warm distilled water (40 °C) to remove the rhodamine B on the surface of the films. Then the films were soaked into distilled water (1 ml, 37 °C) and at pre-designed time intervals, all the dissolution medium was withdrawn and replaced by distilled water (1 ml, 4 °C). After a pre-calculated time period, all of dissolution medium was replaced by same amount of distilled water (37 °C). Any residual drug remaining in the samples was then extracted by injecting distilled water at 4 °C into the wells (30 min). A thermal plate (IC22XT, Torry Pines Scientific) and hot-plate combined with magnetic stirrer were used to help maintain the film at the desired temperature. The results of this experiment were compared with simulations of the mathematical model for values of the diffusion coefficients obtained from the fixed temperature release experiments at 4 °C and 40 °C, 25 °C and 37 °C, 28 °C and 37 °C, 30 °C and 37 °C were chosen to minimise the difference between high temperature and low temperature whilst pulsatile release profile still is obtainable.

4. Results and discussion

4.1. Drug loading

The drug loading efficiency was found by calculating (one minus rinsed drug/total drug), expressed as a percentage (Table 1). The total drug was composed of three parts: the rinsed fraction prior to release, the fraction released during the experiments, and the fraction extracted upon completion of the experiments.

4.2. Drug distribution

The thickness of the films was measured using confocal microscopy, with images of the film being acquired as z-stacks through the film depth. The measurement was carried out at six random locations on the film and in each case the thickness of the dry films was found to lie in the range 5.0 μm ± 0.2 μm. z-Stack images were used to determine the three dimensional distribution of the fluorescent drug in the dry film; the results (Fig. 2) confirmed that the loaded drug was uniformly distributed initially.

4.3. Fixed temperature release

The fraction of total drug released from the system as a function of time was measured for various temperatures, ranging from 4 °C to 40 °C. It is emphasised that the temperature is kept fixed for
Table 1: Calculation of drug loading efficiency.

<table>
<thead>
<tr>
<th>The desired loading amount (mg)</th>
<th>Rinsed off (mg)</th>
<th>Total released (mg)</th>
<th>Loading efficiency [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.0007 ± 0.0003</td>
<td>0.009 ± 0.002</td>
<td>92 ± 6</td>
</tr>
</tbody>
</table>

Fig. 2. 3D-fluorescent images of rhodamine B distribution in a dry film. The grey dots represent the fluorescent signal of rhodamine B.

Fig. 3. Influence of temperature (a) 4°C, (b) 25°C, (c) 32°C, (d) 37°C and stirring on the release of rhodamine B (20 nmol/film) from 5 μm thick UV-crosslinked polymer films (16 mm in diameter) in distilled water for 120 min. (a) Release profile with stirring. (b) Release profile without stirring. The continuous line curves correspond to solutions (4) of the mathematical model which have been fitted to the experimental data.

Fig. 4. Lifetime of the system. (a) Release profile of rhodamine B at 4°C over 2 h. (b) Release profile of rhodamine B at 37°C over 48 h.

Each release experiment; the pulsatile release behaviour in which the temperature is varied in the experiment is discussed separately below. It is clear from this data that the rate at which drug releases from the system decreases significantly as the temperature is increased through the range from 27°C to 34°C. However, the release rate is less sensitive to changes in temperature for ranges below 27°C or above 34°C.

In Fig. 3, a selection of the experimental release profiles is displayed, together with theoretical curves obtained from our mathematical model fitted to the experimental data. The selection includes examples where the film is fully collapsed, fully swollen, and in an intermediate state between these two extremes. For each of these release experiments, the temperature is held fixed so that the appropriate theoretical release profile is given by Eq. (4) above. This expression was fitted to the experimental data using the method of least squares, with $D/H^2$ being the unknown parameter to be estimated. Once the estimate for $D/H^2$ has been obtained, $D$ follows immediately if $H$ is known. The nonlinear equation for
can be accounted for by noting that a non-negligible fraction of the drug has been retained by the collapsed polymer even after 40 h of release; a better fit can be obtained if the data is re-scaled so that the last data point of Fig. 4(b) corresponds to 100% release. This suggests that for the collapsed polymer, a small fraction of the drug releases extremely slowly, if at all. However, we have not attempted to incorporate this effect in the current modelling.

In 1 h, 98.6 ± 0.4% of rhodamine B was released at 4°C and 91.1 ± 1.7% of model drug was release in 42 h at 37°C.

4.5. Temperature dependence of the drug diffusivity across the LCST

In Fig. 5, transmittance is plotted versus temperature for the heating process of the copolymer pNIPAm-co-AlbPh aqueous solution, and this data shows that the transition temperature lies in the range of 28–30°C. The scaled diffusivities D/H2 were estimated at different temperatures by fitting the experimental data with the theoretical release profile given by Eq. (4), and the results are displayed in Fig. 5. This data reveals that the scaled diffusivity D/H2 decreases by approximately one order of magnitude as the temperature is increased through the LCST, which conforms to our intuitive expectations since this temperature change corresponds to the transition from the swollen to the shrunken state for the film. The volume phase transition of the films used in this study was in the range of 22–30°C. This transition temperature range is 6°C wider than that for the uncrosslinked polymer in aqueous solution. It should be noted, however, that the LCST for the polymer in aqueous solution was found to be 3°C higher than that for the crosslinked film (Fedl et al., 1993; Zhang and Zhu, 2001).

4.6. Pulsatile release

In Fig. 6, experimental drug release data is displayed for a system in which the temperature is repeatedly switched between 40°C (above the LCST, slow release) and 4°C (below the LCST, fast release). The resulting release profile has a clear on/off pulsatile character as would be expected. In the experiment, the system was held in the “off” state for a fixed 3 min in each cycle, but the time it was left in the “on” state was increased with increasing cycle number so as to achieve an approximately uniform dose for each cycle. However, for the first cycle, a larger dose was delivered. In Fig. 6, a theoretical curve based on Eq. (3) is also displayed which has been fitted to the experimental data using the method of least squares, with D/coH2, D/coH2 being the unknown parameters to be estimated. The results of the analysis reveal that D/coH2...
is one order of magnitude smaller than $D_o/H^2$, which is consistent with the results of the fixed temperature experiments. The fit is again quite good indicating that our model, despite the simplifying assumptions made in its development, is adequate for capturing the essential features of the release behaviour. In this case, a total of six pulses were obtained. It should be noted that it is very difficult to realise a temperature transition of 36 °C (from 4 °C to 40 °C) in vivo. However, we shall show that the system is capable of successfully exhibiting pulsatile release for temperature transitions as modest as 7 °C (from 30 °C to 37 °C), which is clearly a much more realistic scenario from the point of view of potential applications.

Having established that the model adequately fits the experimental data, we used it to aid in the design of two further pulsatile release experiments. Specifically, we used the model to estimate when the temperature of the polymer should be changed in the experiments so as to ensure that there would be five cycles, each of duration of 20 min approximately, and that between 10% and 20% of the total drug would be released in each cycle. The results of the experiments are displayed in Fig. 7, and we see that these objectives were broadly achieved, except for the first temperature drop where less than 10% of the drug was released. It is also noteworthy that there is a much smaller jump in the temperature for these experiments, such as 12 °C for the pulsatile release of 25 °C and 37 °C, 9 °C for the pulsatile release of 28 °C and 37 °C, and only 7 °C for the pulsatile release 30 °C and 37 °C.

5. Conclusion

This study has shown that the copolymer of poly(NIPAm-co-AbzPh) is capable of incorporating and pulsatile releasing rhodamine B at designated time points. Two stages of release were observed: slow diffusion when the temperature is above the LCST range and swelling followed by more rapid diffusion when the temperature drops below the LCST. The mathematical model, which was developed based on Fick’s law, has shown that the behaviour of the system can be adequately described by a time dependent diffusivity, and a formula for the drug fraction released as a function of time was constructed. Pulsatile release was observed and successfully modelled by our release formula by choosing appropriate diffusivities. As the results show, by carefully controlling the release time at low and high temperature, the release dosage of each cycle can be relatively stable at 10%. However, the minimum practicable release time is about 30 s, and this is too long to keep the dose delivered below 10% in the first release cycle. The model also provided an estimate for the effective life time of this system, and this was found to lie in the approximate range of 1–42 h. In our view, pulsatile systems such as the one developed and analysed here will form important components of future technologies that realise the goal of controlled drug release in vivo.

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dog of muscle 97–114.

277–288.


49, 47–58.


