Department of Chemical Engineering

Synthesis and Characterization of Thermosensitive Macroporous Hydrogels for Controlled Drug Delivery Applications

Yuli Setiyorini

This thesis is presented for the Degree of
Master of Philosophy
of
Curtin University of Technology

February 2010

Declaration

To the best of my knowledge and belief this thesis contains no materials previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature : ……..

Date : February 19th, 2010

Dedication

This thesis is dedicated to

my husband, my children and my parents.

ABSTRACT

The purpose of this research was to synthesize novel macroporous thermosensitive hydrogels and to characterise the produced hydrogel materials for controlled drug delivery applications.

Twelve hydrogel polymers were synthesized based on the homo-, co- and/or ter-polymers of N-isopropylacrylamide (NIPAAm), 2-hydroxyethyl methacrylate (HEMA) and ethyl methacrylates (EMA). The polymers were produced in the presence of varying amounts of water so as to generate porous structures of the hydrogels through a phase separation polymerization process. *N*, *N'*-methylenebisacrylamide (mBAAm) was used as a crosslinking agent and ammonium persulfate (APS) was used an initiator.

The morphology of these hydrogels was examined using scanning electron microscopy (SEM). The porous structure of the hydrogels was future evaluated by the polymer volume fraction at the equilibrium state at various temperatures. The swelling properties of hydrogels were also studied. These include the equilibrium swelling ratio (ESR) and the normalised volume change at different temperatures, the swelling kinetics, and the deswelling kinetics.

Based on the porosities and the swelling properties, their responsiveness to the temperature changes, nine hydrogels were selected for the assessment of drug loading capacity and drug diffusion properties using a conventional anti-inflammatory drug, predinisolone 21-hemisuccinate sodium salt. The influence of temperature, porosity and drug concentrations on the drug loading capacity and diffusion kinetics was also investigated.

BRIEF BIOGRAPHY OF THE AUTHOR

Yuli Setiyorini graduated in Materials and Metallurgy Engineering of Sepuluh November Institute of Technology (ITS) Surabaya, Indonesia in 2003. She has three and a half years experience as a junior lecturer at ITS. She commenced her Master of Philosophy in January 2008 under the support of an Australian Development Scholarship (ADS).

Publication written in support of this thesis:

Conferences:

Setiyorini, Y., Li, C. and Lou, X., "*Controlled delivery of prednisolone derivatives using temperature sensitive hydrogel polymers*", in 19th Annual Conference of the Australasian Society for Biomaterials and Tissue Engineering (ASBTE) Sydney, Australia, January 21-23, 2009.

Setiyorini, Y. and Lou, X., "*Synthesis and characterization of porous poly(HEMA-co-NIPAAm) for controlled drug delivery"*, in International Conference on Materials for Advanced Technologies, Singapore, 28 June-3 July, 2009.

Setiyorini, Y and Lou, X., "*Macroporous Thermosensitive Hydrogels for Controlled Release of Prednisolone 21-Hemisuccinate Sodium Salt*", in 20th Annual Conference of the Australasian Society for Biomaterials and Tissue Engineering (ASBTE) Brisbane, Australia, February 10-12, 2010.

ACKNOWLEDGEMENT

I would like to deeply thank to God about everything that I received, especially opportunity to study in overseas. I thank to my thesis advisor Associate Professor Xia Lou for her patience and insight. I also thank to Professor Moses O. Tede for his encouragement and many help. I thank all staff at Chemical Engineering for supplying everythings.

I thank Elaine Miller for SEM examination and editing the thesis. Also, Karen, Zino and Aan for helped me a lot as well in laboratory of Chemical Engineering.

I thank my husband, daughter, sister Nurul and her family also my parents for all their care, support and encouragement. I thank my best friends Yenny and Chao for listening to me when I want to talk, giving me an advice when I need it, and more. I also thank for all my friends Fonny, Thu, Monica, Nadia, Depak and etc for their support during my study. Thank you for being with me.

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CHAPTER 1 INTRODUCTION

1.1. Controlled Drug Delivery

Controlled drug delivery (CDD) systems have made a significant impact in medical applications and have been developed and progressed further with the advancement of polymer science and engineering. The emphasis on the development of novel controlled drug delivery systems is in response to the discovery and production of new drug carrier devices. A major limitation in the pharmaceutical industry (conventional drug delivery) is that the current methods for drug delivery such as injections, tablets/pills, and sprays, are very ineffective for certain drugs and multiple administrations may be required to maintain the concentration of drug in the body at a therapeutically effective level for a prolonged period of time, causing inconvenience to the patient.

Another disadvantage of conventional drug delivery systems is that it is difficult to control the concentration of drug inside the body. The drug level may rise above the maximum desired therapeutic level, called overdosing, which leads to toxic side-effects, and fall off to a minimum value, called underdosing, which leads to lack of efficacy (Figure 1.1).

The objective of developing controlled drug delivery systems is to successfully engineer systems that can deliver the drug at a specified rate and within a particular time period. The desired drug release pattern from such devices is shown in Figure 1.1 with respect to rate and duration. Drug diffusion is also one of the drug release mechanisms. CDD occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner (Robinson and Lee, 1987), (Langer, 1998). The release of the active agent may be constant or cyclic over a long period, or it may be triggered by the environment or other external events. In any case, the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing (Chien, 1982).

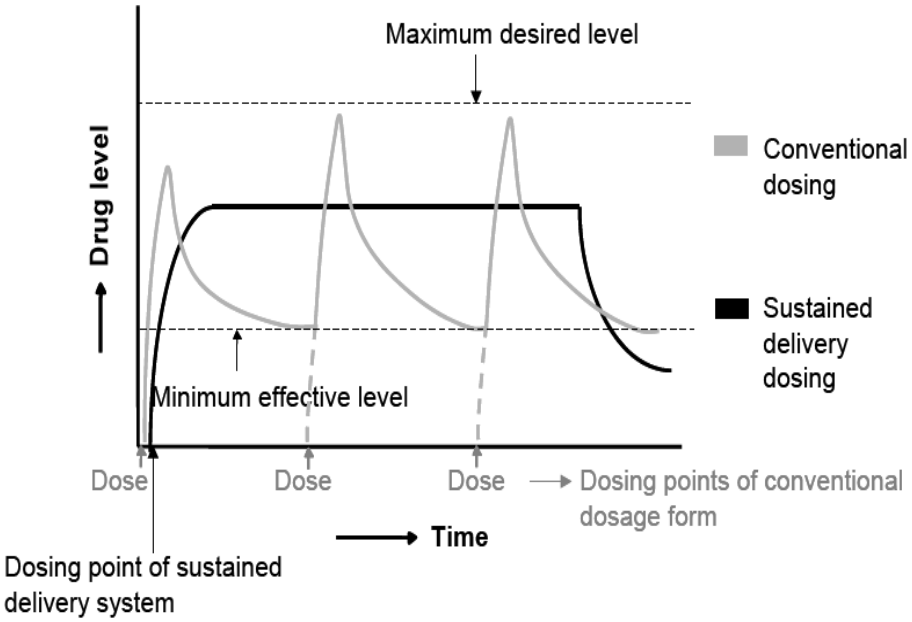


Figure 1.1 Conventional dosing versus controlled delivery dosing
(Adapted from: Chien, 1982)

CDD offers four principal advantages over conventional formulations that deliver all of the active agents over a short period of time including: more constant drug level, more efficient utilization of drug, localized delivery, and less frequent administration (Baker, 1987). There are many ways to classify the controlled drug delivery systems. The following systems are classified based on the mechanism by which the polymer controls the drug release from the device.

Diffusion Controlled Drug Delivery Systems

Diffusion is the most common mechanism controlling the release of drugs from hydrogel-based delivery systems, which is illustrated in Figure 1.2. In these systems, the drug release occurs by diffusion through the macromolecular mesh or through the water-filled pores. They are divided into two types: reservoir systems and matrix systems. A reservoir type consists of drugs within a polymeric matrix surrounded by a film or membrane (Mathiowitz, 1999), (Li and Jasti, 2006), (Chien, 1992). The drug is released due to the different drug concentrations between the reservoir and the environment. To maintain a constant release rate from the reservoir, the concentration difference must remain constant. This can be achieved by designing a

device that may contain excessive amounts of drugs. Under this condition, the solution inside the membrane will remain saturated (Galaev and Mattiasson, 2008). The release rate is dependent on the membrane thickness, area and permeability. Meanwhile, in matrix systems, the drugs are dissolved or dispersed throughout the polymer matrix. The drug release occurs by the diffusion of the drug or agent from the polymer (Ranade and Hollinger, 2004), (Chien, 1992).

Some diffusion controlled drug delivery systems are made of glassy polymers in which the drugs are dissolved and dispersed when water penetrates into the glassy polymer. They form a gel and the drugs are released through the swollen layer (Mathiowitz, 1999). The low permeability of such systems determines that they are more suitable to low molecular weight solutes (Ranade and Hollinger, 2004).

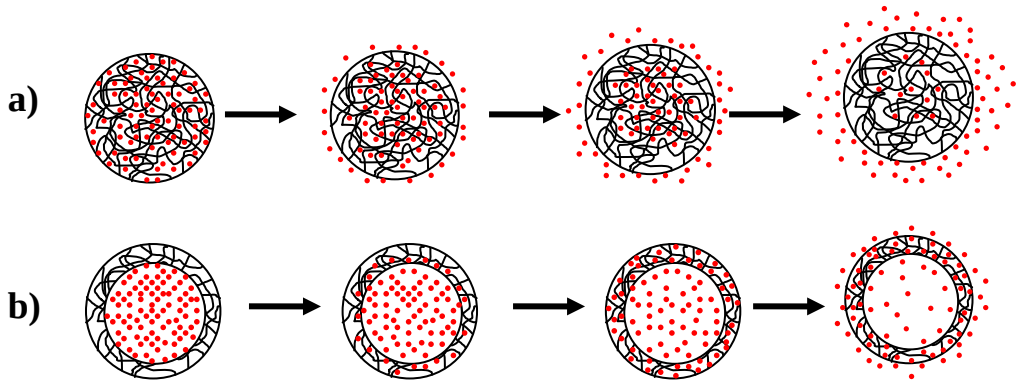


Figure 1.2 Diffusion controlled drug delivery systems: (a) matrix and (b) reservoir

Chemically Controlled Drug Delivery Systems

Chemically controlled systems are also divided into two groups: bioerodible or biodegradable systems and pendent-chain systems, which are shown in Figure 1.3. In biodegradable or erodible systems, the drug release occurs due to degradation or dissolution of the polymer (Mathiowitz, 1999), (Ranade and Hollinger, 2004). Meanwhile, in pendent chain systems, the drug molecules are chemically linked to the backbone of the polymer (Ranade and Hollinger, 2004). They are released from the polymer by hydrolysis or enzymatic degradation of the linkages (Mathiowitz, 1999).

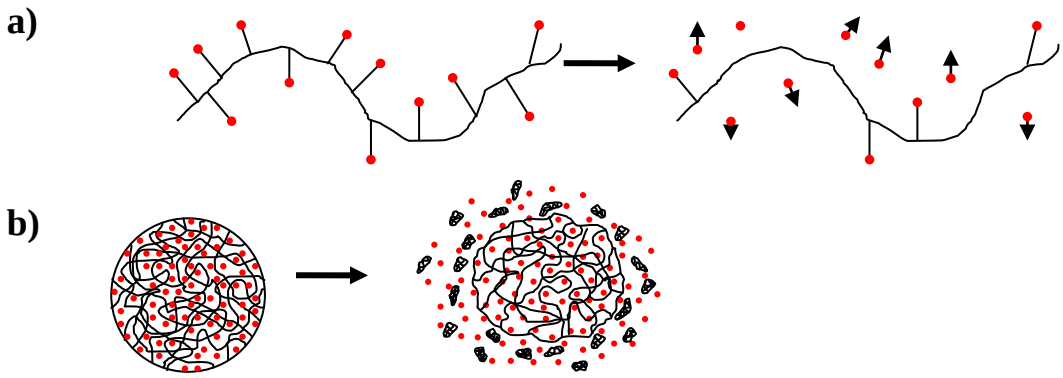


Figure 1.3 Chemically controlled drug delivery systems: (a) pendent-chain and (b) bioerodible

Environmentally Responsive Drug Delivery Systems

Environmentally responsive systems are physiologically responsive to changes in the external environment, as shown in Figure 1.4. The three most commonly studied environmentally sensitive systems are pH, temperature, and magnetic sensitive systems. For pH and temperature sensitive systems, the release of drugs is controlled by a critical pH or temperature value or range at which the swelling behaviour, network structure, permeability and mechanical strength of the materials change dramatically (Mathiowitz, 1999). The drug release is not only a function of time but also a function of the external conditions such as pH and temperature (Galaev and Mattiasson, 2008). In magnetic sensitive systems, the materials contain magnetic microbeads which are often made of polymer-coated iron oxide nanoparticles; the release of the drugs is controlled by the magnetic sensitivity of the materials (Ranade and Hollinger, 2004) and the magnetic field applied on the materials (Mathiowitz, 1999).

Other stimuli of environmentally responsive CDD systems include chemical species, ionic strength, enzyme-substrate, electrical, and ultrasound irradiation.

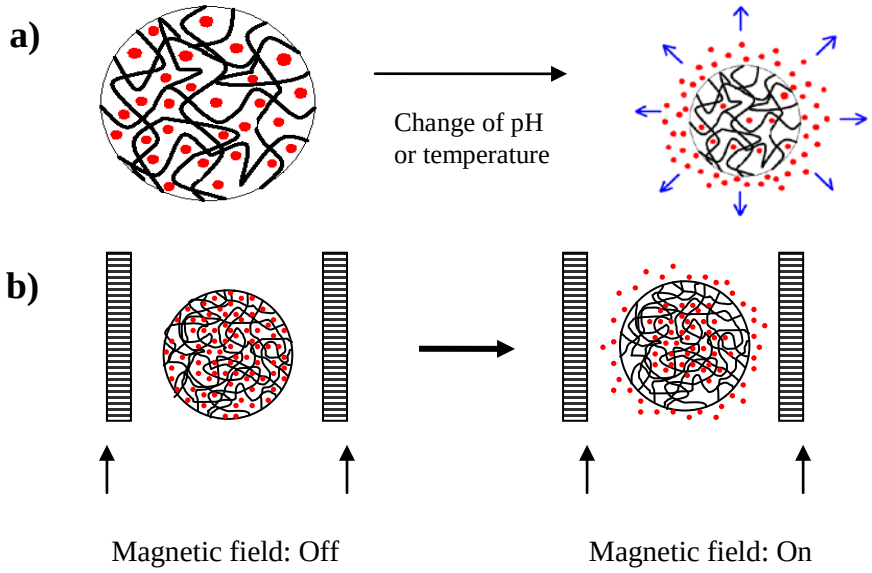


Figure 1.4 Environmentally responsive drug delivery systems: (a) pH and temperature sensitive and (b) magnetic sensitive

1.2. Hydrogels for Controlled Drug Delivery Applicatons

An ideal drug delivery system should be biodegradable, inert, biocompatible, mechanically suitable for intended purpose, comfortable for the patient, capable of high levels of drug loading, safe from pre-matured release, simple to administer and remove, and easy to fabricate and sterilize. Hydrogels meet with most of the requirements and have become one major class of polymers that has been identified for used in controlled release applications.

