Temperature-Sensitive Poly(Acrylamide) Hydrogels for Drug Delivery Applications

Wanwipa Siriwatwechakul
Department of Bio-Chemical Engineering, Sirindhorn International Institute of Technology, Thammasat University, Khlong Luang, Pathumthani, Thailand, 12121
E-mail: wanwipa@siit.tu.ac.th

Abstract

This paper provides an overview of controlled-release mechanisms in temperature-sensitive hydrogels used in drug delivery application, and focuses on the hydrophilic to hydrophobic transition of Poly (*N*-isopropylacrylamide) (PNIAM) as a function of temperature. At a temperature above the lower critical solution temperature (LCST), the hydrophobic interaction becomes stronger causing PNIAM chains to collapse, allowing the captured molecules or drugs to be released from the hydrogel matrix. This study proposed a mechanism to control this transition in order to vary the hydrophobicity of the hydrogel and its LCST. This can be done through modifying polyacrylamide hydrogel with the inclusion of *N*-isopropylacrylamide (NIAM). The modification was done by mimicking micellar polymerization, which resulted in better arrangement of NIAM chains in the polyacrylamide network. The degree of NIAM arrangement is described by N_H number. The hydrophilic to hydrophobic transition was measured through the partition coefficient, K, of Methylene Blue. The study showed that the hydrogel with higher N_H values resulted in better solubility of the dye; yet still retained the temperature-sensitivity of PNIAM.

Keywords: Temperature-sensitive hydrogels, Poly (*N*-isopropyl acrylamide), partition coefficient, the lower critical solution temperature (LCST).

1. Introduction

In recent years, there has been an explosion of advances in the fields of structured and intelligent materials. The variety of chemical structure and control of the molecular architecture and morphology allows numerous uses of polymers in bio-logical applications such as drug delivery and scaffolds for tissue engineering [1]. Figure 1 illustrates the drug concen-tration in plasma as a function of time. With con-ventional drug administration methods such as oral administration or intravenous in-jection, the drug concen-tration in plasma increases initially to reach the maximum concentration and decreases as the drug is metabolized by the liver and the kidney to reduce the drug toxicity to unintended organs.

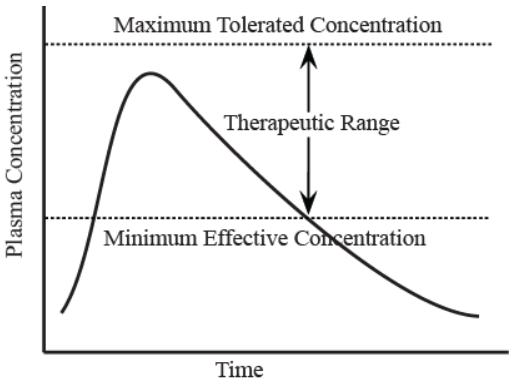

Figure 1 The plot shows the concentration of a typical drug as a function time.

In order to achieve a more effective treatment, it is desirable to maintain the level of drug concentration within the therapeutic range over an extended period of time [2]. Controlled drug delivery strategies are used to achieve this precise level of drug release [1, 3-6]. Figure 2 shows the concentration profile of a controlled release system. With a drug delivery carrier, the drug is prevented from dissolving in the blood plasma all at once. Instead, the drugs is slowly released from the delivery vehicles, thus the concentration of the drug is controlled, and can last a long time.

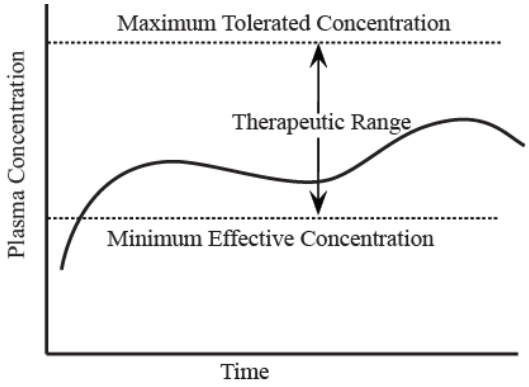

Figure 2 The plot shows the desirable concentration profile of a drug with a controlled –release delivery system.

To achieve this goal, scientists and engineers have recently developed hydrogel systems to use as "drug delivery vehicles" [6]. These vehicles carry drugs to the disease site, after which a change in the chemical environment such as a change in pH [7-9] or temperature [10-12] triggers a release of their content [4, 13-15]. Thus the therapeutic molecules are protected until their release at the target sites, and the healthy organs are protected from exposure to the toxic chemotherapeutic agents. In addition, the drug delivery vehicles allow for maintaining the desirable drug concentration in the body over an extended period of time [2].