Facile synthesis of thermoresponsive block copolymers of N-isopropylacrylamide using heterogeneous controlled/living nitroxide-mediated polymerizations in supercritical carbon dioxide

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1. Introduction

The first preparation of well-defined block copolymers using controlled/living chain growth polymerizations that minimize termination and transfer reactions is attributed to Szwarz et al. by means of living anionic polymerizations and date back to the 1950s [1,2]. The recognised disadvantages of anionic and cationic polymerizations are the low temperatures, scrupulous drying and purification of both monomers and solvents required [3], the non-compatibility of monomer classes with the propagating chain [4], and the requirement for protecting groups (e.g. in the anionic polymerization of N-isopropylacrylamide, NIPAM) [5,6]. Since the early 1990s the more versatile controlled/living radical polymerization (CLRP) techniques have largely superseded living ionic polymerizations for forming block copolymers and other well-defined polymers with narrow molecular weight distributions (MWDs) and various topologies. CLRPs are based on dissociation-combination, atom transfer and degenerative chain transfer mechanisms/techniques. Well-known CLRPs using the first two techniques are nitroxide-mediated polymerization (NMP) [7,8] and atom transfer radical polymerization (ATRP) [9,10], respectively. More recently, atom transfer techniques that reduce the amount of metal salt catalyst used have been popularized [11,12]. The most common degenerative transfer methodologies are reversible addition-fragmentation chain transfer (RAFT) [13,14], and macromolecular design by interchange of xanthate (MADIX) [15]. Organotellurium-mediated radical polymerization (TERP) is thought to achieve living character using more than one of these mechanisms [16]. In all cases, controlled/living chain is achieved by rapid reversible deactivation of propagating radicals (Pd), which is required to minimise the contribution of irreversible bimolecular terminations (and chain transfer), in contrast to a conventional “non-living” radical polymerization where irreversible terminations are the major chain-end forming events [17].
Poly(NIPAM) is a thermoresponsive polymer exhibiting a lower critical solution temperature (LCST) in water of \(\sim 32^\circ C\) leading to biomedical and drug delivery applications [18–20]. The manipulation of the polymer’s LCST by incorporating additional monomers has attracted much interest with block copolymers prepared by a variety of CLRP’s, including solution NMPs of NIPAM [21–24]. Block copolymers of NIPAM and acrylic acid (AA) have attracted much interest, because they form thermo- and pH-responsive micelles, useful in drug-delivery [25–29]. The combination of NIPAM with the hydrophobic monomer N,N-dimethylacrylamide (DMA) gives copolymers with elevated LCST [30–36], which may be closer to physiological temperature of \(\sim 37^\circ C\).

Supercritical carbon dioxide (scCO₂) is a benign reaction medium, which can be used to circumvent the requirement for toxic and hazardous volatile organic compound (VOC). scCO₂ is a continuous phase of CO₂ above its critical point exhibiting liquid-like densities and gas-like diffusivity. As a polymerization medium scCO₂ can generally dissolve most organic molecules (including monomers), but does not dissolve the resulting polymers [37,38]. This makes scCO₂ a useful medium for industrially important heterogeneous polymerizations that allow the synthesis of sub-micron sized polymer particles [39,40]. Controlled/ living precipitation NMPs [41–44] and dispersion (with added stabilizer) NMPs [45–49] in scCO₂ have been reported, as well as dispersion polymerizations using ATRP [50–54] and RAFT [55–57] in scCO₂. The dispersion CLRP allows simultaneous control over particle size distribution and MWD of polymers [38,58].

Using the NMP of styrene (St) and tert-butyl acrylate (t-BA) in scCO₂, polymer chains have been shown to be soluble up to a certain critical degree of polymerization \(J_{crit}\) prior to precipitation, after which the polymerization continues in the monomer rich particle phase [44]. \(J_{crit}\) or the particle nucleation step can be predicted based on the targeted molecular weight and initial monomer loading, and \(J_{crit}\) or polymer solubility can be increased approximately linearly with pressure [44]. Control in terms of MWDs being narrower than in an equivalent nitroxide-mediated solution polymerization can be obtained under conditions where \(J_{crit}\) is high, and is attributed to an enhanced deactivation rate due to predicted increased nitroxide mobility in scCO₂ [42]. However it is not possible to carry out precipitation or dispersion polymerizations of NIPAM in scCO₂ at higher loadings than approximately 5% w/w because of the poor solubility of the monomer. Nevertheless in a recent communication [59], the inverse suspension NMP of NIPAM at loadings 10–40% w/w in scCO₂ using the 2,2’-azoisobutyronitrile (AIBN)/N-tert-butyl-N-[1-dimethylphosphono-(2,2-dimethylpropyl)]nitroxide, SG1 (also known as DEPN) was synthesized according to the literature [65], with purity 96% determined using \(^1 H\) NMR spectroscopy from the reaction of the SG1 radical with pentafluorophenylhydrazine (Aldrich 97%). Macroinitiators (MI) of poly(t-BA)-SG1 \((M_\text{w}/M_\text{n} = 8000 \text{ g mol}^{-1}, M_\text{w}/M_\text{n} = 1.17)\) and poly(St)-SG1 \((M_\text{w} = 6450 \text{ g mol}^{-1}, M_\text{w}/M_\text{n} = 1.13)\) were prepared by precipitation NMP in scCO₂ as outlined in our published procedure [44]. Table 1 details the conditions for the synthesis of MI’s. Sodium hydroxide (Aldrich >97%), N,N-dimethylformamide (DMF, Fisher, GPC grade), tetrahydrofuran (THF, Aldrich, 99.9%), dichloromethane (DCM, Aldrich 99%), anhydrous methanol (MeOH, Corcoran Chemicals 99.9%), acetone (Corcoran Chemicals 99.5%), hexane (Fisher Scientific, reagent grade), benzene (BDH, reagent grade), diethyl ether (Aldrich, reagent grade), trifluoroacetic acid (TFA, Aldrich, 99%), (trimethylsilyl)diazomethane solution in 2.0 M hexanes (Aldrich), lithium bromide (BDH, >97%), and CO₂ (BOC, 99.8%) were all used as received.

### Experimental

#### 2.1. Materials

N-isopropylacrylamide (NIPAM, 98% TCI) was recrystallized from a mixture of 3:2 benzene/hexane before use. Styrene (St, Aldrich, >99%), tert-butyl acrylate (t-BA, Aldrich, 98%) and N,N-dimethylacrylamide (DMA, TCI, >99%) were distilled under reduced pressure before use. 2,2’-Azoisobutyronitrile (AIBN, DuPont Chemical Solution Enterprise) was recrystallized twice from methanol. N-tert-butyl-N-[1-diethylphosphono-(2,2-dimethylpropyl)]nitroxide, SG1 (also known as DEPN) was synthesized according to the literature [65], with purity 96% determined using \(^1 H\) NMR spectroscopy from the reaction of the SG1 radical with pentafluorophenylhydrazine (Aldrich 97%).)

Macroinitiators (MI) of poly(t-BA)-SG1 \((M_\text{w}/M_\text{n} = 8000 \text{ g mol}^{-1}, M_\text{w}/M_\text{n} = 1.17)\) and poly(St)-SG1 \((M_\text{w} = 6450 \text{ g mol}^{-1}, M_\text{w}/M_\text{n} = 1.13)\) were prepared by precipitation NMP in scCO₂ as outlined in our published procedure [44]. Table 1 details the conditions for the synthesis of MI’s. Sodium hydroxide (Aldrich >97%), N,N-dimethylformamide (DMF, Fisher, GPC grade), tetrahydrofuran (THF, Aldrich, 99.9%), dichloromethane (DCM, Aldrich 99%), anhydrous methanol (MeOH, Corcoran Chemicals 99.9%), acetone (Corcoran Chemicals 99.5%), hexane (Fisher Scientific, reagent grade), benzene (BDH, reagent grade), diethyl ether (Aldrich, reagent grade), trifluoroacetic acid (TFA, Aldrich, 99%), (trimethylsilyl)diazomethane solution in 2.0 M hexanes (Aldrich), lithium bromide (BDH, >97%), and CO₂ (BOC, 99.8%) were all used as received.

#### 2.2. Equipment and measurements

All polymerizations in supercritical carbon dioxide (scCO₂) were conducted in a 100 mL stainless steel Thar reactor with 180° inline sapphire windows and overhead Magdrive stirrer with maximum programmable operating conditions.
pressure and temperature of 41.4 MPa and 125 °C, respectively. The pressure was produced and maintained by a Thar P-50 series high-pressure pump to within ±0.2 MPa. The temperature was regulated by a Thar CNS controller to within ±1 °C. The reactor is connected to a Thar automated back pressure regulator (ABPR, a computer-controlled needle valve) for controlled venting.

$M_n$ and polydispersity ($M_D/M_n$) were determined using gel permeation chromatography (GPC) system consisting of a Viscotec DM 400 data manager, a Viscotec VE 3580 refractive-index detector, and two Viscotec Viscogel GMHHHR-M columns. Measurements were carried out at 60 °C at a flow rate of 1.0 mL min$^{-1}$ using GPC grade DMF containing 0.01 M LiBr as the eluent [5,6,23,24,59,61]. The columns were calibrated using six linear poly(St) standards ($M_n = 376–2570000$). The $M_n(GPC)$ values are given as grams per mole throughout, and are not absolute, but relative to linear poly(St) standards. Theoretical ($M_n$) or $M_n(GPC)$ are not quoted due to the use of relative $M_n(GPC)$ values and uncertainties in initiator efficiencies. Control/livingness is assessed using MWD relative shifts, trends and shapes. All GPC data corresponds to polymer before purification, unless otherwise stated.