Hydrogels are three-dimensional hydrophilic polymer networks that are lightly crosslinked (Peppas and Mikos, 1986), (Peppas et al., 2000), (Hoare and Kohane, 2008), (Kopecek, 2007). The networks are composed of homopolymers or copolymers and are insoluble due to the presence of chemical or physical crosslinks. They can be made from virtually any water-soluble polymer, encompassing a wide range of chemical compositions and bulk properties. They can be formulated in a variety of physical forms (Hoffman, 2002), (Freudenberg et al., 2007), (Loh et al., 2008), (Liu, Chakma, and Feng, 2008) including solid moulded forms, pressed powder matrices, microparticles, coatings, membranes or encapsulated solids, films, nanoparticles and slabs. They have been widely used in such applications as artificial organs (Dai and Barbari, 2000), (Kobayashi, Toguchida, and Oka, 2003),

bioadhesives (Chung et al., 2008), biomolecules (Kanazawa et al., 2008), biosensors (Park et al., 2007), (Quinn, Connor, and Heller, 1997), biosynthetic bandages (Pratoomsoot et al., 2008), contact lenses (Uchida et al., 2003), (Kim, Conway, and Chauhan, 2008), soft tissues (Dalton and Shoichet, 2001), and scaffolds in tissue engineering (Kuo and Ma, 2001), (Flanagan et al., 2006), (Linnes, Ratner, and Giachelli, 2007), (Freier et al., 2005), (Park et al., 2005; Park et al., 2007).

Hydrogel polymers are particularly attractive for the development of controlled drug delivery systems due to their highly porous structure, biocompatiblility, deformability, inert surface, nontoxicity, high water contents, and in some cases being responsive to the external stimuli. Many variables are involved in determining the relationship between the amounts of drug administered and that ultimately available at the target sites of action. These include the polymer network permeability and the swelling behaviour that are strongly dependent on the chemical nature of the polymer(s) composing the gel as well as the structure and morphology of the network.

1.2.1. Thermosensitive Hydrogels

Of great interest in polymer therapeutics are hydrogel materials, which respond to environmental changes. These intelligent, stimuli-sensitive materials respond to, for example, physical (temperature, magnetic field), chemical (pH and ionic strength), and biochemical (enzyme) changes (Kopecek, 2003), (Hoffman and Stayton, 2004). These materials undergo large physical changes in properties in response to small changes in the surrounding environmental conditions. Hydrogel materials that are able to react to an environmental temperature change have been considered effective in many medical and drug delivery applications, bioseparation and diagnostics (Hoffman, 1987), (Kost and Langer, 2001), (Kopecek, 2003), (Coughlan, Quilty, and Corrigan, 2004). These materials show temperature dependence when swelling in water and they undergo phase transition at certain temperatures, which results in changes in conformation and hydrophilic-hydrophobic balance (Schmaljohann, 2006). The changes in temperature result in a change in polymer-polymer and water-polymer interactions of the hydrogels therefore leading to a change in the swelling

volume of the materials. The volume change process is called dehydration, when volume is reduced, or rehydration, when the volume is increased.

For positively thermoresponsive hydrogels, the volume increases at low temperature, because the hydrogen bonding between the hydrophilic segments of the polymer chain and water molecules is dominant, leading to enhanced dissolution in water (Qui and Park, 2001). Meanwhile, increasing the temperature causes partial displacement of water from the polymer chain, weakening the hydrogen bonds and increasing the size of the hydrophobic interactions segment of the polymer (Markvicheva et al., 1991). Consequently, the polymer network collapses because the intra- and intermolecular hydrogen bonds between the hydrophobic parts of the polymer molecules are favoured compared to the water molecules, which are reorganized around the non-polar polymers (Figure 1.5).

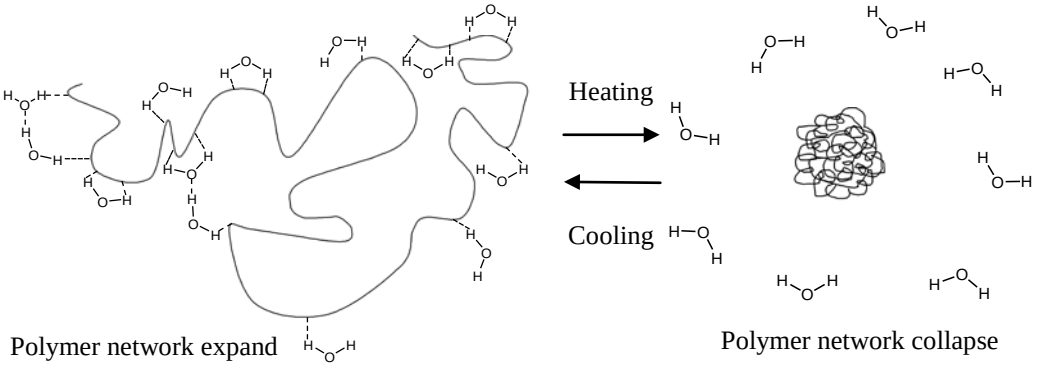


Figure 1.5 Effect of temperature on polymer-polymer and polymer–water interactions

On the other hand, negatively thermosensitive hydrogels swell at high temperature and deswell at low temperature, resulting in increased volume at high temperature and reduced volume at low temperature. The phase transition temperature of negatively thermosensitive hydrogels is called upper critical solution temperature (UCST) and the phase transition temperature of positively thermosensitive hydrogels is called lower critical solution temperature (LCST). It should be noted that both UCST and LCST are occur for linear polymers that are thermally sensitive. When polymers are crosslinked, as seen in CDD applications, another term, volume phase

transition temperature (VPTT) is often used, representing the temperature at which the rapid volume change occurs.

Positively thermosensitive hydrogels are extensively studied for drug delivery applications using temperature as a trigger to control the release of drugs. Some positively thermosensitive hydrogel polymers and their LCSTs are listed in Table 1.1.

Table 1.1 Examples of positively thermosensitive polymers and their LCSTs

Polymer	LCST °C
Poly (N-isopropylacrylamide), PNIPAAm	~32
Poly(vinyl methyl ether), PVME	~40
Poly(ethylene glycol), PEG	~120
Poly(propylene glycol), PPG	~50
Poly(methacrylic acid), PMAA	~75
Poly(vinyl alcohol), PVA	~125
Poly(vinyl methyl oxazolidone), PVMO	~65
Poly(vinylpyrrolidone), PVP	~160
Poly(silamine)	~37
Methylcellulose, MC	~80
Hydroxypropylcellulose, HPC	~55
Polyphosphazene derivatives	33-100
Poly(N-vinylcaprolactam)	~30
Poly(siloxyethylene glycol)	10-60

1.2.2. Positively Thermosensitive Hydrogels for Controlled Drug Delivery

Drug release from positively thermosensitive hydrogels can occur either below or above the LCST, when the polymer is either in a swollen or collapsed state (Figure 1.6). Above the LCST, the net effect of drug release will be a function of

thermosensitivity (deswelling) and the increased drug diffusivity as the temperature rises (Bae et al., 1987). Usually diffusion of the drug from a thermosensitive hydrogel diminishes when the temperature rises, as water uptake is inhibited because of the collapsed structure, whereas at lower temperatures drug diffusion out of the hydrated and more porous structure network is increased (Alvarez-Lorenzo et al., 2005). The inhibited release of the drug at higher temperatures can also be explained by the formation of a dense, less permeable layer on the surface of the hydrogel, which is formed due to the faster collapse of the surface of the hydrogel compared to the interior, upon temperature rise (Bromberg and Ron, 1998).

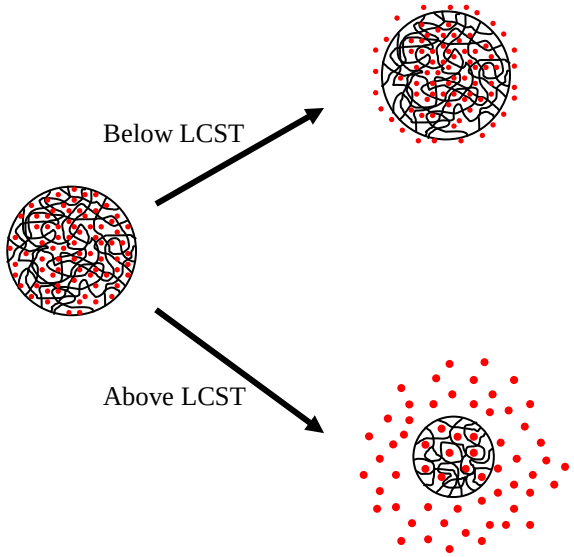


Figure 1.6 Effect of temperature on drug release from a positively thermosensitive hydrogel

On the other hand, this surface layer could also build up a hydrostatic pressure inside the hydrogel, which would eventually squeeze out the drug. The on-off release by altering the heating and cooling has been achieved with thermosensitive materials, and the release rate of drug for the hydrophobic model has been related to the amount of drug in the matrix, the solubility of drug in the polymer, the hydration of the polymer gel, and the swelling and deswelling kinetics of the polymer (Bae et al., 1987). The size of drug molecules also affect the release, as smaller molecules are known to release better than the larger ones. In the case of LCST, the diffusion is

stated to be affected also by physico-chemical properties such as porosity and tortuosity, as the increased temperature reduces drug release due to the membrane shrinking (Park and Hoffman, 1989).

1.2.3. Poly(N-isopropylacrylamide) Hydrogels

Poly(N-isopropylacrylamide is made of monomer *N*-isopropylacrylamide (NIPAAm). It is one of the most well-known thermoresponsive polymers that have been studied and researched for controlled drug delivery applications. Homopolymers of NIPAAm exhibit a lower critical solution temperature (LCST), around 32°C, that is associated with phase behaviour change in aqueous systems ascribed to alterations in the hydrogen-bonding interactions of the amide group. Below LCST, pNIPAAm absorbs and retains a large amount of water; whilst above 32°C, pNIPAAm shrinks to a dense state and loses much water. The chemical structures of NIPAAm and pNIPAAm are depicted in Figure 1.7.

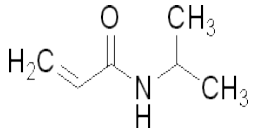
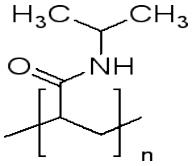
NIPAAm	pNIPAAm
	

Figure 1.7 Chemical structures of NIPAAm and pNIPAAm

As mentioned before, the LCST of a temperature responsive polymer is influenced by hydrophobic or hydrophilic moieties in its molecular chains. In general, LCST can be adjusted with different co-monomers (Taylor and Cerankowski, 1975), (Feil et al., 1993). To increase the LCST of pNIPAAm, a small ratio of hydrophilic monomers must be incorporated into the polymer structure (Shibayama and Tanaka, 1993). In contrast, incorporation of a small amount of hydrophobic constituent can decrease the LCST of NIPAAm and increase its temperature sensitivity (Chen and Hoffman, 1995). More hydrophilic monomers such as acrylamide would make the LCST increase and even disappear, and more hydrophobic monomers such as *N*-butyl acrylamide would induce the LCST to decrease (Hoffman et al., 2000).

Adjustment of LCST near body temperature is essential, especially for drug delivery applications.

Others factors can be used to adjust the LCST. Salts are known to lower the LCST of NIPAAm (Schild, 1990), (Eeckman, Amighi, and Moes, 2001; Eeckman, Moes, and Amighi, 2002). As the salt concentration of the solution increases the LCST decreases, as more ions compete for H-bonding and hydrophobic interaction become available. The presence of proteins such as insulin and bovine serum albumin has been found to increase the LCST of NIPAAm, because of the increased hydrophilicity of the polymer-protein complex. Certain surfactants have been found to either decrease or increase the LCST of NIPAAm depending on their hydrophobic chain length and the concentration (Eeckman, Amighi, and Moes, 2001).

There also are many other factors affecting the physical properties of pNIPAAm. These include the varieties and concentrations of monomer, cross-linking agent, initiator, accelerator and solvent, as well as temperature of the polymerization process. The cross-linking agent plays a pivotal role in polymerization, because it will affect the porosity of the network. By increasing crosslinker content above 5 wt%, the swelling ratio, the deswelling response rate, and the total amount of lost water are decreased (Dogu and Okay, 2006). However, it will increase gel inhomogeneity, (Shibayama et al., 1998), (Kizilay and Okay, 2003), and mechanical properties, with much less deformation (Sayil and Okay, 2001). Temperature is also a critical factor for polymer preparation because it affects the swelling rate of pNIPAAm. For example, pNIPAAM prepared at ambient temperature swells much faster than when prepared at a temperature below or above ambient (Sayil and Okay, 2001, 2002). Another important factor is the concentration of monomer. It has been reported by Sayil and Okay that the concentration of NIPAAm at 5 w/v% results in a weak network structure (Sayil and Okay, 2002).

1.2.4. PNIPAAm for Controlled Drug Delivery

PNIPAAm is one of the most well-known thermoresponsive polymers that have been studied and researched for controlled drug delivery applications. Numerous reviews have reported current progress in the research and development of pNIPAAm as

CDD systems. These include (Khairuzzaman, 2009), (Mano, 2008), (Li, Wang, and Wang, 2006), (Kanazawa, 2004), (Kim, 1996), (Cole et al., 2009), (Jeong, Kim, and Bae, 2002), (Bajpai et al., 2008), (Kopecek, 2007), (Satish, Satish, and Shivakumar, 2006). The major challenges for utilizing this type of material include LCST, drug transportation, porosity, toxicity, biocompatibility and thermosensitivity.

Much research has been conducted to improve the drug release properties of pNIPAAm, so that the LSCT of the polymer can be adjusted to a preferred temperature such as body temperature, 37°C (Cheng, Zhang, and Zhuo, 2003); (Jennifer et al., 2008), (Kim and Lee, 2009), (Zhao et al., 2009), (Zhang and Misra, 2007), (Liu, Tong, and Yang, 2005). Various monomers and polymerisation methods have been used to modify the polymer's porous structure so that the loading and release of various drugs can be well controlled.

Porous thermosensitive hydrogels allow aqueous loading of large size bioactive agents such as protein, protecting the drug from a hostile environment (Ramkissoon-Ganorkar et al., 1999) and can modulate drug release in response to temperature changes, (Zhang, Huang, and Zhuo, 2004), therefore attracting great attention from many researchers (Zhao et al., 2009), (Zhang et al., 2004), (Zhao et al., 2008), (Safrany, 2005), (Wu, Hoffman, and Yager, 1992), (Cheng, Zhang, and Zhuo, 2003). Porous structure has also been used as a parameter to adjust the temperature response rate of hydrogels since the absorption and desorption of water through the interconnected pores are mainly by convection, which is much faster than a diffusion process.

Many methods can be used to create a porous structure in the resultant hydrogels, for example, adjusting the monomer ratio, the type and concentration of a crosslinking agent or initiator, and changing the polymerisation temperature (Galaev and Mattiasson, 2008). Among all methods investigated, polymerising a monomer mixture in the presence of a diluent has proved to be most effective in altering the porous structure of a hydrogel by phase separation (Okay, 2000). This process involves phase separation of polymer chains from the mixture of the diluent and the polymerisation ingredients, leading to the formation of porous hydrogel networks. Depending on the synthesis parameters in polymerization, the resultant pores can be varying on a macroscale or a microscale (see more details in Chapter 2).

Although water is the most commonly used diluent for phase separation polymerisation, others diluents have also been investigated for the preparation of fast responsive macroporous pNIPAAm. These include hydroxypropyl cellulose (Wu, Hoffman, and Yager, 1992), acetone (Zhang, Zhuo, and Yang, 2002), 1,4-dioxane (Zhang, Huang, and Zhuo, 2004), sucrose (Zhang, 2003), silica particles (Serizawa, Wakita, and Akashi, 2002), inorganic salts (Cheng, Zhang, and Zhuo, 2003), surfactant (Zhao et al., 2009), and poly(ethylene glycol)/PEG (Dogu and Okay, 2006); (Cicek and Tuncel, 1998), (Zhang and Zhuo, 2000), (Cheng, Zhang, and Zhuo, 2003), (Zhang, 2001).

1.3. Materials and Methodology

Co-monomers, HEMA and EMA, will be used together with NIPAAm for the purpose of altering the pore structure, pore volume and phase change temperature. The chemical structure of both monomers and their homopolymers are presented in Figures 1.8 and 1.9.

Polymers of HEMA, poly(2-hydroxyethyl methacrylates), or pHEMA is most well known for its applications in the manufacture of contact and intraocular lenses (Opdahl et al., 2003). In most of these applications, pHEMA hydrogels are non-porous.

HEMA **pHEMA**

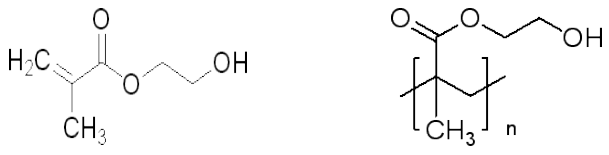


Figure 1.8 Chemical structures of HEMA and PHEMA

Macroporous pHEMA hydrogels can be produced in the presence of varying amounts of water that are higher than the equilibrium capacity of the hydrogel (Wichterle and Lim, 1960). They are a unique type of hydrogel with high water

content and interconnected pores ranging from a few to hundreds of micrometers. These hydrogels often appear opaque or translucent due to the presence of macropores in the materials (Chen, Chirila, and Russo, 1993) which make them chemically identical, but structurally distinctive to the transparent and homogeneous type of pHEMA that are commonly used for applications in which a combination of optical clarity and limited diffusive characteristics is required, such as contact lenses and intraocular lenses (Oxley et al., 1993).

An application of macroporous pHEMA hydrogels has been demonstrated in the novel design of an artificial cornea and an orbit implant in which the porous pHEMA component allows host cells and tissue to grow into the device, thereby preventing extrusion of the implants (Chirila et al., 1998), (Hicks et al., 2006). Their applications as ophthalmic drug delivery systems also have been reported (Hicks, Crawford et al., 2002). The results have shown that macroporous pHEMA hydrogels represent a significant advance over the non-porous types, with a much higher drug loading capacity (Lou, Munro, and Wang, 2004), (Lou, Wang, and Tan., 2007). The loading of drugs can be achieved in ambient conditions through very simple means with less concern about the drug's stability (Wang et al., 2010). The release rate of prednisolone 21 hemisuccinate sodium salt from marocporous pHEMA is comparable to that from the less porous type. As the materials are soft in nature, an improved patient compliance is also expected (for eye patients) (Hicks, Crawford et al., 2002).

Polymers of EMA, poly(ethylmethacrylate) (pEMA), is a hydrophobic polymer. It is used in this study to increase the temperature responsiveness of the hydrogels.

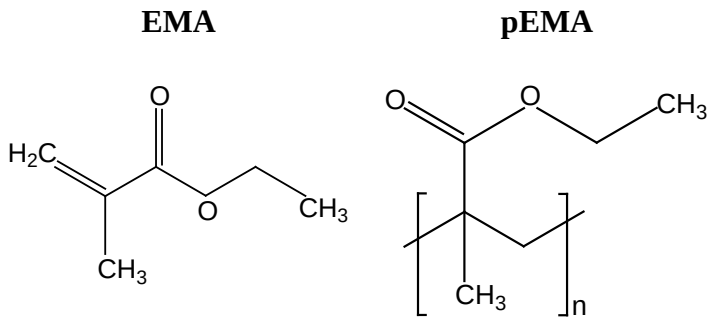


Figure 1.9 Chemical structures of EMA and pEMA

1.4. Aims and Objectives of the Project

This study aims to synthesize novel macroporous thermosensitive hydrogels in the presence of NIPAAm and two co-monomers, 2-hydroxyethyl methacrylate (HEMA) and ethyl methacrylates (EMA), and to characterise the produced hydrogel materials for possible applications as controlled drug delivery systems. Homopolymers, copolymers and terpolymers of the above three monomers will be prepared in the presence of water to generate the porous structure. A conventional anti-inflammatory drug, predinisolone 21 hemisuccinate sodium salt, will be used as a model drug. The monomer-water ratio and the monomer-monomer ratio will be changed to create different porous structures. The hydrogel morphology, the swelling/deswelling kinetics and the equilibrium swelling ratio will be studied. The polymer volume fraction which indicates the porosity of the hydrogel polymers also will be investigated. The temperature dependence of these parameters, as well as the drug loading capacity and drug diffusion properties of selected hydrogels, will be examined. Ultimately, we hope to establish some insight and understanding of the drug release properties of the systems in relation to their chemical structure, the porosity and other physical properties.

To achieve the objective of this research, the following experiments will be carried out:

1. Synthesis of homo-, co- and terpolymers of HEMA, NIPAAm and EMA in the presence of varying amounts of water.

2. Characterisation of the morphology, swelling ratio and polymer volume fraction of the produced hydrogel at various temperatures.

3. Analysis of changes in swelling and deswelling kinetics, as well as in equilibrium volumes against temperature.

4. Investigation of drug loading capacity and drug diffusion kinetics and the effect of different materials (porosity), temperature and drug concentration on the drug loading and diffusion properties.

CHAPTER 2 SYNTHESIS AND CHARACTERIZATION OF HYDROGELS

2.1. Introduction

This chapter will present and discuss the synthesis and the morphological characteristics of macroporous thermosensitive hydrogels that are produced by free-radical polymerisation and examined by SEM. This study will contribute to the knowledge of the synthesis parameters, in particular the diluent and monomer ratios and their effect on the macroporous structure of the hydogels.

Macroporous copolymer networks form as a result of phase separation during the free-radical polymerisation of the monomer and crosslinking in the presence of an inert diluent. Various porous structures can be obtained during or after the polymerisation process by changing the synthetic conditions such as the extent of polymer interaction, the amount of crosslinking agent and diluent, as well as by the initiator concentration and polymerisation temperature. Depending on these synthesis parameters, phase separation takes place on a macrosyneresis or microsyneresis scale (Dusek, 1971).

In phase separation theory as explained by Dusek (1971), in the absence of a diluent, if a small amount of crosslinking agent is used in the network synthesis, an inhomogeneous gel structure is obtained. The gels formed by free-radicals using crosslinking are always inhomogeneous due to the fact that the crosslinking agent has at least two functional groups and, therefore, if one assumes equal functional group reactivity, the reactivity of the crosslinking is twice that of the monomer. As a consequence, the crosslinking molecules are incorporated into the growing copolymer chains much more rapidly than the monomer molecules, so that the final network exhibits a crosslinking density distribution. If a good solvent is included in the free-radical polymersisation, the solvent acts as a diluent and the gel obtained will expand its structure. If the amount of crosslinking in the reaction is increased while the amount of the diluent remains constant, it will result in the formation of a highly crosslinked network which cannot absorb all the diluent molecules present in the reaction. As a result, a phase separation occurs, leading to the formation of

microporous structure, which is shown in Figure 2.1.a. The figure shows the microsyneresis with the blue area as liquid phase and the yellow area as polymer phase. According to the macrosyneresis model, the growing gel collapses at a critical point for phase separation and becomes a microgel, whereas the separated liquid remains as a continuous phase (Figure 2.1.a). The microgels are continuously generated due to the successive separation of the growing polymer. This will produce the formation of a heterogeneous gel which consists of two phases: a gel and a diluent phase. Furthermore, if the amount of diluent is increased, a critical point is passed, causing the system to become discontinuous because the amount of monomer is not sufficient and the growing chains cannot occupy the entire available volume. Increasing the amount of diluent decreases the size of the gel particles and, finally, they are as small as ordinary macromolecules (Figure 2.1.b).

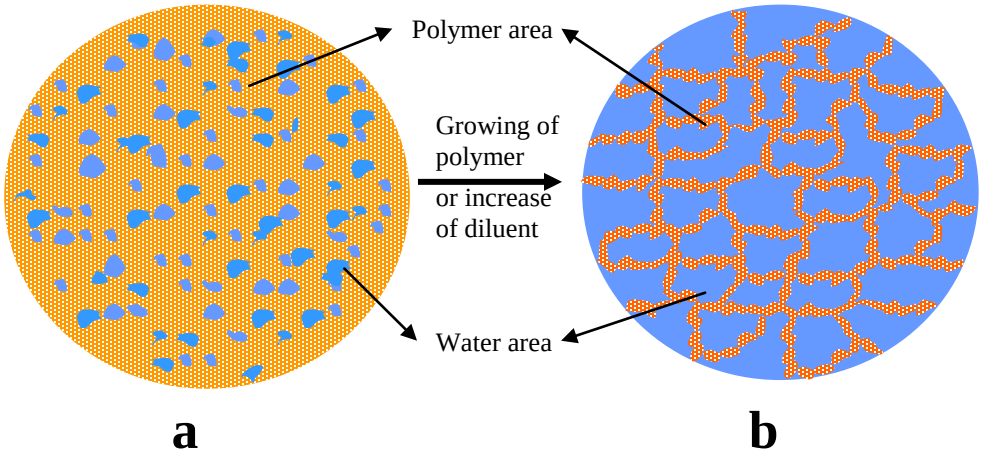


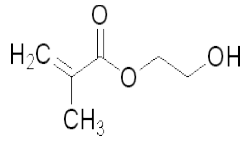
Figure 2.1 Formation of: (a) microporous structure due to the high crosslink and (b) macroporous structure due to the high level of diluents

Several diluents, soluble in the monomer mixture, were reported as inert diluents that produced a hydrophilic macroporous polymer (Chen, Chirila, and Russo, 1993), (Shea et al., 1990). These include water molecules. By varying the amount of water in the crosslinking polymerisation of HEMA, a large number of pores can be attained in the final polymer network (Chen, Chirila, and Russo, 1993). The greater amount of polar solvent (water, methanol) produces materials with higher specific surface area and internal pore volume. Also, decreased crosslinking flexibility may increase

the porosity of a network (Shea et al., 1990). Other parameters also may influence the porous structure of the materials. These include temperature and initiators (Okay, 2000).

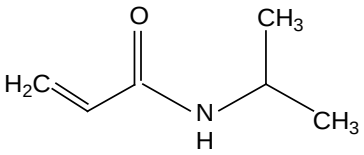
In this study, water will be used as the diluent. HEMA, NIPAAm, and EMA will be used either alone or together in the polymerisation process. *N*, *N'*-methylenebisacrylamide (mBAAm) will be used as a crosslinking agent which is hydrophobic in nature. Ammonium persulfate (APS), will be used together with *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (TEMED) as initiators. Copolymers, poly(HEMA-NIPAAm), as well as, terpolymers, poly(EMA-HEMA-NIPAAm), will be synthesized according to the synthetic schemes which are presented in Figure 2.2. The amounts of crosslinking agent and initiator will be fixed. However, the effects of the water to monomer ratios and monomer to monomer ratios on the morphology of the resultant hydrogel will be investigated.

a



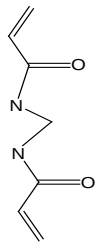
2-Hydroxyethyl methacrylate

+

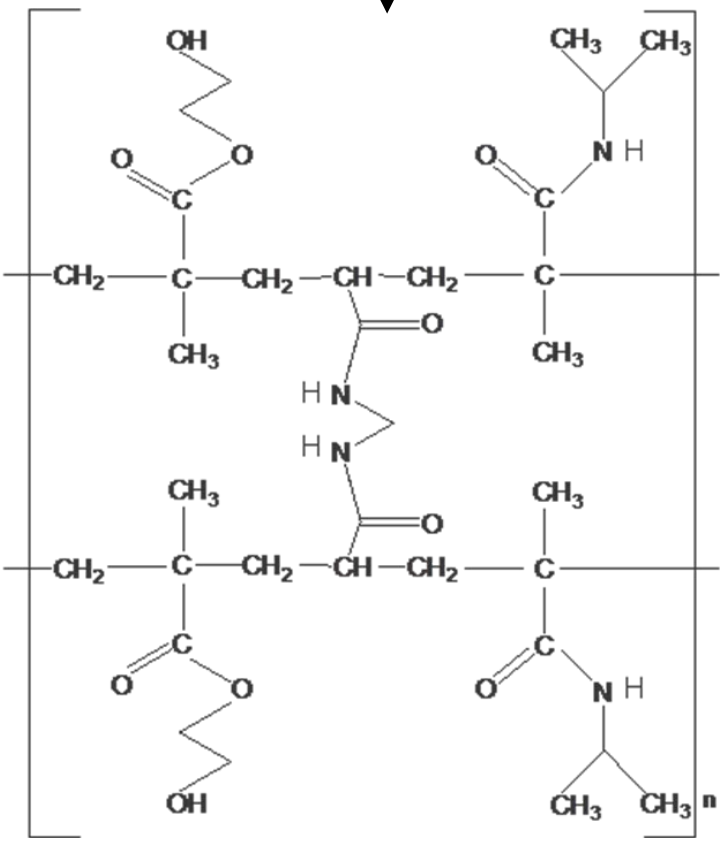


N-isopropylacrylamide

APS/TEMED

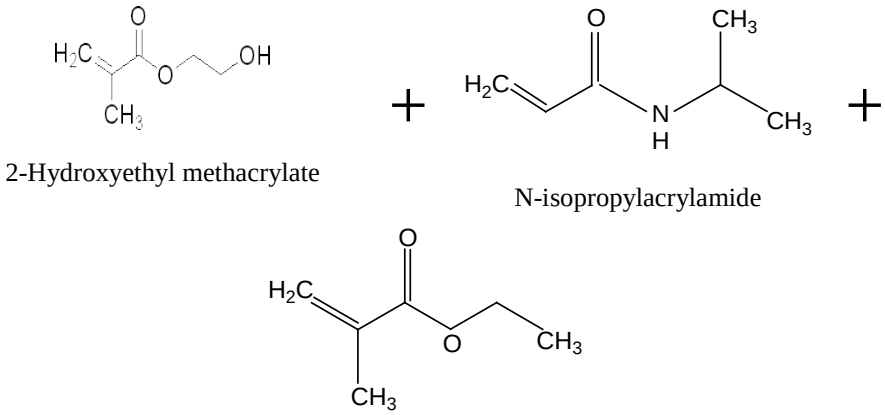


mBAAm



poly(HEMA-NIPAAm)

b



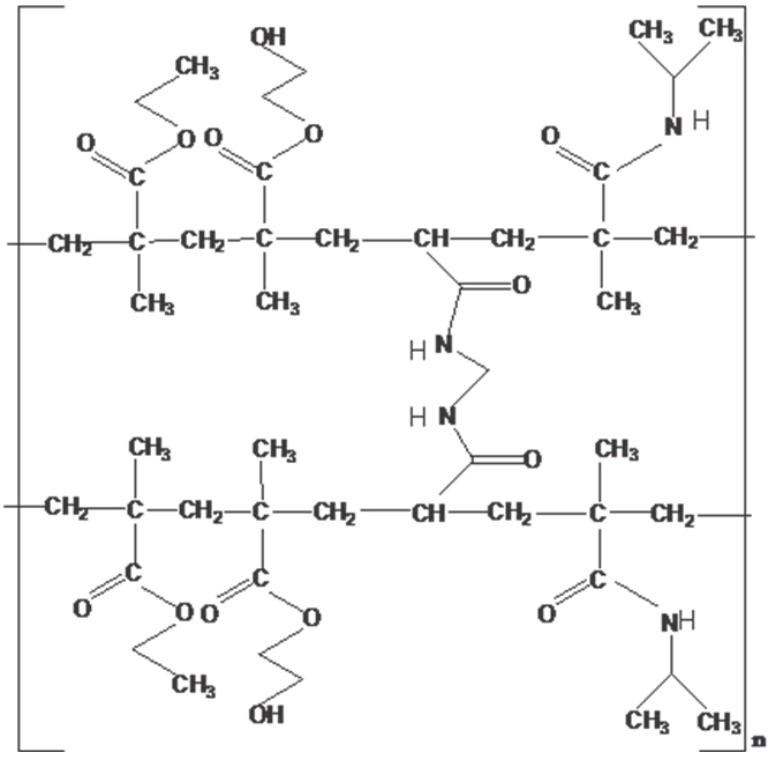
2-Hydroxyethyl methacrylate

N-isopropylacrylamide

Ethyl methacrylate

APS/TEMED

mBAAm



poly(EMA-HEMA-NIPAAm)

Figure 2.2 Synthetic scheme of: (a) poly(HEMA-NIPAAm) and (b) poly(EMA-HEMA-NIPAAm)

2.2. Materials and Methods

2.2.1. Chemicals

Ophthalmic grade 2-hydroxyethyl methacrylate was purchased from Bimax Inc USA and was used as received. Monomer N-isopropylacrylamide (97%), ethyl methacrylate was supplied from Polyscience, crosslinking agent *N, N'*-methylenebisacrylamide (99%) and initiators, ammonium persulfate (98%) and *N,N,N',N'*-tetramethylethylenediamine (99.5%), were supplied by Sigma-Aldrich Co, Australia, and used as received. Deionised water was used for all experiments in this study.