$^1$H NMR spectra were obtained using a JEOL 400 MHz spectrometer. Samples containing poly(acrylic acid, AA) were recorded in (CD$_3$)$_2$SO, and all other samples were recorded in CDCl$_3$ with Me$_4$Si used as the internal standard. $M_n(NMR)$ of purified block copolymers is calculated according to eq. (1): where $DP_n(AM)$ is the average degree of polymerization of each MI obtained from $M_n(GPC) = M_n(NMR)$ divided by the MW of the constituent monomer; $x$ is the ratio of poly(NIPAM) relative to MI incorporated in the block copolymer obtained from integration of poly(NIPAM) N–C–H (δ$_H$ = 3.8–4.1 ppm, 1H) resonance, and resonances at δ$_H$ = 1.0–2.4 ppm due to each individual incorporated MI plus poly(NIPAM) (see Figs. S5–S7 in Supplementary data). E.g. In the case of poly(DMA)-b-poly(NIPAM), δ$_H$ 0.90–2.0 ppm represents 3H (CH–CH$_2$) of poly(DMA) plus 5H (CH–CH$_2$ and 2 × Me) of poly(NIPAM) (Fig. S5), $MW_{(NIPAM)}$ is the molecular weight of NIPAM monomer.

$$\text{Mn(NMR)} = \frac{(DP_{n(AM)} \times x) \times MW_{(NIPAM)} + M_n(AM)}{x}$$

2.3. Precipitation NMP of DMA in scCO$_2$

DMA (20.0 g, 0.20 mol), AIBN (66 mg, 0.40 mmol) and SG1 (0.393 g, 1.33 mmol) were loaded into the scCO$_2$ reactor. The reactor was sealed with the magnetically coupled stirring lid (Magdrive). The mixture was purged for 15 min by passing gaseous CO$_2$ through the mixture to remove oxygen. Liquid CO$_2$ (~5 MPa) was added and the temperature was raised to the reaction temperature of 120 °C followed by the pressure to the reaction pressure (in this case 30 MPa) by the addition of CO$_2$. The reaction mixture was stirred at ~1200 rpm throughout and monitored through the inline sapphire windows. At the start the viewing window indicated a transparent solution, which became opaque at $t_{10}$ or the particle nucleation stage [44]. Heating was stopped and rapid external cooling using a cooling fan applied (this caused the temperature of the reactor to drop to ~80 °C in 10 min). When at approximately room temperature the CO$_2$ was vented slowly from the reactor through DCM to prevent the loss of polymer and opened using the ABPR. The reaction mixture was pipetted directly from the reactor. The polymer was precipitated into excess hexane, filtered and dried prior to conversion measurement by gravimetry. Polymerizations were then carried out to higher conversions beyond $t_{10}$.

2.4. Test for livingness: chain extension of MI with bulk St

MI (0.025 mmol, based on the $M_n$ of the purified polymer), St (2.0 g, 19.2 mmol), and SG1 (5.2 mg, 17.7 µmol) were charged in glass ampoules and subjected to several freeze/thaw degrad cycles. After sealing under vacuum, the ampoules were heated at 110 °C in an aluminium heating block for 15 h. The polymerizations were quenched by immersing the ampoule into an ice-water bath. Each polymer was precipitated into an excess of methanol, filtered and dried prior to conversion measurement by gravimetry. Conversion was obtained from the increase in weight of polymeric material.

2.5. MI-initiated inverse suspension NMP of NIPAM in scCO$_2$

NIPAM (10.0 g, 88.4 mmol), MI (0.14 mmol, based on the $M_n$ of the purified polymer; e.g poly(r-BA)-SG1 = 1.12 g) and SG1 (20.6 mg, 0.07 mmol) were loaded into the reactor. Poly(St)-SG1 initiated polymerization was carried out at 20% w/v NIPAM loading (20.0 g, 0.177 mol) using poly(St)-SG1 (1.81 g, 0.28 mmol) and SG1 (61.8 mg, 0.21 mmol). The polymerizations were carried out as above (Section 2.3) at 120 °C and 30 MPa and stopped at various times. Poly(r-BA)-b-poly(NIPAM) and poly(St)-b-poly(NIPAM) were dissolved in MeOH and precipitated in excess methanol and dried prior to conversion measurement by gravimetry. The livingness was assessed using MWD relative shifts, trends and shapes. All GPC data corresponds to polymer before purification, unless otherwise stated.

$^a$ All polymerizations carried out in scCO$_2$ at 120 °C and 30 MPa using initial monomer loading [Monomer]$_0$.

$^b$ See Figs. 2–5 and Table 2 for GPC data before and after purification of polymers respectively.
acetone respectively and precipitated into excess petroleum ether and poly(DMA)-b-poly(NIPAM) was dissolved in DCM and precipitated into excess hexane. All copolymer samples were filtered and dried prior to conversion measurement by gravimetry. Table 1 details the conditions for the synthesis of block copolymers.

Table 1. Conditions for the synthesis of block copolymers.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(t-BA)-b-poly(NIPAM)</td>
<td>Acetone, precipitated into excess hexane</td>
</tr>
</tbody>
</table>

2.6. Hydrolysis of poly(t-BA)-b-poly(NIPAM)

Poly(t-BA)-b-poly(NIPAM) (1.5 g) was dissolved in DCM (15 mL) and a tenfold molar excess of TFA was added and the mixture stirred at room temperature for 24 h. The reaction was evaporated to dryness and the solid dissolved in methanol. The polymer was precipitated into excess diethyl ether, filtered and dried under vacuum. $^1$H NMR spectra verified the hydrolysis of the tert-butyl groups (~1.4 ppm) had occurred quantitatively (see Fig. S6).

2.7. Aqueous cloud point measurements of block copolymers

These were carried out using a Cary 100 UV–vis spectrophotometer at 500 nm, with temperature ramping at 0.1 °C/min. Polymers solutions (0.1% w/v) were prepared in Millipore water. NaOH solutions were used to adjust the pH of samples containing poly(AA) blocks. Samples were allowed to equilibrate for several hours before analysis. Commercial poly(NIPAM) with $M_n = 56,000$ and $M_w/M_n = 2.70$ using the above GPC conditions, was used as reference.

3. Results and discussion

3.1. Precipitation NMP of N,N-dimethylacrylamide (DMA) in scCO$_2$

DMA has a large propagation rate constant ($k_p$), and NMP with good control/living character is reported with bulk monomer in the presence of an excess of the nitroxide, SG1 [66,67]. For precipitation and dispersion NMP of St in scCO$_2$, a larger amount of free nitroxide is necessary compared to the solution or bulk polymerization of St because of the partitioning of some of the mediating nitroxide away from the locus of polymerization (i.e. the monomer rich particle) upon precipitation of the propagating radical from the continuous phase [41,43,47]. Polymerizations of 20% w/v DMA (i.e. 20 g DMA in 100 mL reactor) in scCO$_2$ with initial ratios of $[\text{SG1}]_0/[\text{AIBN}]_0 = 2.5$, 3.0 and 3.3 were carried out at 120 °C and 30 MPa, in order to give an optimal ratio required to achieve control/living character, as well as reasonable polymerization rates (Fig. 1). The highest ratio of $[\text{SG1}]_0/[\text{AIBN}]_0$ was found to give the narrowest MWD with negligible effect on rate and was used in subsequent polymerizations. The solubility of DMA in scCO$_2$ using these polymerization conditions was deciphered by observation of the reaction through the inline sapphire windows. The monomer was found to be soluble beyond 40% w/v loading and thus amenable to a precipitation polymerization. At 20% w/v monomer loading, precipitation occurred after 37 min at the above conditions with $f_{\text{cat}}$ occurring at 6% conversion ($M_w = 1600$ g/mol and $M_n = 122$). Poly(DMA) has comparable solubility to poly(t-BA) in scCO$_2$, which has been shown to be more soluble than poly(St) under similar conditions [44]. A series of polymerizations were then performed to high conversion and despite the large excess of nitroxide used, the NMP remained relatively fast reaching 66% conversion in about 12 h (Fig. 2). Controlled/living character is demonstrated by uniform MWDs remaining relatively narrow throughout (1.22–1.37) and $M_n$ increasing linearly with conversion. It is interesting that the polydispersity index also increases approximately linearly with conversion (over this narrow range) with the initial rise in $M_n/M_m$ presumably because of the partitioning of the nitroxide between the continuous and particle phase causing a small depreciation in control (see earlier discussion). Livingness was assessed by chain extension of the isolated poly(DMA)-SG1 at 24% conversion with bulk styrene (Fig. 3). After conversion of the chain extended GPC trace, to its number distribution curve (Fig. 3b), [47,62,68] and integration of the distinctive low molecular weight peak (non-extended chains, $M_n = 4750$ g/mol, $M_m = 1.45$, 16% conversion; $M_n = 6100$ g/mol, $M_m = 1.32$, 28% conversion; and $M_n = 5450$ g/mol, $M_m = 1.26$, 24% conversion; respectively.

3.2. MI-initiated inverse suspension polymerizations of N-isopropylacrylamide (NIPAM) in scCO$_2$

The block copolymers were prepared by inverse suspension NMP of NIPAM in scCO$_2$ at 120 °C and 30 MPa [59]. The latter optimised conditions (particularly pressure) are thought to reduce the influences of NIPAM solubility in scCO$_2$, as well as reagent partitioning between the two phases. The poly(DMA)-SG1 sample at 24% conversion in Fig. 2 was used as MI, and the poly(t-BA)-SG1 MI synthesis is previously reported [44]. Since the MIs were obtained using precipitation NMPs in scCO$_2$, it follows that the polymeric initiators would be expected to have a lower
solubility in the continuous phase, and would be located mostly in the monomer-rich suspended droplets at the start of the polymerization. NMP of 10% w/v NIPAM was initiated by the MIs in the presence of a 50 mol% excess of free SG1 (Fig. 4). There is an induction period in the polymerizations using two different concentrations of poly(t-BA)-SG1, which is absent when using poly(DMA)-SG1, as MI (Fig. 4a). This however is not due to the dispersed nature of the polymerization mixture, but is a feature of the kinetics of this particular NMP, given that a similar ~12 h delay in polymerization was observed in the solution NMP of NIPAM in DMF using a similar poly(t-BA)-SG1 MI (with 25% excess free SG1) [24]. This may be attributed to the dissociation rate constant ($k_d$) of the MI, which is likely to be about two times greater for poly(DMA)-SG1 compared to poly(t-BA)-SG1, based on literature $k_d$ data available for analogous small molecule alkoxyamines of SG1 at 120 °C [69]. It is thus expected that in the polymerizations initiated by poly(t-BA)-SG1, there is a greater tendency for the initiation equilibrium to lie towards the dormant state (i.e. non-dissociated MI). Halving the ratio of [NIPAM]/[poly(t-BA)-SG1] by doubling the number of initiated chains, with the [MI]/[free SG1] ratio maintained, gives no increase in the rate of polymerization (Fig. 4a). This indicates that stationary state kinetics apply, whereby the $[P^*]$ is not affected by [MI] [17]. The rate of spontaneous thermal initiation is negligible in this system [24], and rate which is proportional to $[P^*]$ (evidenced by the linear first order plot) will be dictated by the high excess of free nitroxide available for a given [MI]/[SG1] [41,43,47]. It is also evident from the identical rates of polymerization for the two different [poly(t-BA)-SG1]$_0$

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Fig. 2. Precipitation NMPs of 20% w/v initial loading of DMA in scCO$_2$ at 120 °C and 30 MPa, where [SG1]$_0$/[AIBN]$_0$ = 3.3. (a) First order rate plot (b) MWDs (normalized to peak height) and (c) GPC $M_n$ (●) and $M_w/M_n$ (□) versus conversion plots. $I_{lu}$ is at 6% conversion.

Fig. 3. Chain extension of poly(DMA)-SG1 with bulk St at 110 °C for 15 h: (a) GPC traces of purified MI (dashed line) from precipitation NMP of DMA in scCO$_2$ at 24% conversion (see Fig. 2), $M_n = 5700$ g/mol, $M_w/M_n = 1.23$ and chain extension in bulk St in the presence of 70% free SG1 (solid line) $M_n = 60,250$ g/mol, $M_w/M_n = 1.78$ and (b) $P(M)$ vs $M$ (number distribution curve) of the chain extended polymer. All peaks normalized to peak height.
initiated polymerizations that the level of partitioning of SG1 between phases is proportionate. Fig. 4b shows the increase in the poly(t-BA)-SG1 concentration by a factor of two resulted in a decrease in $M_n$ by close to a factor of two, as expected for a controlled/living system. This increase in $[MI]_0$ has however no effect on the rate of monomer consumption because of an increase in the average number of activation-deactivation cycles for the higher $[MI]_0$ is accompanied by a proportional decrease in the number of monomer units incorporated into each living polymer chain per cycle, in accordance with ideal CLRP kinetics [17]. The monomodal MWDs demonstrated that the polymerizations took place mainly in the suspended particles and that polymerization in the scCO$_2$ phase is less significant (see Supplementary Figs. S1–S3). Therefore the polymerizations initiated by poly(DMA)-SG1 and poly(t-)

![Fig. 4. Inverse suspension NMPs of 10% w/v initial loading of NIPAM in scCO$_2$ at 120 °C and 30 MPa initiated by poly(t-BA)-SG1 = 0.14 mmol (■), poly(t-BA)-SG1 = 0.28 mmol (△) and poly(DMA)-SG1 = 0.14 mmol (○, □) in the presence of 50 mol% free SG1. (a) First order rate plot and (b) GPC $M_n$ (closed symbols with trend lines) and $M_w/M_n$ (open symbols) versus conversion plots.](image)

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characterization of macroinitiators (MI) and poly(NIPAM) containing block copolymers used in aqueous cloud point analysis.</th>
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</thead>
<tbody>
<tr>
<td>Polymers</td>
<td>$M_n$(GPC)</td>
</tr>
<tr>
<td>Poly(DMA)-SG1 (MI)</td>
<td>5700 (1.23)</td>
</tr>
<tr>
<td>Poly(DMA)(58)-b-poly(NIPAM)(82)</td>
<td>13400 (1.48)</td>
</tr>
<tr>
<td>Poly(DMA)(58)-b-poly(NIPAM)(117)</td>
<td>21400 (1.55)</td>
</tr>
<tr>
<td>Poly(DMA)(58)-b-poly(NIPAM)(217)</td>
<td>25250 (2.07)</td>
</tr>
<tr>
<td>Poly(t-BA)-SG1 (MI)</td>
<td>8000 (1.17)</td>
</tr>
<tr>
<td>Poly(t-BA)(62)-b-poly(NIPAM)(81)</td>
<td>20600 (1.42)</td>
</tr>
<tr>
<td>Poly(t-BA)(62)-b-poly(NIPAM)(192)</td>
<td>30750 (1.33)</td>
</tr>
<tr>
<td>Poly(t-BA)(62)-b-poly(NIPAM)(254)</td>
<td>34550 (1.31)</td>
</tr>
<tr>
<td>Poly(St)-SG1 (MI)</td>
<td>6450 (1.13)</td>
</tr>
<tr>
<td>Poly(St)(62)-b-poly(NIPAM)(266)</td>
<td>34200 (1.51)</td>
</tr>
</tbody>
</table>

$^a$ $D_P$ of block copolymers is calculated from $M_n$(GPC) for the first block relative to overall $M_n$(NMR) of the block copolymer.

$^b$ After purification.

$^c$ Calculated from $^1$H NMR according to Eq. (1).

![Fig. 5. Inverse suspension NMPs of NIPAM in scCO$_2$ at 120 °C and 30 MPa initiated by (a) 0.14 mmol and (b) 0.28 mmol poly(St)-SG1 (dashed lines, $M_n = 6450$, $M_w/M_n = 1.13$). MWDs for (a) 10% w/v initial monomer loading with 50 mol% free SG1 (solid line, $M_n = 18450$, $M_w/M_n = 2.00$, 14% conv.) and (b) 20% w/v initial monomer loading with 75 mol% free SG1 (solid line, $M_n = 30700$, $M_w/M_n = 1.63$, 51% conv.). All peaks normalized to peak height.](image)
BA)-SG1 (at two different [MI]₀) proceeded with good controlled/living character, since Mₙ increases linearly with conversion starting from each Mₙ(MI). Polydispersities remain reasonably low, although some broadening occurs up to intermediate conversion especially for the poly(-DMA)-SG1 initiated system.

The initial inverse suspension polymerization of NIPAM in scCO₂ initiated by poly(St)-SG1 was carried out using the same conditions that proved successful using the polyamide and polyacrylate MIs (shown above). However poor control/livingness was obtained, as indicated by a prominent low MW shoulder corresponding to non-extended MI (Fig. 5a). Simultaneous doubling of the monomer loading, concentration of MI, and an increase in the excess free SG1 (Fig. 5b) gave a clear shift in the MWD of MI (at Mₙ = 6450 and Mₚ/Mₙ = 1.13) to higher MW block copolymer (at Mₙ = 30,700 and Mₚ/Mₙ = 1.63). The minor low MW tail in Fig. 5b is attributed to mainly dead poly(St) chains that did not initiate the polymerization of NIPAM, based on a 70% calculation of livingness of the MI (Fig. S4).

Prior to aqueous cloud point analysis of block copolymers, any traces of unreacted MI, and NIPAM were removed by selective precipitation procedures using an anti-solvent for the block copolymers, which dissolved monomer and any non-extended MI (see Section 2). Mₙ(GPC) of purified block copolymers are in reasonable agreement with Mₙ(NMR) in Table 2, indicating good accuracy in the estimation of the average degrees of polymerization of block copolymers (¹H NMR spectra of block copolymers are given in Supplementary data, see Figs. S5–S7).

3.3. Hydrolysis of Poly(t-BA)-b-Poly(NIPAM)

The required pH-sensitive thermal response polymer, poly(AA)-b-poly(NIPAM) was obtained through hydrolysis of the tert-butyl groups of poly(t-BA)-b-poly(NIPAM) with an excess of TFA (Fig. S6). The poly(AA) copolymer was then methylated using Me₃SiCH = N₂, and its MWD shown to be similar to that of the poly(t-BA) precursor before hydrolysis, indicating both negligible loss of polymer chains, and decomposition of the poly(NIPAM) part during the hydrolysis process (Fig. S8). The average degrees of polymerization for each poly(AA)-b-poly(NIPAM) are assumed to be the same as the t-BA precursor prior to hydrolysis (Table 2).

3.4. Aqueous cloud point analysis of block copolymers

This involved the preparation of purified and dried block copolymer samples (0.1% w/v) in de-ionised water. Upon heating at pH 7, the solutions became turbid above
the LCST, which corresponds to 50% transmittance on the heating curves in Figs. 6–8, with commercially available high MW polydisperse poly(NIPAM) (LCST = 31.7 °C) used as a reference. Our study assumes negligible end-group influences and similar relative GPC error for all samples (see Section 2). The MI incorporated part of the AB poly(NIPAM) block copolymer is of similar length in each case, and this study shows the effect of changing the chemical composition of the MI block on the observed LCST of poly(NIPAM). Fig. 6 shows the block copolymer solutions containing a hydrophobic poly(S) and poly(β-BA) parts become turbid at lower temperatures than those block copolymers incorporating hydrophilic poly(AA) and poly(ε-DMA) parts. The formation of aggregates or micelles for the poly(S) and poly(β-BA) containing block copolymers prior to the LCST is possible [27,28], and could influence cloud point measurements shown in Fig. 6. The controlled/living polymerization technique allowed us to analyse the effect of extending the poly(NIPAM) block length, while maintaining identical MI incorporated block sizes. Fig. 7a and b, respectively show this for the poly(DMA) and poly(ε-DMA) containing block copolymers. As a greater number of NIPAM monomeric units are incorporated in each case, the copolymers reach the cloud point at temperatures closer to that of commercial poly(NIPAM). The differences in polydispersity have less effect on cloud points, as indicated upon examination of Table 2. Strong decreases in the phase transition temperature with increasing MW of poly(NIPAM) have been reported by Stöver and co-workers for narrow dispersity homopolymers prepared using room temperature ATRP [70]. Poly(ε-DMA)-b-poly(NIPAM) is a well-studied dual responsive copolymer [25,27–29], and it is now observed that the cloud point is lowered significantly at pH4 close to the pKa (≈4.5–5.0) of the poly(AA) part, where it is the most hydrophobic (Fig. 8). This is in agreement with the findings of Kulkarni et al. [26] with the dependence of cloud point measurements on pH being small once the pKa is exceeded.