2.2.2. Preparation of Hydrogels

HEMA, NIPAAm, EMA and mBAAm were mixed with water according to the formulae listed in Table 2.1. The solution was purged with nitrogen gas for 20min prior to the addition of appropriate amounts of the APS solution and TEMED. The monomer mixtures were then dispensed into a mould to produce hydrogel discs for morphological examinations. (Some were used for swelling behaviour and drug loading capacity measurements - see Chapter 3 for details). Hydrogel membranes of 1-2mm thickness were also cast for drug diffusion experiments (Chapter 4). The detailed casting methods are given below. The preparation of hydrogel was done at room temperature.

For hydrogel discs, 10g of monomer mixture were prepared for each formulation. The monomer mixtures were distributed equally, 1mL in each well, in a Perspex mould which is shown in the figure below. The diameter of each button was 15.66 ± 0.01mm.

Figure 2.3 The Perspex mould which was used in the casting of discs

In order to cast a batch of membranes, 20g of monomer mixture were prepared for each formulation. The monomer mixtures were poured into a glass mould, which is separated by silicon rubber gasket. It is shown in the figure below. The thickness of each membrane was 1mm.

Figure 2.4 The glass mould which was used in the casting of membranes

All samples were cured at room temperature for 24h. After the curing process, the samples were removed from the moulds and stored in deionised water, with daily water exchange for 2 weeks to remove residual monomers and impurities. Samples

for morphological examination and drug loading characterisation were freeze-dried prior to the measurements. Others were kept in deionised water for further processing.

2.2.3. SEM Examination of Hydrogels

The cross-sectioned swollen hydrogel samples, after reaching their maximum ratio in deionized water at room temperature, were quickly transferred to a freezer at -40°C for overnight cooling then freeze-dried for 2 days until all water was sublimed to reveal the pore morphology.

The freeze-dried hydrogels were then coated with gold and examined using a SEM (Scanning Electron Microscopy) (Zeiss EVO 40XVP, Germany) at 10 kV.

2.3. Results and Discussion

2.3.1. Preparation of Hydrogels

Three homopolymers (20HEMA, 30HEMA and 20NIPAAm) were prepared as control hydrogels. Six copolymers (5HEMA15NIPAAm, 10HEMA10NIPAAm, 15HEMA5NIPAAm, 10HEMA20NIPAAm, 15HEMA15NIPAAm and 20HEMA10NIPAAm) and two terpolymers (1.25EMA2.5HEMA15NIPAAm and 2.5EMA5HEMA20NIPAAm) were prepared for this study. The chemical composition of these materials is summarised in Table 2.1. Numerical parts of the sample codes represent the weight percentages of monomers used in the polymerisation. For instance, 5HEMA15NIPAAm represents a polymer that was produced from 5wt% HEMA, 15wt% NIPAAm and 80wt% water. Meanwhile, 2.5EMA5HEMA10NIPAAm represent a polymer that was produced from 2.5wt%EMA, 5wt% HEMA, 10wt% NIPAA, and 82.5wt% water.

All polymers appeared opaque when they were fully hydrated, which is shown in Figure 2.5. This indicates a porous polymer network. The formation of the porous structure of these materials was a result of phase separation, since water was used as the diluent for polymerisation. The effect of the amount of water on the resultant

porous structures of the hydrogels is displayed and discussed in the following section.

Figure 2.5 Sample hydrogel disc and membrane

2.3.2. Morphology of Hydrogels

Figure 2.6, Figure 2.7, and Figure 2.8 show the interior morphologies of freeze-dried hydrogels. Copolymer hydrogels made from 80wt% water (Figure 2.6) were more porous than those made from 70wt% water (Figure 2.7), indicating a clear influence of the water content upon the phase separation process (Chen, Chirila, and Russo, 1993), (Okay, 2000), (Dusek, 1971). The same trend was observed in terpolymer hydrogels, i.e., the polymers made from higher water content displayed greater pore size. Pores and the polymer textures were well defined and evenly distributed in 20HEMA, 30HEMA, 15HEMA15NIPAAm and 10HEMA20NIPAAm, but more random in others. Polymer textures in 15HEMA5NIPAAm were quite different from all others.

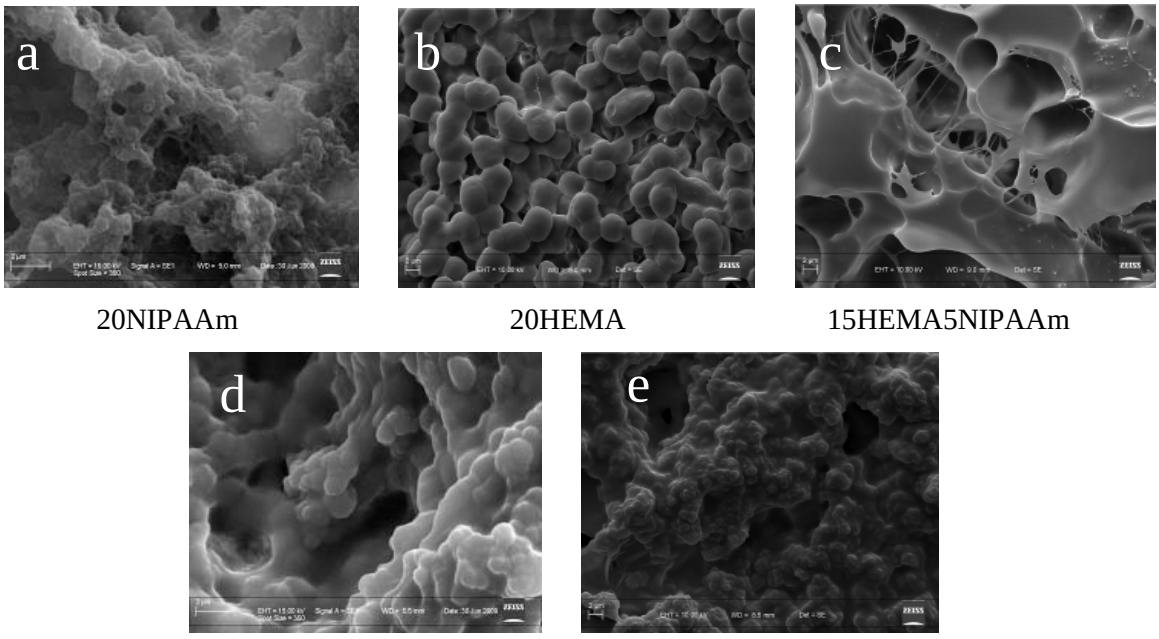
These results can be well explained using the phase separation theory discussed in the previous section (Dusek, 1971). When the hydrogels were produced with 80wt% water, monomers first reacted to each other and/or reacted with the crosslinker until passing the critical point at which the amount of the unreacted monomers was not sufficient to dissolve the growing polymer chains and the system became discontinuous, i.e. the phase separation occurred and pores formed. When less water (70wt%) was used, the phase separation was slightly delayed due to the presence of a

relatively larger amount of monomer, therefore, the formation of smaller pores and smaller polymer particles. When the amount of diluent was increased to 80wt%, a critical point was passed which caused the structure to become discontinuous due to the amount of monomer being insufficient, thus the growing chains could not occupy the entire available volume. Increasing the amount of diluent decreases the size of the polymer phase, and finally they are as small as ordinary macromolecules, then the liquid phase area is increased which results in an interconnected pore structure network(Okay, 2000), (Dusek, 1971).

As demonstrated by the above results, increasing the concentration of monomer HEMA in copolymer hydrogels produced an interconnected pore structure. However, increasing the concentration of NIPAAm led to an increase in the pore size because the polymerisation was initiated by the decomposition of APS and TEMED molecules, so the primary radicals that formed started to grow with the involvement of the monomers and the crosslinking agent. Initially, the primary polymer chains contained HEMA, NIPAAm, and mBAAm. As time went on, more and more primary molecules formed so that the intermolecular crosslinking reaction between the primary polymer chains also could occur. The solubility of NIPAAm is less than HEMA because it has two moieties, hydrophilic and hydrophobic, which probably induced the phase separation more quickly. In addition, incorporating EMA as a hydrophobic component into the hydrogel caused the terpolymers to become even more porous. This is probably due to quicker phase-separation, induced by the presence of EMA which has low solubility in water.

Table 2.1 Chemical composition of macroporous thermosensitive hydrogels

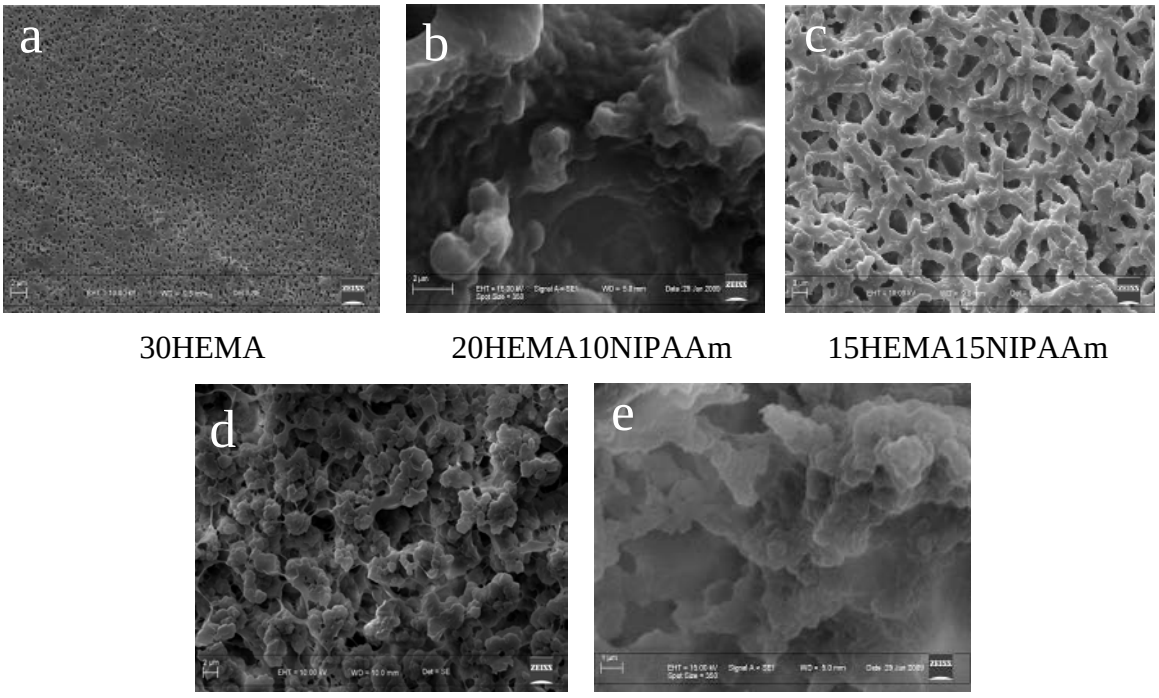
Sample Codes	HEMA	NIPAAm	EMA	mBAAm	Water	APS (25 wt %)	TEMED
	mmol	mmol	mmol	mmol	g	µl	µl
20NIPAAm	0	17.7	0	0.65	8	50	20
5HEMA15NIPAAm	3.8	13.3	0	0.63	8	50	20
10HEMA10NIPAAm	7.7	8.8	0	0.61	8	50	20
15HEMA5NIPAAm	11.5	4.4	0	0.58	8	50	20
20HEMA	15.4	0	0	0.57	8	50	20
30HEMA	23.1	0	0	0.85	7	75	30
20HEMA10NIPAAm	15.4	8.8	0	0.89	7	75	30
15HEMA15NIPAAm	11.5	13.3	0	0.91	7	75	30
10HEMA20NIPAAm	7.7	17.7	0	0.93	7	75	30
1.25EMA2.5HEMA15NIPAAm	1.9	13.3	1.1	0.60	8	50	20
2.5EMA5HEMA10NIPAAm	3.8	8.8	2.2	0.54	8	50	20
2.5EMA5HEMA20NIPAAm	3.8	17.7	2.2	0.87	7	75	30



20NIPAAm 20HEMA 15HEMA5NIPAAm

10HEMA-10NIPAAm 5HEMA15NIPAAm

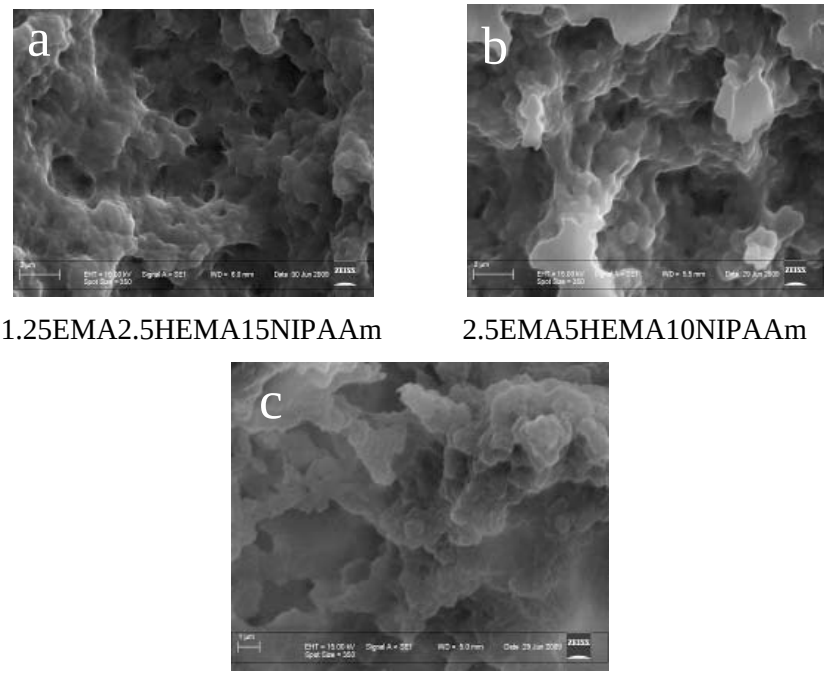
Figure 2.6 Interior morphology of copolymer hydrogels made from 80wt% of water



30HEMA 20HEMA10NIPAAm 15HEMA15NIPAAm

10HEMA20NIPAAm 2.5EMA5HEMA20NIPAAm

Figure 2.7 Interior morphology of copolymer hydrogels made from 70wt% of water



1.25EMA2.5HEMA15NIPAAm 2.5EMA5HEMA10NIPAAm

2.5EMA5HEMA20NIPAAm

Figure 2.8 Interior morphology of terpolymer hydrogels

2.3.3. Conclusions

In this study, twelve hydrogel polymers were synthesized. SEM examination indicated the presence of macroporous structure in all hydrogels. The porous structure of the resultant hydrogels was largely dependent on the amount of water used in the polymerisation process. The interior morphology of these materials also was affected by the ratio of monomers used in the polymerisation. In general, the higher the water content the more porous was the structure. Both copolymers and terpolymers showed greater pore size and denser polymer textures than the homopolymers. Increasing NIPAAm content has resulted in increased pore sizes and extended connection of the polymer skeleton, whilst the presence of a small amount of hydrophobic EMA had more effect on the pore sizes. Overall, the results from this study demonstrated that the porous structure of the hydrogel polymers can be well-tuned by simply adjusting the chemical composition in the formula.