4. Conclusion

DMA is found to be appreciably soluble in scCO2 and amenable to a precipitation polymerization. Precipitation NMP of 20% w/v DMA was carried out in scCO2 at 120 °C and 30 MPa with good control/living character demonstrated. Under these conditions the polymer becomes insoluble at the critical degree of polymerization (f_{c}}) at 6% conversion, M_{n0} = 1600 g/mol and M_{n}/M_{n0} = 1.22. This indicates a similar solubility to polyacrylates in scCO2 [44]. Poly(DMA) at 24% conversion was used as MI, which was estimated to contain ~82% living chains based on chain extension with bulk styrene. Inverse suspension NMP of 10% w/v NIPAM in scCO2 using various MIs (prepared by precipitation NMPs) was then carried out with controlled/living stationary state kinetics observed, when using poly(ε-BA)-SG1 (at two different [MI]0). Different conditions were required to establish control for poly(S)-SG1 initiated inverse suspension polymerization of NIPAM in scCO2, in comparison to poly(DMA)-SG1 and poly(ε-BA)-SG1 initiated polymerizations. The MIs incorporated are shown to greatly influence the aqueous cloud point temperature, and cloud points approach commercial polydisperse high MW poly(NIPAM) as a greater number of NIPAM units are incorporated. Future work will focus on stabilizing heterogeneous NMPs of hydrophilic NIPAM and DMA monomers, which is not possible in aqueous or organic solution environments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2012.04.011.

References


A drug delivery system based on a thermoresponsive polymer and a cooling device: a theoretical assessment

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Abstract

A mathematical model is developed to evaluate the feasibility of an in vivo implanted drug delivery system. The delivery device consists of a cooling material coated by a drug-loaded thermoresponsive polymer film. Drug release is initiated by remotely dropping the temperature of the cooling material sufficiently for the temperature throughout the polymer coating to drop below its lower critical solution temperature (LCST), causing the polymer to swell and release the drug. Drug release switches off again when heat conduction from an external fluid medium raises the polymer temperature to above the LCST causing the polymer to collapse. Candidate cooling mechanisms based on endothermic chemical reactions, the Peltier effect, and the magnetocaloric effect are discussed. In the thin polymer film limit, the model provides an upper bound for the temperature the cooling material must be dropped to for drug release to be initiated. Significantly, the model predicts that the duration a thin polymer will continue to release drug in a single cycle is proportional to the square of the thickness of the cooling material. It is found that the system may be realised for realistic parameter values and materials. A simple illustrative calculation incorporating the presence of a heat source is also presented, and the results suggest that conduction due to the initial temperature difference between the water and the cooling material can make the dominant contribution to heat transfer in the polymer as it reheats to its LCST.

Keywords: Thermoresponsive polymer, Local drug delivery, Pulsatile release, Cooling device, Mathematical model, Theoretical model

1. Introduction

Thermoresponsive polymers undergo a volume phase transition in aqueous solution when the temperature of their fluid environment is varied across a critical value. If the polymer goes from being in a swollen, hydrophilic state to a shrunken, hydrophobic state as the temperature is increased through the critical value, the critical temperature is referred to as the lower critical solution temperature (LCST). The LCST of a thermoresponsive polymer can be readily varied by, for example, changing the pH or composition of its fluid environment, by varying the molecular weight of the polymer, or by the incorporation of hydrophilic or hydrophobic groups [1, 2]. LCST values as low as 7.5°C [1] and as high as 80°C [2] have been observed experimentally. Poly(N-isopropylacrylamide) (pNIPAm) is a thermoresponsive polymer that has been extensively studied [3, 4]. Its LCST is close to 32°C and it has a sharp phase transition in a narrow temperature region [5]. Furthermore, the LCST of pNIPAm can be easily increased to near human physiological temperature (37°C) [6, 7].
Thermoresponsive polymers have numerous potential applications in areas such as drug delivery \([8,9,10]\), gene delivery \([11]\), tissue engineering \([12]\), chemical valve technology \([13]\), and catalysis \([14]\). However, a biomedical device based on a thermoresponsive polymer that enables the pulsatile release of drug \textit{in vivo} has yet to be developed, principally because of the difficulty of locally decreasing the temperature of human tissue \textit{in vivo}. Nevertheless, candidate mechanisms for cooling polymers \textit{in vivo} have been proposed, and some of these are now briefly discussed.

A potential chemically-based cooling mechanism is to add a substance to the polymer’s fluid environment that gives rise to an endothermic chemical process. In an endothermic process, heat is absorbed from the environment as the reactions involved proceed causing a drop in its temperature. The dissolution of ammonium nitrate in water is an example of an endothermic process; when ammonium nitrate is dissolved in water at room temperature at a concentration of 1 M, a drop in fluid temperature of 6 K is observed. However, the choice of substances that could be safely \textit{in vivo} is limited since most endothermic reactions involve toxic chemicals \([15]\).

Another candidate cooling mechanism is based on the Peltier effect, which is a thermoelectric phenomenon \([16]\). The Peltier effect arises at the junction of two dissimilar conductors through which a current is flowing, and refers to the transfer of heat from one of the conductors to the other across the junction. The Peltier effect allows for the transfer of heat at the junction against the temperature gradient, and cooling devices have been manufactured based on the effect \([17,18]\). Bi$_2$Te$_3$ and Bi$_2$Se$_3$ \([19]\) are amongst the best performing thermoelectric materials at room temperature, and exhibit a temperature-independent thermoelectric effect \([20,21]\). A thermoelectric cooling device based on the Peltier effect has recently been developed by Morizane \textit{et al.} \([22]\) for the treatment of spinal cord injury in rats, although the cooling component of their device was deployed outside of the body as it was too large to be implanted \textit{in vivo}. However, extremely thin thermoelectric cooling devices can now be fabricated that are less than 200 \(\mu\)m thick \([23]\), making feasible the development of devices that may be implanted \textit{in vivo}.

The magnetocaloric effect (MCE) provides another possible mechanism for externally lowering the temperature of the cooling material. The MCE refers to the heating or cooling of magnetic materials due to the application of time-varying magnetic fields. The effect, which has been substantially understood for a long time \([24]\), is caused by the change in magnetic entropy of a material due to the application or removal of an external magnetic field. Specifically, the cooling of a magnetic material is caused by an increase in its magnetic entropy when an external magnetic field is removed. The MCE has been a subject of considerable interest since 1997 when Pecharsky and Gschneidner \([25]\) discovered a giant MCE in some Gd$_x$(Si$_{1-x}$Ge$_x$)$_4$ alloys. Many alloys have subsequently been found to possess a colossal MCE \([24,26]\), although only some of these were found to undergo their maximum temperature change close to room temperature and ambient pressure. Of these, only a very few were found to possess their MCE peak near physiological temperature (37°C). De Campos \textit{et al.} \([27]\) reported that the MCE peak of Mg$_{1-x}$Fe$_x$As is at 37°C when \(x=0.003\).

The three cooling technologies just described can in principle be used to locally decrease the temperature of tissue in the body. It should be emphasised, however, that a system will only be of practical value if it can be manufactured at...
In this paper, a mathematical model is formulated to investigate the feasibility of developing a pulsatile drug release system that is based on dropping the temperature of a thermoresponsive drug-loaded polymer coating below its LCST. The candidate cooling mechanisms discussed above are not incorporated in the modelling presented here. Rather, the model is used to determine the properties a cooling system should have for a drug release device to produce practically useful pulsatile release profiles.

2. The mathematical model

Introduction

The proposed release system is composed of a thermoresponsive drug-loaded polymer bonded to a cooling device which consists of a slab of cooling material. The device and polymer are immersed in water that is initially held at a temperature above the polymer's LCST, so that the polymer is collapsed and drug release is negligible; see Fig. 1 (a). Drug delivery is initiated by quickly lowering the temperature of the cooling material sufficiently for the temperature throughout the adjacent polymer to drop to below its LCST, thereby causing the polymer to swell. Drug then releases from the swollen polymer. Drug release spontaneously switches off again when heat conduction from the fluid environment raises the polymer temperature to above its LCST causing it to collapse. In the current theoretical study, mathematical modelling is used to assess the feasibility of a system of this kind.

Modelling assumptions

(i) Heat conduction in the cooling material, polymer and water is governed by Fourier's law of conduction.

(ii) The thermal conductivity and diffusivity of the cooling material, polymer and water are taken to be constant. The thickness of the cooling material and polymer are also assumed constant and the water is taken to occupy an infinite domain. Although these are reasonable assumptions for the cooling material and the water, they are not valid for a thermoresponsive polymer since its properties and thickness change during the swelling and collapsing processes. However, for the important case of a thin polymer film, these assumptions are frequently acceptable since it will be shown in this study that the polymer temperature can then be dominated by the properties of the cooling material and the water. For polymers whose thickness is comparable to that of the cooling material, the assumptions of constant polymer conductivity, thermal diffusivity and thickness may be viewed as providing a starting point for the analysis of the system.

(iii) The temperature and heat flux are taken to be continuous at the cooling material-polymer and polymer-water interfaces.

(iv) It is assumed that the presence of drug does not affect the thermal properties of the polymer or the water. The model thus does not incorporate drug concentrations, and is used instead to predict the evolution of the temperature profiles in the cooling material, polymer and water. This is sufficient to establish the feasibility of the system.
The temperature of the cooling material may be instantaneously dropped to the same value throughout its volume. There is no further cooling of the system subsequent to this. These assumptions will not affect the conclusions of the modelling presented here since allowing for a finite cooling period will only serve to delay the reheating of the system by the fluid environment, thereby prolonging the time the polymer remains swollen.