CHAPTER 3 SWELLING PROPERTIES

3.1. Introduction

The aim of the experiment was to be presented in this chapter is to study the influence of water content, the monomer ratio, and the temperature upon swelling characteristics of hydrogels based on copolymers poly(HEMA-NIPAAm) and terpolymers poly(EMA-HEMA-NIPAAm). This study will contribute to the knowledge of the behaviour and properties of these materials, both in equilibrium and dynamic state, and their responses to the change of temperature. The information collated will be used to determine the formulations for further studies on the loading capacity and sustained release properties of the model drug from the hydrogel matrices.

Thermosensitive hydrogels may respond to the temperature changes negatively or positively. The response can be reversible or irreversible depending upon the properties of the hydrogel polymers. Negatively thermosensitive hydrogels deswell when the temperature decreases and swell when the temperature increases. Some negatively thermosensitive hydrogels include those made of poly(acrylic acid) and polyacrylamide (PAAm) or poly(acrylamide-co-butyl methacrylate). On the other hand, positively thermosensitive hydrogels swell at low temperature and deswell at high temperature. Examples of positively thermosensitive hydrogels were given in Table 1.1 in Chapter 1. The macroporous hydrogel polymers produced in this study are pNIPAAm based and are positively thermosensitive.

As was discussed in Chapter 1, pNIPAAm is one of the most well known negatively thermosensitive hydogels. The VPTT of pNIPAAm can be adjusted by adding a hydrophobic (Bae, Okano, and Kim, 1990) and/or a hydrophilic (Chen et al., 2005) co-monomer to the reaction mixture so that the phase transition occurs at a more desirable temperature. Hydrogels based on pNIPAAm show reversible swelling and deswelling behaviour in response to change in temperature. The swelling occurs at temperatures below the VPTT when the hydrophilic moieties (–CONH–) interact with water molecules

through hydrogen bonding and lead to water up-take by the hydrogen bonding, resulting in expanded structure network. When the external temperature increases, the deswelling occurs because the hydrogen-bonding interactions become weakened or destroyed. Thus, the hydrophobic interactions among the hydrophobic moieties ($–CH(CH_3)_2$) grow to be strong, which induces the freeing of the entrapped water molecules from the network then results in collapse of the structure network.

In this chapter, the equilibrium swelling ratio, polymer volume fraction and normalized volume change of each of the synthesized homo-, co- and ter-polymers were measured at selected temperatures. The swelling and deswelling kinetics of all hydrogels were also investigated. The relationships between the chemical composition, the microporous structure and the swelling properties of the hydrogel polymers, as well as the temperature dependence of these properties are also discussed.

3.2. Experimental

3.2.1. Measurement of Equilibrium Swelling Properties

The hydrogels prepared in Chapter 2 were stored in deionized water at 10°C for one week until the samples reached equilibrium. The samples then were removed from water. The weights of the samples, both in air and in water, were measured using an analytical balance after the excess water on the surface of each sample was wiped off with wet filter paper. The sample weight in air was taken as $W_{w,a}$ and the weight in water was noted as $W_{w,w}$. After the measurements, the samples were put back into the deionized water and kept at 22°C for one week. The same measurements were carried out to obtain $W_{w,a}$ and $W_{w,w}$ at 22°C. The measurements were repeated at 30°C, 37°C, 45°C, 50°C, and 60°C respectively in order to examine the sample responsiveness to temperature changes in the equilibrium state. At the completion of these measurements, the samples were removed from the water and dried in an oven for 2 days at 50°C, following which, the weights of the dry samples in air and water were measured as $W_{d,a}$ and $W_{d,w}$.

The following parameters then were calculated using various equations (Okay, 1971).

Equlibrium swelling ratio (ESR) of each specimen at various temperatures was calculated using eq. (1):

$$ESR = \frac{W_{w,a} - W_{d,a}}{W_{d,a}} \quad (1)$$

Polymer volume fraction, Φ, was determined using equation (2) in order to determine polymer porosity quantitively, where V_p represents volume of polymer after dehydration and V_{total} represents volume of the same hydrogel in an equilibrium swelling. V_p and V_{total} were calculated using eq. (3) and (4) respectively, where ρ_w is the water density at the measured temperature.

$$\phi = \frac{V_p}{V_{total}} \quad (2)$$

$$V_p = \frac{W_{d,a} - W_{d,w}}{\rho_w} \quad (3)$$

$$V_{total} = \frac{W_{w,a} - W_{w,w}}{\rho_w} \quad (4)$$

In order to compare the experimental results of all samples, the volume of each sample at various temperatures was normalized to the value of 10°C, using equation (5).

$$Normalized\ volume\ change = \frac{V_{total.T}}{V_{dtotal.10°C}} \quad (5)$$

Five measurements were carried out for each hydrogel. The results displayed in Section 3.3 are the average values of the five measurements.

It should be noted that measuring the sample weight in water was to determine the sample volume on the basis of Archimedes' buoyancy principle in room temperature (Fogiel, 1995). The measurement method is shown in Figure 3.1 and the details of the method can be found in previous studies (Lou, Chirila, and Clayton, 1997; Lou, Dalton, and Chirila, 2000).

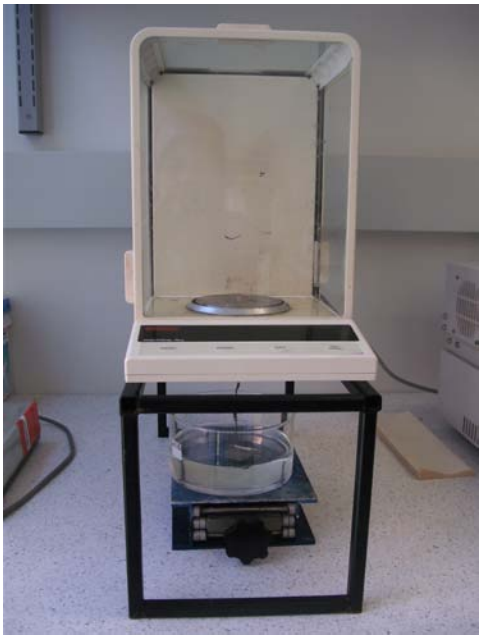


Figure 3.1 Experimental set-up

3.2.2. Measurement of Dynamic Swelling Properties

Swelling kinetics of the produced hydrogels was investigated at ambient temperature (22°C) using conventional gravimetric method. In brief, a hydrogel polymer of known dry weight, $W_{d,a}$, was put into water and taken out at a chosen time point to record the weight, W_t. The equilibrium swelling weight, $W_{w,a}$, also was recorded. The percentage water uptake capacity, W_u, then was calculated using equation 6. Similarly the deswelling kinetics of the hydrogels, at both 37°C and 50°C, was investigated. In this experiment, an equilibrium hydrogel at 22°C was put into water at preferred temperature and taken out for weighing at regular time intervals. Equation 6 then was used to determine the water retention capacity *(W_r)* respectively.

$$W_u / W_r = \frac{W_t - W_{d,a}}{W_{w,a} - W_{d,a}} \times 100 \quad (6)$$

3.3. Results and Discussion

3.3.1. Equilibrium Swelling Ratio

The equilibrium swelling ratio of the hydrogels at various temperatures is shown in Figure 3.2. Control homopolymers, 20HEMA and 30HEMA, showed no significant change in ESR within the investigated temperature range, 10°C - 60ºC, indicating their thermally independent nature. For hydrogels made from 80wt% of water (Figure 3.2a), a significant change in ESR was observed in the range of 10°C and 50ºC among which 5HEMA15NIPAAm shows the greatest change of ESR, from approximately 9 to approximately 1. For hydrogels made from 70wt% of water (Figure 3.2b), little change in ESR was observed. These results were attributed by both water concentration and the monomer ratio. Whilst the change in ESR was clearly dependent on the NIPAAm-HEMA ratio in each copolymer, the degree of ESR change was dominantly controlled by the microporous structure formed, which is largely dependent on the amounts of diluent. This phenomenon also has been noticed by Tuncel and Huang and their coworkers (Cicek and Tuncel, 1998), (Lee and Huang, 2000) and tentatively explained by a theory of equilibrium macrosyneresis and microsyneresis (Dusek, 1971) and discussed in Chapter 2.

Incorporation of hydrophobic monomer EMA into the hydrogels has led to a shift of the PVTT to the lower temperature and a more rapid volume change in a monomer range of , 20°C -40°C (Figure 3.2c). This is consistent with the hydropohobic polymer interaction theory (Hoffman et al., 2000).

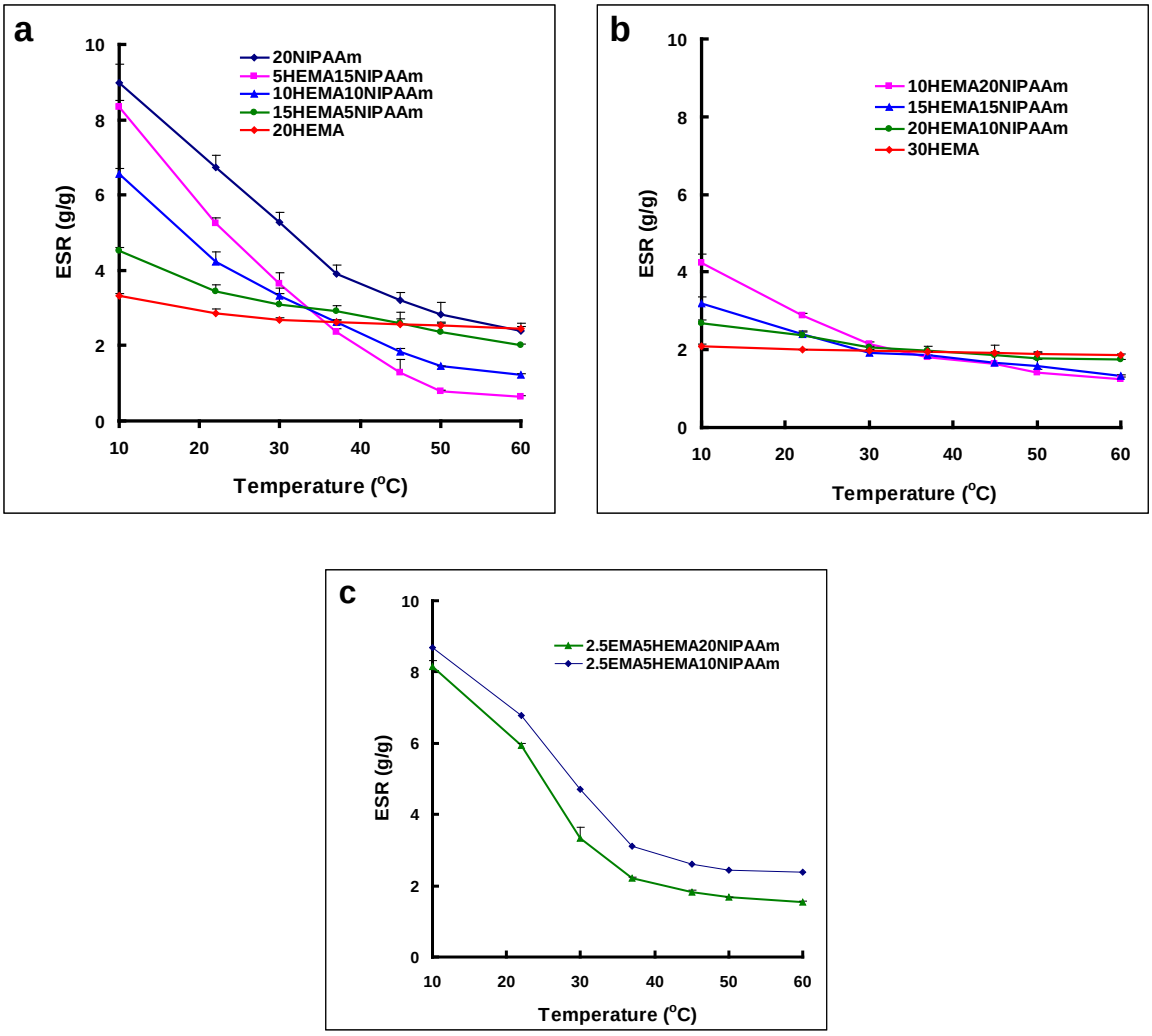


Figure 3.2 Temperature dependence of ESR of hydrogels: (a) copolymers made from 80wt% of water; (b) copolymers made from 70wt% of water; and (c) terpolymers

3.3.2. Polymer Volume Fraction and Volume Change

Table 3.1 shows the polymer volume fraction values and their dependence on temperature changes. In general, the higher is the water content in the monomer mixtures, the lower is the polymer volume fraction, which indicates a higher porosity of the hydrogel polymer. For the hydrogels made of the same water concentration, the higher is the NIPAAm, the lower is the value of polymer volume fraction, i.e., the higher is the porosity, which is consistent with the observations made in SEM (Chapter 2). For all polymers containing

NIPAAm, increasing the temperature has led to increasing polymer volume fraction, indicating a decrease in the porosity of hydrogel matrices. These trends can be more clearly manifested in Figure 3.3 which shows the normalised polymer volume change, in percentage, based on the value of polymer volume fraction at 10°C.

The normalised volume change in response to the change in temperature, shown in Figure 3.3, also is consistent with those presented in Figure 3.2, indicating a significant contribution of the porous structure to the degree of volume change of the hydrogels.

Table 3.1 Polymer volume fraction of hydrogels at various temperatures

Samples Codes	**Polymer Volume Fraction (v/v)**						
	10 °C	**22 °C**	**30 °C**	**37 °C**	**45 °C**	**50 °C**	**60 °C**
20NIPAAm	0.095	0.123	0.152	0.195	0.224	0.251	0.285
5HEMA15NIPAAm	0.097	0.146	0.198	0.277	0.423	0.556	0.591
10HEMA10NIPAAm	0.116	0.170	0.204	0.249	0.319	0.376	0.411
15HEMA5NIPAAm	0.158	0.199	0.213	0.226	0.247	0.265	0.298
20HEMA	0.196	0.220	0.232	0.236	0.237	0.241	0.247
10HEMA20NIPAAm	0.177	0.241	0.298	0.336	0.359	0.392	0.425
15HEMA15NIPAAm	0.219	0.273	0.318	0.326	0.352	0.364	0.401
20HEMA10NIPAAm	0.241	0.264	0.293	0.303	0.313	0.322	0.326
30HEMA	0.283	0.293	0.293	0.298	0.299	0.300	0.304
1.25EMA2.5HEMA15NIPAAm	0.086	0.105	0.166	0.234	0.264	0.278	0.296
2.5EMA5HEMA10NIPAAm	0.092	0.113	0.158	0.221	0.252	0.265	0.270
2.5EMA5HEMA20NIPAAm	0.101	0.132	0.216	0.294	0.337	0.354	0.372

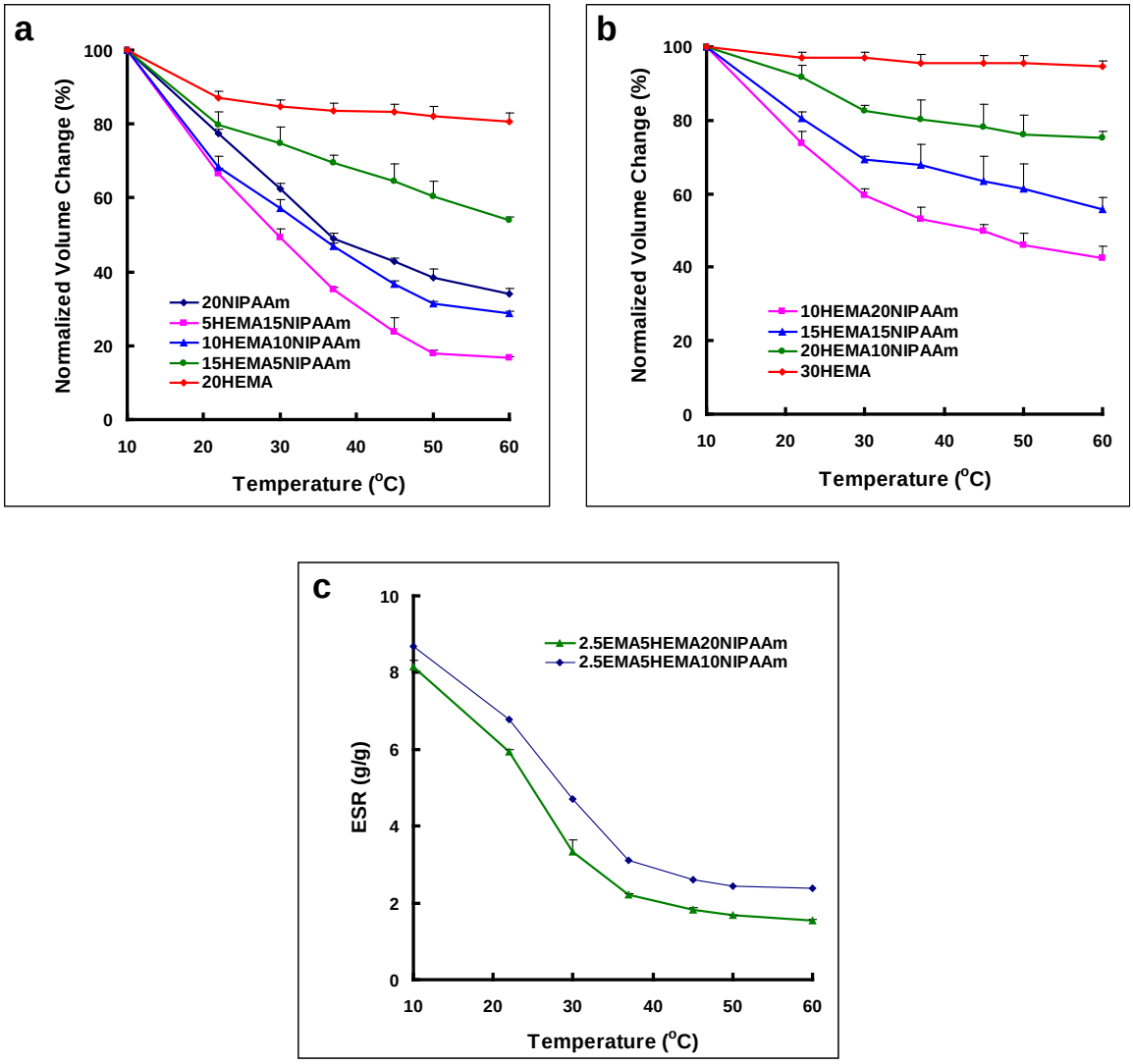


Figure 3.3 Temperature dependence of normalized volume changes: (a) copolymers made from 80wt% of water; (b) copolymers made from 70wt% of water; and (c) terpolymers

3.3.3. Swelling Kinetics

One method of measuring the response rate of the materials is to look at the swelling kinetics, which shows the swelling rate of hydrogels response to certain temperature changes due to the transformation from hydrophobic to hydrophilic chains in the hydrogel. Swelling kinetics was observed for all hydrogels within 30 hours and the results are displayed in Figure 3.4. Interestingly, the fastest swelling kinetic rate was

demonstrated in homopolymers 20HEMA and 20NIPAAm, which were followed by the copolymers made from 70wt% of water and then the terpolymers. Copolymers made from 80wt% of water displayed the slowest swelling kinetics rate.

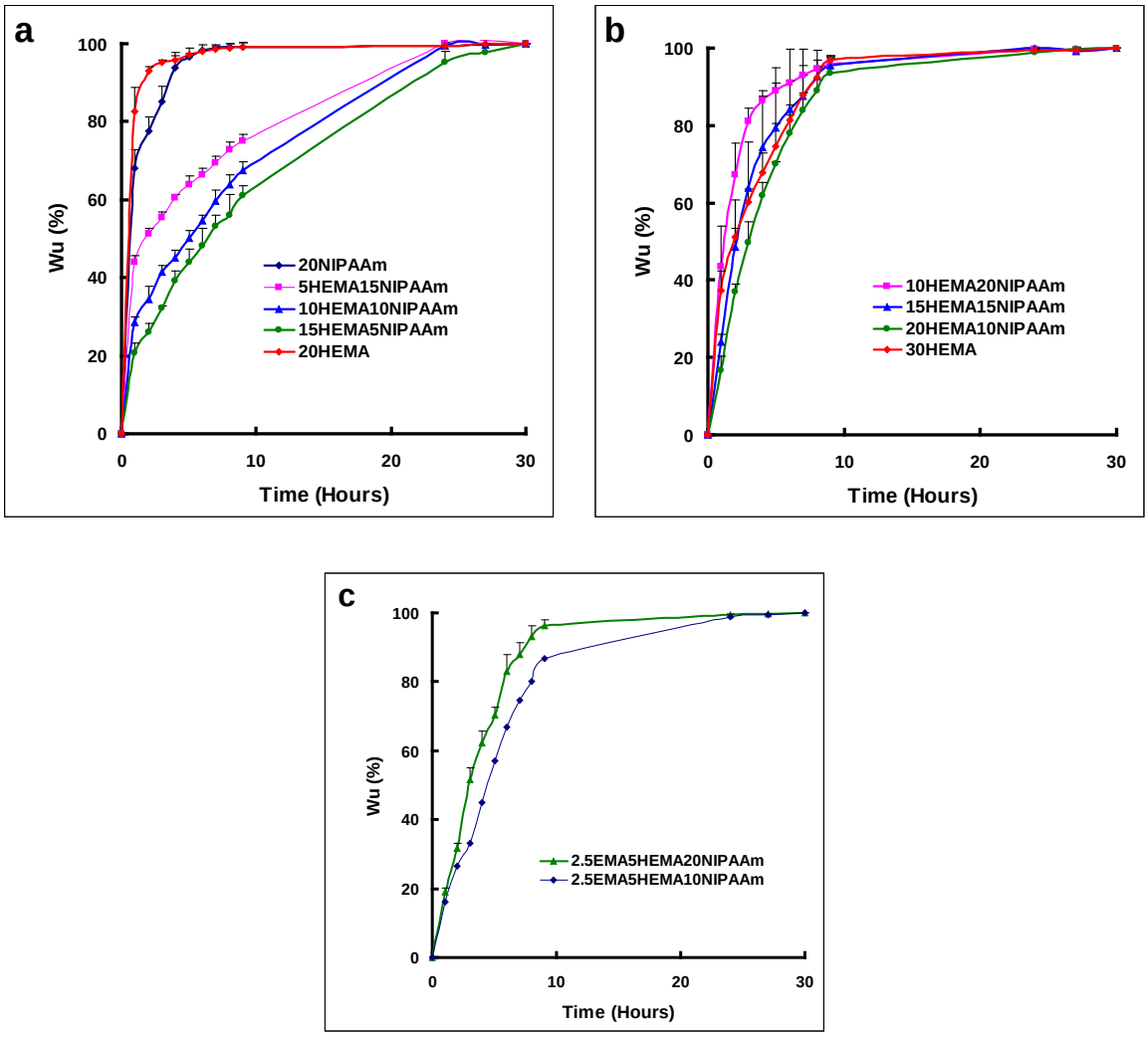


Figure 3.4 Swelling kinetics at room temperature: (a) copolymers made from 80wt% of water; (b) copolymer made from 70wt% of water; and (c) terpolymers

Whilst the faster swelling kinetic rate can be due to the presence of greater pores, such as those observed in both the homo- and ter-polymers, the slower swelling kinetic rate shown by copolymers made in 80wt% water could be due to the skin effect of these

polymers. As they respond quickly to the change in temperature, the surface of the materials might have changed their porosity (shrank) in the early stage of swelling, therefore preventing further swelling (Zhao et al., 2009).

It should be noted that prior to the swelling kinetics experiment, the hydrogel samples were pre-dried at 50°C for 2 days. The drying process may have affected the internal structure of the samples which in turn would influence the swelling kinetics rate.

3.3.4. Deswelling Kinetics

After having reached equilibrium swelling at room temperature, the hydrogels were quickly transferred to a hot water environment of 37°C and 50°C, respectively, to measure their deswelling kinetics, the results of which are shown in Figures 3.5 and 3.6. A decrease in water retention was observed for all polymers at both temperatures.

At 37°C, the water retention in the copolymers made in 80wt% water, and in the terpolymers, was lower than that in copolymers made of 70wt% water, indicating that the higher is the porosity, the lower is the water retention. The decrease in water retention of copolymers was faster than the decrease of water retention in terpolymers. This could also be due to the skin effect as a consequence of the initial fast shrinking of the hydrogel surface that prevents the diffusion of water out from the hydrogel matrix.

At 50°C, the water retention of all polymers was lower than that at 37°C due to a complete collapse of the porous structure. The deswelling rate of the copolymers made of 80wt% water and the terpolymers was much faster than that of copolymers made from 70wt% water due to the greater porosity in the former hydrogels. For homopolymers, 20HEMA and 30HEMA, approximately 90% water was retained at 37°C, and 80% at 50°C, indicating their non-temperature dependence nature. Water contained in 10HEMA20NIPAAm and 5HEMA15NIPAAm was less than 20% at 50°C (Figure 3.6a&b).

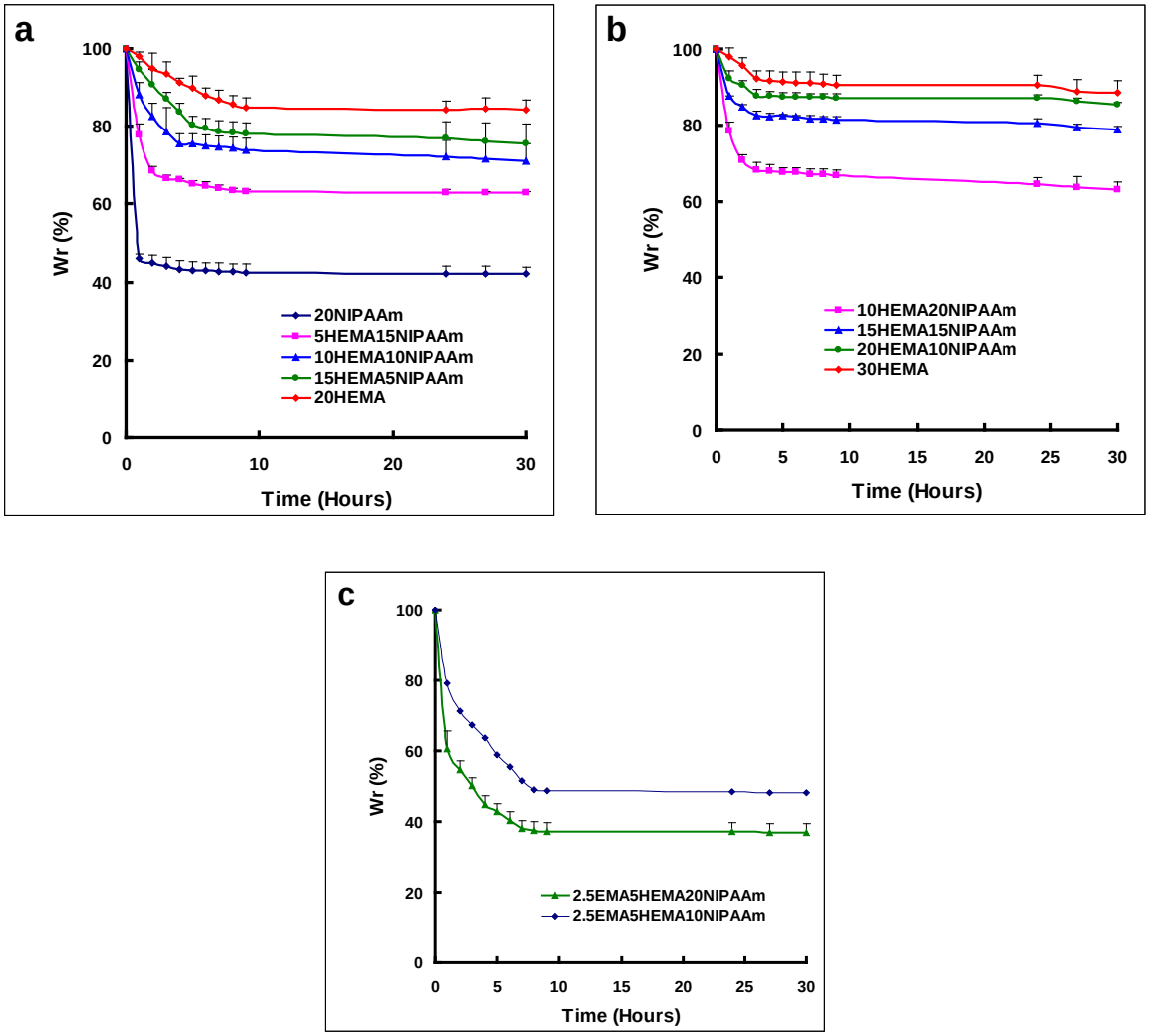


Figure 3.5 Deswelling kinetics at 37°C: (a) copolymers made from 80wt% of water; (b) copolymers made from 70wt% of water; and (c) terpolymers