The phase transition for the polymer is assumed to be perfectly sharp, so that for temperatures immediately above its LCST, the polymer collapses fully.

Geometry and model equations

For simplicity, the cooling device is taken to be composed of an infinite slab of homogeneous material of thickness $2H_M$ that occupies $-2H_M < x < 0$, $-\infty < y, z < \infty$. At $x = -2H_M$ and $x = 0$, the cooling material is taken to be in thermal contact with polymer films of thickness $H_P$. At $x = -H_M - H_P$ and $x = H_M$, the polymer films are in turn taken to be in thermal contact with water that occupies $-\infty < x < -2H_M - H_P$ and $H_P < x < \infty$. Water is chosen as the fluid medium here since it is the dominant component of biological fluids. The system is clearly symmetric about the centreline of the cooling material at $x = -H_M$, and so a symmetry condition is imposed at $x = -H_M$ and the semi-infinite domain $-H_M < x < \infty$ only is considered; see Fig. 1(b).

The temperatures in the cooling material, polymer and water at location $x$ and time $t$ are denoted by $T_M(x, t)$, $T_P(x, t)$ and $T_W(x, t)$, respectively. The initial temperature of the entire system is taken to be at the constant temperature of the water, $T_W$, say. Since the behaviour of the device in the human body is being modelled, $T_W$ is taken to be $37^\circ C$ in the numerical calculations of this paper. The LCST of the thermoresponsive polymer is denoted by $T_L$, and it is supposed that $T_L < T_W$ so that the polymer is initially in its collapsed state. At time $t = 0$, it is assumed that the temperature throughout the cooling material is instantaneously lowered to $T_L$. The purpose of the analysis of this paper is to investigate the subsequent temperature evolution for $t > 0$ in the adjoining polymer, and in particular, the possibility of the polymer temperature dropping to below its LCST throughout its thickness so that it can swell and release drug. The subsequent reheating of the polymer above its LCST due to heat conduction from the adjacent fluid medium will also be tracked.
In view of the discussion immediately above and the modelling assumptions of the previous section, the temperature in the cooling material $T_M(x,t)$ satisfies:

\[
\frac{\partial T_M}{\partial t} = k_M \frac{\partial^2 T_M}{\partial x^2} \quad \text{for } -H_M < x < 0, \quad t > 0,
\]
\[
\frac{\partial T_M}{\partial x} = 0 \quad \text{on } x = -H_M, \quad t > 0,
\]
\[
T_M = T_M^0 \quad \text{for } -H_M < x < 0, \quad t = 0,
\]

where $k_M$ is the constant thermal diffusivity of the material. The boundary condition (1) is the symmetry condition that allows the spatial domain to be halved.

The temperature in the polymer $T_P(x,t)$ satisfies:

\[
\frac{\partial T_P}{\partial t} = k_P \frac{\partial^2 T_P}{\partial x^2} \quad \text{for } 0 < x < H_P, \quad t > 0,
\]
\[
T_P = T_P^0 \quad \text{for } 0 < x < H_P, \quad t = 0,
\]

where $k_P$ is the constant thermal diffusivity of the polymer, and the temperature in the water medium $T_W(x,t)$ satisfies:

\[
\frac{\partial T_W}{\partial t} = k_W \frac{\partial^2 T_W}{\partial x^2} \quad \text{for } H_P < x < +\infty, \quad t > 0,
\]
\[
T_W = T_W^0 \quad \text{for } H_P < x < +\infty, \quad t = 0,
\]
\[
T_W \to T_W^0 \quad \text{as } x \to +\infty, \quad t > 0,
\]

where $k_W$ is the constant thermal diffusivity of the water. Imposing continuity in temperature and heat flux at the cooling material-polymer and the polymer-water interfaces gives:

\[
T_M = T_P, \quad -K_M \frac{\partial T_M}{\partial x} = -K_P \frac{\partial T_P}{\partial x} \quad \text{on } x = 0, \quad t \geq 0,
\]
\[
T_P = T_W, \quad -K_P \frac{\partial T_P}{\partial x} = -K_W \frac{\partial T_W}{\partial x} \quad \text{on } x = H_P, \quad t \geq 0,
\]

where $K_M$, $K_P$, $K_W$ give the constant thermal conductivities of the cooling material, polymer, and water, respectively. The thermal diffusivities are related to the thermal conductivities via:

\[
k_M = \frac{K_M}{\rho_M c_M}, \quad k_P = \frac{K_P}{\rho_P c_P}, \quad k_W = \frac{K_W}{\rho_W c_W},
\]

where, using an obvious notation, the $\rho$'s give the densities and the $c$'s the specific heat capacities of the three media. The ratio of the conductivity to the thermal diffusivity, $K/k$, is referred to as the volumetric heat capacity of a material.

The mathematical model is now complete and consists of equations (1)-(4).

3. Results and discussion

3.1. The thin polymer film limit

In the appendices, a non-dimensionalisation of the governing equations (1)-(4) is presented, and the asymptotic limit $H_P/\sqrt{k_P t} \to 0$ is considered, with the other independent dimensionless parameters arising being taken to be $O(1)$. This limit is of practical relevance since it corresponds to the case of a thin polymer film coating. It is found that $T_P \sim T_P^0(\tau)$ as $\epsilon \to 0$, where

\[
T_P(\tau) = T_P^0 - \frac{1}{2}(T_P^0 - T_W^0)(1 - \alpha) \sum_{n=0}^{\infty} \alpha^n \left( \text{erfc} \left( \frac{H_P}{\sqrt{\tau k_P}} \right) + \text{erfc} \left( \frac{H_P(a+n) + 1}{\sqrt{\tau k_P}} \right) \right),
\]

with:

\[
\alpha = \frac{\sqrt{K_M \rho_W c_W} - \sqrt{K_P \rho_W c_W}}{\sqrt{K_M \rho_W c_W} + \sqrt{K_P \rho_W c_W}} \frac{I_M - I_{WP}}{I_{WP} + I_M},
\]

where:

\[
l_M = \sqrt{K_M \rho_M c_M} \quad l_P = \sqrt{K_P \rho_P c_P} \quad l_W = \sqrt{K_W \rho_W c_W}
\]

give the thermal inertias of the cooling device and water, respectively. It is clear from (7) that $|\alpha| < 1$. It is noteworthy that at leading order, the temperature in the
polymer does not depend on position, and is also independent of the conductivity, thermal diffusivity and thickness of the polymer. Hence, for a thin polymer film, the temperature in the polymer will frequently be dominated by the properties of the cooling material and the water medium, as would be expected.

An elementary calculation shows that $T_p$ is an increasing function of time for $T_i^W$, so that the polymer has its minimum temperature at leading order immediately after the temperature in the cooling material has been lowered.

From equation (6), this minimum temperature is given by:

$$T_p(t) \rightarrow \frac{1}{2}[(1 + \alpha) T_i^M + (1 - \alpha) T_i^W] = \frac{L_T}{T_m} + \frac{T_i^W}{T_m} \text{ as } t \rightarrow 0^+,$$

which is a weighted average of the initial temperatures of the cooling material and the water, with the weights being given by the thermal inertias of the two media. As time progresses, the leading order temperature of the polymer increases toward the initial water temperature, $T_i^W$. Hence, in the thin film limit, a necessary condition for the polymer to fully swell is that:

$$\frac{L_T}{T_m} + \frac{T_i^W}{T_m} < T_i^L \quad \text{or} \quad T_i^M < T_i^W \frac{T_i^M}{T_i^W} (T_i^L - T_i^M).$$

The second inequality in (8) is of considerable practical value since it provides an upper bound for the temperature the cooling material must be dropped to for drug release to be initiated.

If inequality (8) is satisfied, it is of interest to estimate the time $t = t_0$ by which the polymer has heated back up to its LCST, as $t_0$ then provides an estimate for the duration the polymer releases drug. If $T_i \rightarrow T_i^L$ as $t \rightarrow 0$, then $T_p(t_0) = T_i^L$, and using equation (6):

$$\sum_{n=0}^{\infty} \alpha^n \left( e^{R/T_{i}^L} \right) = \frac{2}{1 - \alpha} \frac{T_i^W - T_i^L}{T_i^W - T_i^M}.$$

Elementary calculations show that equation (9) has a unique solution $0 < t_0 < \infty$ provided $|\alpha| < 1$, $T_i^M < T_i^L$, and inequality (8) is satisfied. It also follows from equation (9) that $t_0$ has the general structure:

$$t_0 = \frac{H_0^2}{2k_m} \left( \frac{T_i^W - T_i^M}{T_i^W - T_i^L} \right) \left( \frac{1}{T_i^L - T_i^M} - \frac{1}{T_i^W - T_i^L} \right),$$

for some function $F$, or:

$$t_0 = t_M F(\theta, \alpha),$$

where:

$$t_M = \frac{H_0^2}{2k_m} \left( \frac{T_i^W - T_i^M}{T_i^W - T_i^L} \right) \left( \frac{1}{T_i^L - T_i^M} - \frac{1}{T_i^W - T_i^L} \right).$$

Note that $0 < \theta < 1$ for $T_i^M < T_i < T_i^W$ and that inequality (8) corresponds to $\theta > (1 - \alpha)/2$. In Table B.3, values are displayed for $F(\theta, \alpha)$ in the range $1 - \alpha/2 < \theta < 1$, $|\alpha| < 1$, which were found by numerically solving equation (9) using the mathematical package MAPLE.

The formula (10) is instructive because it implies that in the thin film limit, the duration the polymer is held below its LCST is proportional to the square of the thickness of the cooling material. Since $F(\theta, \alpha) > 0$ for $1 - \alpha/2 < \theta < 1$, $|\alpha| < 1$, it is in principle possible to adjust the thickness of the cooling material so as to maintain the polymer in the swollen state for a desired time interval for any parameter pair $\theta, \alpha$ with $1 - \alpha/2 < \theta < 1, |\alpha| < 1$. Furthermore, since the dependence on the thickness of the cooling material is quadratic, it is an effective parameter to adjust to tune the system. It is also noteworthy in equation (10) that the three temperature parameters $T_i^M, T_i^L, T_i^W$ appear only in a single divided difference ratio. However, it remains to show that the system is realisable for realistic parameter values, and this issue is now addressed.
3.2. Cooling materials

The feasibility of a particular system may be evaluated using the formula (11) since $t_{L0}$ estimates the time the polymer will remain in the swollen state. In (11), it is seen that $t_{L0}$ depends on the three parameters $t_M$, $\theta$, and $\alpha$. The parameter $t_M$ depends on the properties of the cooling material only, $\theta$ depends on the three temperature scales that arise in the system (one each for the cooling material, polymer and water), and $\alpha$ depends on the ratio of the thermal inertias of the cooling material and water. It is noteworthy that in the thin film limit, the properties of the polymer only enter at leading order via its LCST in the parameter $\theta$. However, it is clear that the properties of the cooling material are critical to the behaviour of the system.

In Table 1, parameter values for four potential cooling materials are displayed. However, only two of these materials, water and copper, are evaluated in detail here. Water is chosen because it may be used as the medium for a cooling system based on endothermic chemical reactions. Copper has a large thermal and electrical conductivity, and has been chosen to represent metallic materials; thermoelectric and magnetocaloric cooling technologies typically involve metals.

<table>
<thead>
<tr>
<th>Material</th>
<th>Volumetric Heat Capacity $\alpha$</th>
<th>Thermal Conductivity $K$ (J m$^{-1}$K$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>$4.2 \times 10^6$</td>
<td>0.6</td>
</tr>
<tr>
<td>Copper</td>
<td>$3.4 \times 10^6$</td>
<td>386.0</td>
</tr>
<tr>
<td>Aluminum</td>
<td>$2.4 \times 10^6$</td>
<td>204.0</td>
</tr>
<tr>
<td>Stainless Steel 316</td>
<td>$3.9 \times 10^6$</td>
<td>13.4</td>
</tr>
</tbody>
</table>

Figure 2: (a) The leading order temperature of the polymer as a function of time, $T_{p0}(t)$, with copper being the cooling material. Here the initial temperature of cooling device and water are $T_{iM} = 32^\circ C$ and $T_{iW} = 37^\circ C$, respectively, and various cooling material thicknesses, $2H_{M}$, have been used. (b) The leading order time it takes the polymer to heat back up to its LCST as a function of $T_{iM}$ for various cooling material thicknesses. Here, the LCST is $T_L = 35.5^\circ C$, the initial temperature of the water is $T_{iW} = 37^\circ C$, and plots for both copper and water being the cooling material are displayed.
In Fig. 2 (a), the leading order polymer temperature, $T_{P_0}(t)$, has been plotted as a function of time, with copper being the cooling material. In the plot, $T_u = 32\,^\circ C$, $T_i = 37\,^\circ C$ (physiological temperature), and various thicknesses for the cooling material are used, ranging from 2 mm to 8 mm. The strong dependency of the behaviour on cooling material thickness is evident in these plots. If, for example, the LCST of the polymer is 35.5 $^\circ C$, then it is clear from the figure that a drug release time of some minutes may be achieved for a device thickness of some millimetres if a temperature drop of the order of 5 $^\circ C$ can be induced in the cooling material. A drug release duration of a couple minutes for a single dose can be appropriate for many systems of practical interest; see, for example [32].

In Fig. 2 (b), plots for the leading order time it takes the polymer to reheat to its LCST, $T_{L_0}$, are given as a function of $T_i$, the temperature to which the cooling material is initially dropped. In these plots, parameter values for both copper and water being the cooling material are used, and $T_L = 35.5\,^\circ C$, $T_i = 37\,^\circ C$. It is evident from this figure that the system is realisable for reasonable parameter values for both materials.

### 3.3. Thicker polymers

The case where the polymer thickness is not small compared to that of the cooling material is now very briefly considered. For this case, the full set of equations (1)-(4) are integrated numerically. However, it should be emphasised again here that some of the modelling assumptions may not now be valid; see Section 2.

In Fig. 3, numerical solutions for the temperature of the polymer at its interface with the water medium are plotted as functions of time, that is, $T_{P_0}(H_p, t)$ versus $t$. The model predicts that the polymer will remain swollen while $T_{P_0}(H_p, t) < T_i$, and that it will then reheat to its LCST, $T_L$, over a period of time that depends on the initial temperature of the cooling material and the water, $T_i$, and the ratio of the polymer thickness $H_p$ to half the cooling device thickness $H_M$, with $H_M = 3\,\text{mm}$. The cooling material is water in (a), and copper in (b). The thermal diffusivity and conductivity chosen for the polymer are $k_p = 1 \times 10^{-2} \,\text{m}^2\,\text{s}^{-1}$ and $K_p = 0.15\,\text{J}\,\text{m}^{-1}\,\text{K}^{-1}\,\text{s}^{-1}$, respectively. The initial temperature of the cooling device and the water are $T_u = 32\,^\circ C$ and $T_i = 37\,^\circ C$, respectively.

![Figure 3: Plots of the temperature at the polymer-water interface as a function of time for various ratios of the polymer thickness $H_p$ to half the cooling device thickness $H_M$, with $H_M = 3\,\text{mm}$](image-url)
APPENDIX A

since \( T(x,t) \) has its maximum value at \( x = H_s \). In the figure, parameter values for poly(methyl methacrylate) [33] have been used for the polymer; poly(methyl methacrylate) is known to have similar properties to pNIPA in its collapsed state.

The cooling material is water in Fig. 3 (a), and copper in Fig. 3 (b). The model predicts, as would be expected, that thicker polymers are more difficult to cool thoroughly, although the results also indicate that the system can be feasible for ratios \( H_p/H_s \) as large as \( \mathcal{O}(1/10) \).

3.4. Incorporating a heat source in the modelling

A limitation of the modelling presented here is that no attempt has been made to incorporate the possible presence of heat sources or sinks. In the human body, tissues generate heat via metabolic processes, and blood flowing in or nearby to tissue can exchange thermal energy with it. The subject of bioheat transfer has been extensively studied in the past, and numerous mathematical models have been proposed to describe it [34,35,36,37,38].

The model presented here can be justified for systems where heat conduction due to the initial temperature difference between the cooling material and the water makes the dominant contribution to heat transfer in the polymer as it reheats to its LCST. Fourier’s law implies that heat transfer via conduction at a given location is proportional to the temperature gradient at that location. In our system, the temperature gradients in and near a thin polymer are large for times \( t \ll H_s^2/k_w \) and \( t \approx \mathcal{O}(H_s^2/k_w) \) compared to these gradients for \( t \gg H_s^2/k_w \) (with the dimensionless parameters other than \( H_s/H_w \) being \( \mathcal{O}(1) \)), so that heat conduction in the polymer is strong in this sense for \( t = \mathcal{O}(H_s^2/k_w) \) or smaller. However, it should be emphasised that the model presented here cannot be used to estimate the time taken for a polymer to reheat to 37°C in the human body. This is because the model predicts that \( T \to 37°C \) as \( t \to \infty \), and since heat conduction becomes weaker as time progresses, other factors that can contribute to heat transfer, such as the presence of blood vessels or metabolism, can become significant, or even dominant, as time goes on.

A simple illustrative calculation is now presented that models the presence of a constant heat source at 37°C that is at a distance \( H_w \) from the polymer. This is achieved by replacing the boundary condition \( T_w \to 37°C \) by:

\[
T_s = 37°C \quad \text{on} \quad x = H_s + H_w. \tag{13}
\]

This condition could serve as a crude model for the presence of a major artery at \( x = H_s + H_w \), for example; more realistic models for heat transfer in the body can be found in the literature cited above. Numerical solutions corresponding to the boundary condition (13) are presented in Fig. 4, and the results are broadly as expected. It is seen that for \( H_w \leq 6 \text{ mm} \), the profiles for the first few minutes are close to the \( H_w \to \infty \) curve, but that the curves with \( H_w = 1, 6, 12 \text{ mm} \) recover to close to 37°C much more quickly than the \( H_w \to \infty \) curve. This suggests that for some geometries and materials, heat conduction due to the initial temperature mismatch between the cooling material and the water can make the major contribution to heat transfer in the polymer in the first few minutes. In Table 2, numerical estimates for the time taken for the polymer to reheat to its LCST and to 36.9°C are displayed for various separations between the polymer and the constant heat source, \( H_w \).

4. Conclusions

A mathematical model has been developed to evaluate the feasibility of a proposed local drug delivery that may be implanted in vivo. The delivery system is...
Figure 4: Numerical solutions for the temperature at the polymer-water interface as a function of time, and for various separations $H_W$ between the polymer and a constant heat source held at $37^\circ C$. The polymer is of thickness $H_P = 150 \mu m$, and the cooling material is water of thickness $H_M = 3 \, mm$. The thermal diffusivity and conductivity chosen for the polymer are $k_P = 1 \times 10^{-7} \, m^2 \, s^{-1}$ and $K_P = 0.15 \, J \, m^{-1} \, K^{-1} \, s^{-1}$, respectively. The initial temperatures of the cooling device and water are $T_i^M = 32^\circ C$ and $T_i^W = 37^\circ C$, respectively.