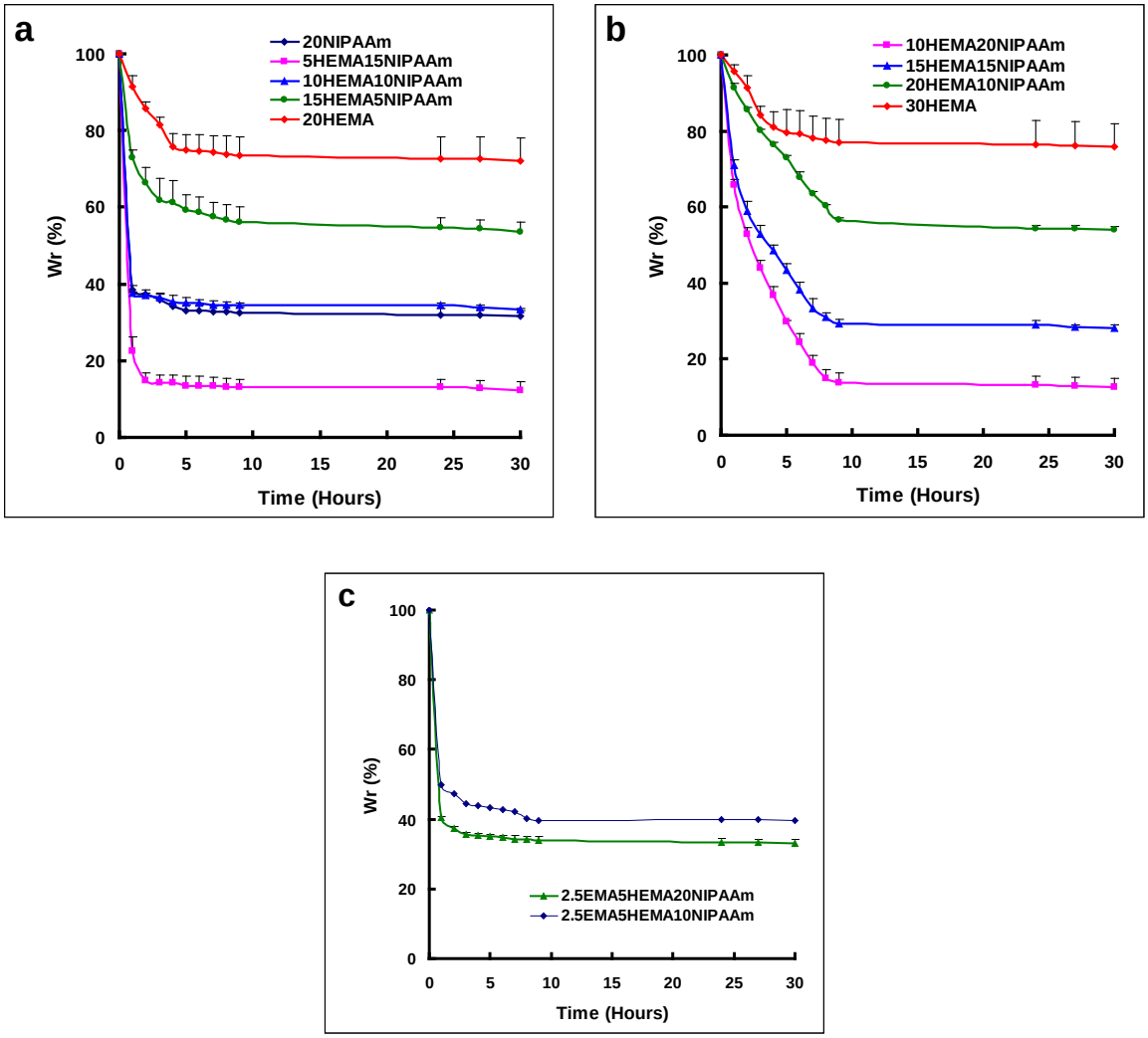


Figure 3.6 Deswelling kinetics at 50°C: (a) copolymers made from 80wt% of water; (b) copolymers made from 70wt% of water; and (c) terpolymers

3.3.5. Conclusions

This study demonstrates that swelling capacities of hydrogels are affected by the presence of a large amount of pores. The temperature responsiveness is affected by several parameters. This includes the porous structure, the presence of NIPAAm and the hydrophobic component EMA. To facilitate the investigations on the drug loading capacity and the drug release characteristics of the hydrogel materials, only polymers that

contain large pores and respond quickly to the change of temperature should be chosen for further study.

CHAPTER 4 DRUG LOADING CAPACITY AND DIFFUSION KINETICS

4.1. Introduction

In this chapter, we studied the drug loading capacity and the diffusion kinetics of prednisolone 21-hemisuccinate sodium salt, from various thermosensitive macroporous hydrogels. The effects of porosity, temperature and concentration on the drug loading capacity and diffusion kinetics will be reported and discussed.

Diffusion is the process by which particles are transported from one part of a system to another as a result of random movement. For the macroporous hydrogel drug delivery systems produced in this study, both the drug loading and release are influenced by diffusion properties of the drugs through the hydogel matrix which, in turn, are largely determined by the porosities of the polymeric networks (Wang et al., 2010). The diffusion mechanism of molecules through macroporous and microporous structured polymers is relatively simpler than through nonporous polymer, since the molecules diffuse only through the solvent-filled pores. The process can be described by the theories of Faxen (Faxen, 1922) and Renkin (Renkin, 1954). These theories often have been used to describe the pore-solute diffusion process, and were developed based on hydrodynamic analysis of diffusion through porous systems in the absence of a pressure gradient. The Renkin theory has been applied to diffusion through biological membranes (Stein, 1967) and through swollen hydrophilic membranes (Lakshminarayanaiah, 1969). The basic equations of diffusion were put forth by Fick in 1855 as an analogy to the heat-conduction equation developed by Fourier in 1822. Fick's first law is used in steady state diffusion where the concentration within the matrix does not change with time. The negative sign arises because the direction of molecular movement is opposite to the increase in the concentration. The first law is given by:

$$J = \frac{dQ_t}{dt} = -D\frac{dC}{dx} \quad (1)$$

Where J is the rate of diffusion, D is the diffusion coefficient, and dC/dx is the concentration gradient.

Fick's first law of diffusion incorporates the difference in concentration as an important factor for modifying the rate of diffusion. The greater this difference is, the greater the rate of diffusion.

For the macroporous thermosensitive hydrogels produced in this study, the swelling volume changes with the change in the temperature, which in turn causing the changes in pore sizes. This has been discussed in Chapter 3. In this chapter, we investigated the drug diffusion properties of selected hydrogels and their dependence on temperature and drug concentrations. As an important parameter, the drug loading capacity of these hydrogels is also investigated.

4.2. Materials and Experiments

4.2.1. Materials

Listed in Table 4.1 are the hydrogel polymers selected for diffusion kinetic studies and drug loading capacity investigations. All samples were used to investigate the effect of materials, meanwhile for drug loading capacity only copolymer hydrogel were used as comparator because they revealed sharp change in polymer volume fraction due to the temperature change. Prednisolone 21-hemissucinate sodium salt powder was purchased from Sigma Chemical Co., Belgium, and stock solutions of 0.5, 1 and 2wt% drug were prepared by weighing appropriate amounts of drug powder and dissolving into deionised water in a volumetric flask. Deionised water was used for all experiments in this study.

Table 4.1 Hydrogels selected for diffusion and drug loading capacity

Hydrogel	Drug Diffusion Kinetics			Drug Loading Capacity
	Effect of Materials	Effect of Temperature	Effect of Drug Concentration	
5HEMA15NIPAAm	X	X		X
10HEMA10NIPAAm	X			X
20HEMA	X			X
10HEMA20NIPAAm	X	X	X	X
15HEMA15NIPAAm	X			
15HEMA5NIPAAm	X			X
30HEMA	X			X
2.5EMA5HEMA10NIPAAm	X			
2.5EMA5HEMA20NIPAAm	X	X		

4.2.2. UV-Visible Spectroscopy

UV-Visible Spectroscopy is a method used to measure the amount of UV and visible light absorbed by a solution. The term absorbance is used to describe the amount of light absorbed. The concentration of an unknown solution can be determined through Beer-Lambart's law which is given by:

$$A = \varepsilon.b.c$$

where A is the absorbance, ε is the extinction coefficient, b is the path length through the sample, and c is the concentration of the absorbing solution.

A GBC 916 UV-vis spectrometer (GBC Scientific Equipment, Australia) was used to measure the absorbance of the prednisolone 21 hemisuccinate sodium salt at a fixed wavelength of 247nm (Kenkel, 2003). It operates at a slit width less than 2nm and an

integration time of 10 seconds. A 10mm thick quartz cell was used in all tests and deionised water was used as a blank. Each of the readings was tested twice to ensure stable and accurate results. The experiment was carried out at 22°C.

4.2.3. The Calibration Curve

Using the UV-Vis Spectometer, a calibration curve was first established by plotting absorbance versus the concentration of the drug, using a series of known concentration solutions. A linear equation then was generated from the calibration curve using linear regression analysis. With the linear equation generated, the absorbance of an unknown solution was measured and the concentration calculated from the equation (Figure 4.1).

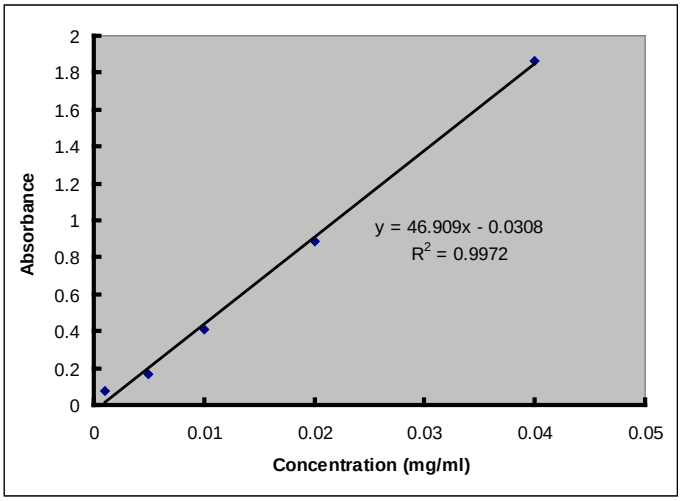


Figure 4.1 Calibration curve

4.2.4. Measurement of Drug Loading Capacity

The drug loading was carried out through a simple diffusion procedure at the selected temperature. In brief, a freeze-dried hydrogel sample was weighed and kept in a drug solution that was approximately 2 times its ESR for 4 days, until it became fully swollen.

The remaining drug was diluted in a known volume of deionized water and its absorbance determined using a UV-Vis spectrometer. With the linear equation generated from the calibration curve, the absorbance of an unknown solution was measured and the concentration was calculated to measure the mass of the drug (W_{drug}). The loading capacity was calculated using the following equation:

$$\text{Loading capacity \%} = \frac{W_{drug}}{W_{polymer}}$$

Where W_{drug} is the weight of drug loading in the hydrogel and $W_{polymer}$ is the weight of the freeze dried hydrogel.

4.2.5. Drug Diffusion Experiment

Drug diffusion experiments were carried out using a diffusion cell consisting of two 25cm^3 compartments that were separated by a circular hydrogel membrane of 22mm in diameter. A photograph of the diffusion cell and the cross-sectional sketch of the diffusion cell after the assembly are shown in Figure 4.2.

Both compartments of the diffusion cell were first filled with deionised water and kept at the selected temperature for 24 hours, thus allowing the hydrogel membrane to reach its equilibrium. After that, water in one compartment was removed and refilled with prednisolone 21-hemmisuccinate sodium salt solution of the same temperature. The volume samples were taken out in both compartment. The changes of absorbance in both compartments were then monitored regularly for 15 days using a UV-Visible spectrometer in order to determine the concentration of the drug and the cumulative amounts of drug. Drug diffusion kinetics were established using the time dependence of the cumulative amounts of drug in the water compartment.

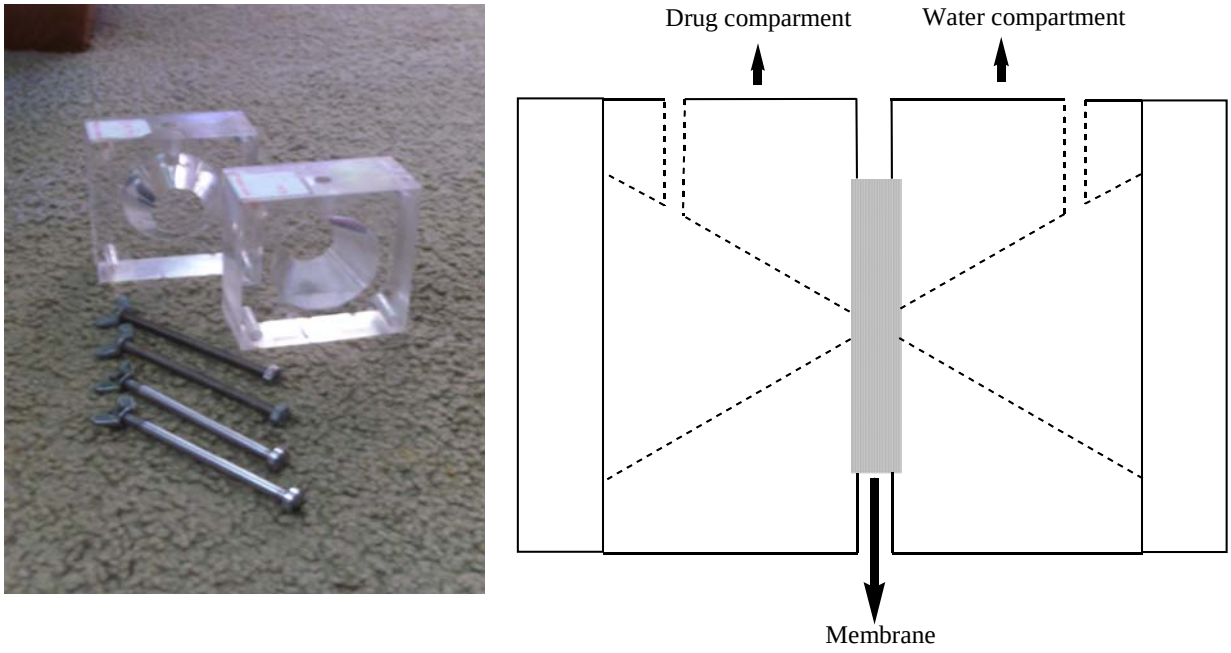


Figure 4.2 Drug diffusion cell (L) and a cross-sectional sketch after the assembly of the diffusion cell (R)

4.3. Results and Discussion

4.3.1. Drug Loading Capacity

The drug loading capacity of each hydrogel is presented in Table 4.1.

The effect of the hydrogel porosity on the drug loading capacity is apparent. For example, at the same temperature (22°C) and using the same drug concentration solution, the drug loading capacity was greatest in 5HEMA15NIPAAm, followed by 10HEMA10NIPAAm, 15HEMA5NIPAAm, 20HEMA, 10HEMA20NIPAAm, and 30HEMA. This follows the same trend as the polymer volume fraction (Chapter 3) which was caused by the decrease in porosity of these hydrogel polymers at the ambient temperature. This result is consistent with the reported experimental results of porous pHEMA hydrogels (Lou, Munro, and Wang, 2004).

The effect of temperature on drug loading was even more significant. For instance, using a 2wt% drug stock solution, the loading capacity of 5HEMA15NIPAAm increased from

10.4 to 17.5wt% when the temperature was decreased from 22°C to 10°C. A similar effect also was observed in 10HEMA20NIPAAm hydrogels.

The significantly increased drug loading capacity at lower temperature indicates that the hydrogels contain greater pore volumes at the lower temperature due to their thermosensitive nature. The increased pore volumes cause the increased mass uptake of drugs into hydrogels as they provide more spaces for molecules to occupy during the diffusion (Lou, Munro, and Wang, 2004), (Yasuda and Lamaze, 1971), (Yasuda et al., 1969). The increased pore volumes have been demonstrated by the reducing polymer volume fraction reported in Chapter 3.

It also is evident that as the concentration of drug solution increases, the drug loading capacity increases as well. This can be seen from both 5HEAMA15NIPAAm and 10HEMA20NIPAAm hydrogels (Table 4.2). Increasing the drug concentration from 1wt% to 2wt%, the drug loading capacity of 5HEMA15NIPAAm was increased from 8.3 to 17.5 and the drug loading capacity of 10HEMA20NIPAAm was increased from 4.4 to 12.6.

Table 4.2 Drug loading capacity of selected hydrogels at various temperatures

Samples Codes	**Drug Loading Capacity**		
	10 °C		**22 °C**
	1 wt% (drug solution)	2 wt% (drug solution)	2 wt% (drug solution)
5HEMA15NIPAAm	8.3	17.5	10.4
10HEMA10NIPAAm	-	-	9.08
15HEMA5NIPAAm	-	-	7.42
20HEMA	-	-	8.51
10HEMA20NIPAAm	4.4	12.6	5.41
30HEMA	-	-	4.96

4.3.2. Drug Diffusion Kinetics

Shown in Figure 4.3 are drug diffusion profiles of various hydrogels at 37°C using a 1wt% drug stock solution. For copolymer hydrogels made from 80wt% of water (Figure 4.3.a), there was no significant difference between the diffusion profiles of investigated materials, although a slightly more rapid initial burst in the first 5 days was seen in the hydrogels containing higher HEMA ratio. A lot of drugs diffused through the membrane within 12 days. For hydrogels made from 70wt% of water, a much slower diffusion was observed in comparison to the hydrogels made from 80wt% water. At day 15, less than 90mg of drugs diffused through the 10HEMA20NIPAAm membrane, and only about 50mg diffused through the 30HEMA and 15HEMA15NIPAAm membranes. A similar trend was seen in the terpolymer hydrogels.

In general, incorporation of NIPAAm into HEMA reduced the diffusion rate of the drugs. However the reduction of the diffusion rate in hydrogels by the change of water content was equally significant. Both results are a consequence of the reduced pore sizes and/or number of pores.

Figure 4.4 shows the effect of temperature on the drug diffusion properties of hydrogels. Three hydrogels including 5HEMA15NIPAAm, 10HEMA20NIPAAm, and 2.5EMA5HEMA20NIPAAm, and three temperatures including 22°C, 37°C and 50°C were selected for this study. For all selected hydrogels, the lower the temperature, the faster was the drug diffusion. At 22°C, a fast diffusion was seen for all hydrogels. Increasing the temperature to 37°C resulted in a reduced diffusion rate in both the 10HEMA20NIPAAm and 2.5EMA5HEMA20NIPAAm. However change in 5HEMA15NIPAAm was more significant due to the more rapid temperature responsiveness of 5HEMA15NIPAAm in the investigated temperature range.

The drug diffusion through the hydrogels was faster when the temperature was low due to the expansion of the pores. Meanwhile, increasing the temperature of diffusion kinetics experiments resulted in slow drug diffusion because the pore structure network collapsed and entrapped drug molecules.

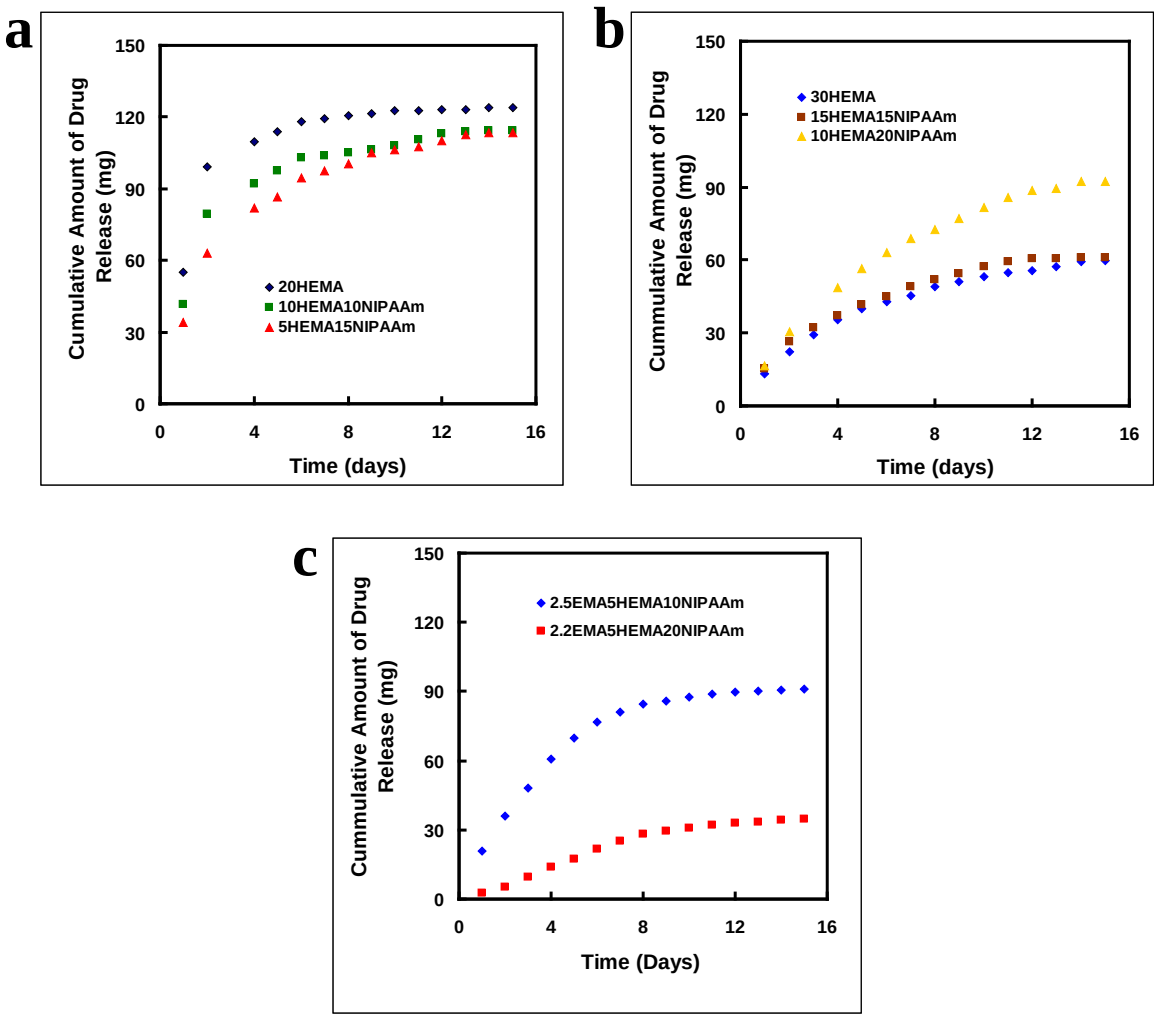


Figure 4.3 Drug diffusion kinetics of different hydrogels at 37°C: (a) copolymers made from 80wt% water; (b) copolymers made from 70wt% water; and (c) terpolymers

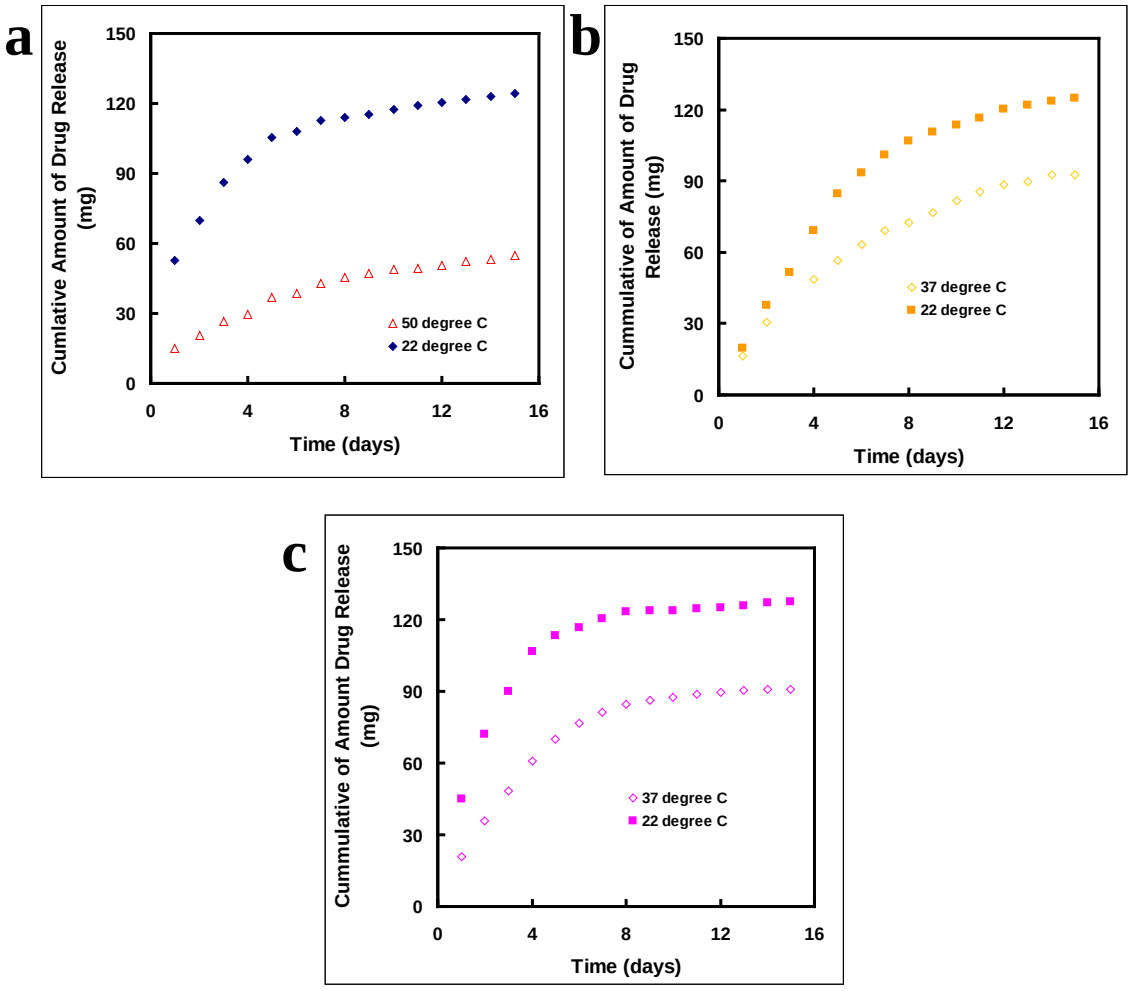


Figure 4.4 Drug diffusion kinetics at various temperatures: (a) 5HEMA15NIPAAm, (b) 10HEMA20NIPAAm, and (c) 2.5EMA5HEMA20NIPAAm

Finally, shown in Figure 4.5, is the effect of the concentration of the drug solution on the release rate of 10HEMA20NIPAAm hydrogel. Faster drug diffusion was observed when the concentration of the drug solution increased, which is not uncommon based on Fick's law. As shown in Equation 1, the net movement of diffusing molecules depends on the concentration gradient. The rate of drug diffusion is directly propotional to the concentration of the solution (Beals, Gross, and Harrell, 1999).

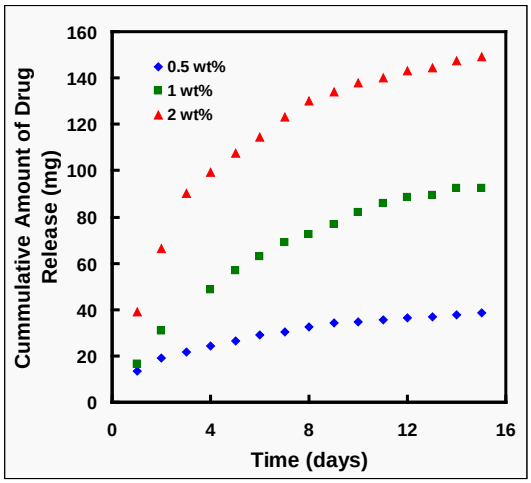


Figure 4.5 Drug diffusion kinetics of 10HEMA20NIPAAm at varying drug concentrations

4.3.3. Conclusions

This study demonstrates that drug loading capacity and drug diffusion kinetics are influenced by the porosity of hydrogels, temperature, and drug concentration.

In general, the more porously structured hydrogels showed high drug loading capacity and faster diffusion kinetics because more drug molecules can reside in the porous hydrogel matrix and diffusion is easier through a more porous structure. Since the hydrogels investigated were positively themosensitive, (i.e., the lower is the temperature, the higher is the porosity), the drug loading could be conducted at a temperature below body temperature, thus a higher drug content could be achieved and the release at an elevated temperature could be retarded due to the shrinkage of pores. Increasing drug concentration also resulted in higher drug loading.

CHAPTER 5 GENERAL CONCLUSIONS

Twelve homo-, co- and ter-polymers of HEMA, NIPAAm and EMA based hydrogels were synthesized in the presence of varying amounts of water. SEM examination indicated the presence of macroporous structures in all hydrogels. The porous structure of the resultant hydrogels was largely dependent on the amount of water, and the ratio of monomers used in the polymerisation process. In general, the higher the water content the more porous was the structure. Increasing NIPAAm content has resulted in increased pore sizes and extended connection of the polymer network, whilst the presence of a small amount of hydrophobic EMA had more effect on the pore sizes.

The investigation also showed that the presence of large amounts of pores have led to a great equilibrium swelling capacity of these hydrogels at ambient and below ambient temperatures. The swelling capacity of the hydrogels changed dramatically with the temperature change when the thermosensitive component NIPAAm was incorporated into the polymer structure. This was well demonstrated by the copolymers made from 80wt% of water and the terpolymers (Figure 3.2a&c). The measurement of the polymer volume factions at various temperatures and the normalised volume changes of these hydrogels demonstrated that the change of swelling capacity was strongly associated with the change in equilibrium volumes (polymer volume fraction) which revealed the changes in the porosity of the hydrogels (Figure 3.3). The study has also shown that the incorporation of EMA has made the change of equilibrium swelling ration more rapid with the change of temperature. For instance, the apparent change of ESR was observed within the temperature range of 10°C – 50°C for the copolymers, and 10°C – 37°C for the terpolymers. Results from swelling and deswelling kinetic studies demonstrated that polymers containing higher NIPAAm contents, such as 5HEMA15NIPAAm and the terpolymers, responded faster to the changes of temperature.

The presence of the porous structure and the thermoresponsiveness of the produced hydrogels had a significant impact on the drug loading and release characteristics. In general, the more porously structured hydrogels showed high drug loading capacity

and faster diffusion kinetics because more drug molecules can reside in the porous hydrogel matrix and diffusion is easier through a more porous structure. Since the hydrogels investigated were positively themosensitive, (i.e., the lower is the temperature, the higher is the porosity), the drug loading could be conducted at a temperature below body temperature, thus a higher drug content could be achieved and the release at an elevated temperature could be retarded due to the shrinkage of pores. It should be noted that loading drugs at a low temperature can also prevent the decomposition of the drugs which is often a great concern of the drug formulation preparation.

Finally, two hydrogels 5HEMA15NIPAAm and 1.25EMA2.5HEMA15NIPAAm are recommended for further studies on the drug stability and ex-vivo release of the selected drug in order to further develop the hydrogel materials for possible applications in the treatment of various eye conditions which was one of the initiatives of this study (Hicks, Lou et al., 2002), (Lou, Munro, and Wang, 2004; Lou, Wang, and Tan., 2007), (Wang et al., 2010). Although it is not always possible to translate laboratory researches into eventual applications, the fundamental understanding about the materials produced in this study is valuable, both scientifically and practically, for the development of smart hydrogels for the controlled drug delivery applications.

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