Table 2: Estimated times taken for the polymer to heat back to its LCST, $T_L = 35.5^\circ C$, and to $36.9^\circ C$, with water being cooling material. The times are estimated for various separations $H_W$ between the polymer and the heat source. The thickness of the cooling material and the polymer here are $2H_M = 6 \, mm$ and $H_P = 150 \, \mu m$, respectively. The initial temperature of the cooling material is $T_i^M = 32^\circ C$, and of the water is $T_i^W = 37^\circ C$.

<table>
<thead>
<tr>
<th>$H_W$ (mm)</th>
<th>$t_L$ (min)</th>
<th>$t_{36.9,^\circ C}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>2.85</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>6.5</td>
</tr>
<tr>
<td>6</td>
<td>2.4</td>
<td>13.3</td>
</tr>
<tr>
<td>12</td>
<td>3.0</td>
<td>32.0</td>
</tr>
<tr>
<td>18</td>
<td>3.0</td>
<td>56.0</td>
</tr>
<tr>
<td>30</td>
<td>3.0</td>
<td>115.0</td>
</tr>
</tbody>
</table>

The independent non-dimensional parameters governing the behaviour of the system have also been identified. For thin polymers, a useful formula has been derived to estimate the duration the polymer will remain swollen in a single cycle; in particular, it is found that this duration is proportional to the square of the cooling material thickness. A calculation that incorporates a constant heat source in the modelling has also been presented, and the results indicate that while the presence of heat sources in the body cause the polymer to heat more quickly, the overall process of heating and cooling remains similar in both cases.

The cooling device is composed of an appropriate cooling material coated by a drug-loaded thermoresponsive polymer film. Some candidate cooling mechanisms for the device have been proposed based on endothermic chemical reactions, thermoelectric effects, and the magnetocaloric effect. In the thin polymer film limit, the model provides an upper bound for the temperature the cooling material must be dropped to for drug delivery to be initiated. The thickness of the cooling material and the polymer have been estimated for various separations $H_W$ between the polymer and the heat source. The thickness of the cooling material and the polymer here are $2H_M = 6 \, mm$ and $H_P = 150 \, \mu m$, respectively. The initial temperature of the cooling material is $T_i^M = 32^\circ C$, and of the water is $T_i^W = 37^\circ C$. 

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<tbody>
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</tr>
<tr>
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<tr>
<td>6</td>
<td>2.4</td>
<td>13.3</td>
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<tr>
<td>12</td>
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<td>32.0</td>
</tr>
<tr>
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to recover to 37°C on a realistic time scale, heat conduction due to the initial

temperature difference between the water and the cooling material can make the
dominant contribution to heat transfer in the polymer for sufficiently small times.

Finally, it has been established that the system may be realised for realistic mate-

trials and parameter values.

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dustry (MACSI, SFI Grant 06/MI/005).

Declarations

Competing interests: None.

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and Industry (MACSI, SFI Grant 06/MI/005).

Ethical approval: Not required.

Appendix A. Non-dimensionalisation and non-dimensional parameters

The independent non-dimensional parameters that may be varied to tune the

system are identified by non-dimensionalising the governing equations (1)-(4).

Introducing the dimensionless variables:

\[ \tilde{x} = \frac{x}{H_m}, \quad \tilde{t} = \frac{t}{(H_m \rho \alpha)}, \quad \tilde{T}_m = \frac{T_m - T_i}{T_w - T_i}, \quad \tilde{T}_p = \frac{T_p - T_i}{T_w - T_i}, \]

the following dimensionless equations are obtained:

**Cooling Material.**

\[ \frac{\partial \tilde{T}_m}{\partial \tilde{t}} - \frac{k}{\rho \alpha} \frac{\partial^2 \tilde{T}_m}{\partial \tilde{x}^2} \quad \text{for} \quad -1 < \tilde{x} < 0, \quad \tilde{t} > 0, \quad \tilde{T}_m(\tilde{x}, \tilde{t}) \quad \text{on} \quad \tilde{x} = 0, \quad \tilde{t} \geq 0 \quad \text{(A.1)} \]

**Cooling material/polymer interface.**

\[ \tilde{T}_m = \tilde{T}_p, \quad \frac{\partial \tilde{T}_m}{\partial \tilde{x}} = -K_{np} \frac{\partial \tilde{T}_p}{\partial \tilde{x}} \quad \text{on} \quad \tilde{x} = -1, \quad \tilde{t} \geq 0; \quad \tilde{T}_m(\tilde{x}, \tilde{t}) \quad \text{on} \quad \tilde{x} = -1, \quad \tilde{t} \geq 0 \quad \text{(A.2)} \]

**Polymer.**

\[ \frac{\partial \tilde{T}_p}{\partial \tilde{t}} = \frac{k}{\rho \alpha} \frac{\partial^2 \tilde{T}_p}{\partial \tilde{x}^2} \quad \text{for} \quad 0 < \tilde{x} < \epsilon, \quad \tilde{t} > 0, \quad \tilde{T}_p(\tilde{x}, \tilde{t}) \quad \text{on} \quad \tilde{x} = 0, \quad \tilde{t} \geq 0 \quad \text{(A.3)} \]

**Polymer/water interface.**

\[ \tilde{T}_p = \tilde{T}_w, \quad \frac{\partial \tilde{T}_p}{\partial \tilde{x}} = -K_{pw} \frac{\partial \tilde{T}_w}{\partial \tilde{x}} \quad \text{on} \quad \tilde{x} = \epsilon, \quad \tilde{t} \geq 0 \quad \text{(A.4)} \]
APPENDIX A

Water.

\( \frac{\partial T}{\partial t} = k_w \frac{\partial^2 T}{\partial x^2} \) for \( \varepsilon < x < \infty, \ t > 0 \)

\( T = 1 \) for \( \varepsilon < x < \infty, \ t = 0 \), \hspace{1cm} (A.5)

\( T \rightarrow 1 \) as \( x \rightarrow \infty, \ t \geq 0 \),

where the over bars have been dropped for convenience, and where:

\[ \begin{align*}
T_{m0} = \frac{T_m}{T_0}, & \quad \varepsilon = \frac{H_p}{H_m}, \quad k_m = \frac{k}{k_0}, \quad k_w = \frac{k_w}{k_w}, \\
k_m = \frac{k_m}{k_m}, & \quad k_w = \frac{k_w}{k_w}, \quad k_m = \frac{k_m}{k_m}, \quad k_w = \frac{k_w}{k_w}, \\
\end{align*} \]

(A.6)

are the non-dimensional parameters arising.

It is noteworthy from (A.6) that for a given polymer and cooling material, there are two parameters that may be independently varied to tune the system, namely the temperature ratio, \( T_{m0} \), and the geometrical parameter, \( \varepsilon \). The remaining four quantities appearing in equation (A.6) are material parameters, and are fixed for a given choice of polymer and cooling material. If both the cooling material and the polymer are allowed to change, then in principle all six parameters in equation (A.6) may be independently varied.

The problem defined by equation (A.1)-(A.5) is linear and analytical progress is possible using, for example, the method of Laplace transforms [28]. However, the algebra arising is quite heavy and it is more convenient to solve the full problem numerically. Fortunately, a convenient analytical solution may be written down at leading order in the thin polymer film limit (which is probably the most important case from the point of view of applications), and this is considered now.

Appendix B. The thin polymer film limit

The asymptotic limit \( \varepsilon = H_p/H_m \rightarrow 0 \) is considered, which corresponds to the thickness of the polymer being small compared to the thickness of the cooling material. The remaining parameters are taken to be \( O(1) \), and as \( \varepsilon \rightarrow 0 \) the following expansions are posed for \( t = O(1) \):

\[ T_{m0} \sim T_{m0}(x,t) \quad \text{in} \quad -1 < x < 0, \quad T_{w0} \sim T_{w0}(x,t) \quad \text{in} \quad x > 0. \]

It is easily shown that \( T_{m0} \) satisfies (A.1), \( T_{w0} \) satisfies (A.5) with \( \varepsilon = 0 \), and:

\[ T_m = T_{m0}, \quad \frac{\partial T_m}{\partial t} = -K_m \frac{\partial T_m}{\partial x} \quad \text{on} \quad x = 0, \ t \geq 0, \]

where \( K_m = K_m K_w = K_m. \) This leading order problem can be readily solved using Laplace transforms (similar problems are considered in the book [28], for example) to obtain:

\[ T_{m0} = \frac{1}{2(1 - \alpha)(1 - \varepsilon) \sum_{m = 0} \alpha^m \left( \text{erf} \left( \frac{2k_m}{2\sqrt{t}} \right) + \text{erf} \left( \frac{2k_m + 1}{2\sqrt{t}} \right) \right)}, \]

where:

\[ \alpha = \frac{\sqrt{\varepsilon - K_m}}{\sqrt{\varepsilon + K_m}} \]

so that \( |\alpha| < 1 \). The leading order temperature in the polymer film, \( T_{m0} \) (say), is then given by:

\[ T_m(0,t) = T_m(0,t) = \frac{1}{2(1 - \varepsilon) \sum_{m = 0} \alpha^m \left( \text{erf} \left( \frac{H_p}{H_m} \right) + \text{erf} \left( \frac{H_p + 1}{H_m} \right) \right)}, \]

or, in dimensional terms:

\[ T_m(0,t) = T_m(0,t) = \frac{1}{2(1 - \varepsilon) \sum_{m = 0} \alpha^m \left( \text{erf} \left( \frac{H_p}{\sqrt{t}} \right) + \text{erf} \left( \frac{H_p + 1}{\sqrt{t}} \right) \right)}. \]
References


APPENDIX A


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