Hydrogels are three-dimensional hydrophilic polymer networks with the ability to imbibe large amount of solvent [16]. Due to their biocompatibility, hydrogels are suitable for the use in biological systems [3]. As a result, hydrogel technology has been widely studied in many biomedical applications over the past few decades [11]. The high water content in hydrogel allows it to be flexible and resemble biological tissues [3]. The water content of the hydrogel fills up the hydrogel pore space allowing selective diffusion of solutes through the hydrogel matrix. This characteristic of the hydrogel is required for controlled release drug delivery systems [1, 6, 16].

Since the diffusion of drug in hydrogels occurs in the space between the polymer chains, any factor that affect the size of this space also affects the diffusion of drug through the hydrogel matrix [10]. As a result, it is important to control the swelling rate of hydrogels in order to control diffusion through the matrix [1, 6, 7, 16].

2. Temperature-sensitive controlled release systems

In recent development, temperature-sensitive hydrogels have gained considerable attention because they provide a controlled-release system whose on-off switch is triggered by the temperature of the environment [15]. This is achieved from the change in hydrophobicity of the polymer hydrogels as the temperature changes [10]. These polymers demonstrate a transition from hydrophilic to hydrophobic structures at the temperature known as the Lower Critical Solution Temperature (LCST) [17]. When the temperature is below LCST, the hydrophilic chains are hydrated, and the hydrogels become swollen, allowing the drugs to diffuse into the hydrogel void space. As the temperature increases past LCST, the hydrophobic interaction becomes stronger, thus the balance between hydrophilic/ hydrophobic interactions break down, causing the gel to

collapse. This allows the solute to diffuse outside the gel [10, 13].

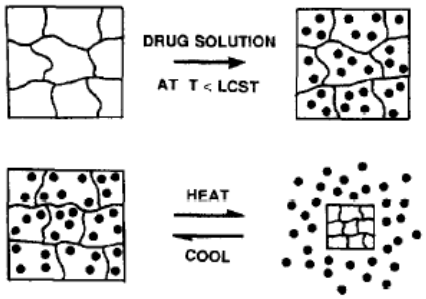

Figure 3 Schematic of drug loading and drug release with temperature-sensitive Poly (*N*-isopropylacrylamide) system (excerpt taken from [10]).

Table 1 provides some examples of the polymers exhibiting temperature-sensitive properties with LCST ranges from 32 – 125 ºC.

Table 1 Examples of polymers showing a LCST in water [15].

Polymer	***LCST (ºC)***
Poly(*N*-isopropylacrylamide), PNIAM	~ 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

In the past few decades, the hydrogels of Poly (*N*-isopropylacrylamide) (PNIAM) has been extensively studied because the normal range of the LCST for PNIAM is close to physiological condition and make it applicable in biomedical applications [4, 12-14]. The hydrophilic-to-hydrophobic transition is due to the presence of the hydrophilic amide groups and the hydrophobic isopropyl group on its side chain.

In addition, the LCST of PNIAM can be further tuned by adding ionic polymers such as Poly(acrylamide) (PAM) or Poly(acrylic acid) (PAA) [14, 18]. LCST of PNIAM hydrogel increases as more acrylamide is incorporated in the PNIAM network [14]. Thus the LCST can be tuned by varying the amount of acrylamide in the polymer network. This approach also helped to overcome the strength setback posed by using PNIAM homopolymer, which tends to collapse when highly swollen. In order to retain the temperature-sensitive properties but to correct for the low mechanical strength problem, PNIAM is trapped in a PAM hydrogel network to form a semi-interpenetrating network, (semi-IPNs). This is proven to be stable and preserves the temperature-sensitive property [11].

For the control of the molecular release mechanism, the hydrophobic structure in the PNIAM plays a crucial role. Since, the semi-IPNS of PNIAM trapped in PAM appears in random orientation, the use for wider applications that can be achieved through a better control of PNIAM arrangement will help achieve a better control of release mechanism and a wider range of LCST.

We proposed a better method to control the arrangement PNIAM chains in PAM hydrogels through mimicking micellar polymerization [19, 20], which is a technique used to synthesize hydrophobically-modified polyacrylamide (hmPAM) used as a thickening material [21]. The proposed material is shown in Figure 4.

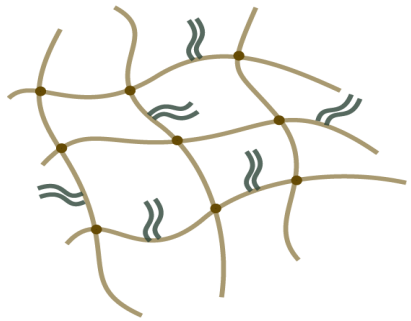

Figure 4 Schematic of Polyacrylamide (PAM) hydrogel with *N*-isopropyl acrylamide polymerized with micellar polymerization technique. Refered to as modified-PAM hydrogels

The principles of micellar polymerization were first described by Candau *et al.* [20] based on the technique developed by Turner *et al.* [22] for water-based polymer syntheses with hydrophobic comonomer by using surfactants.

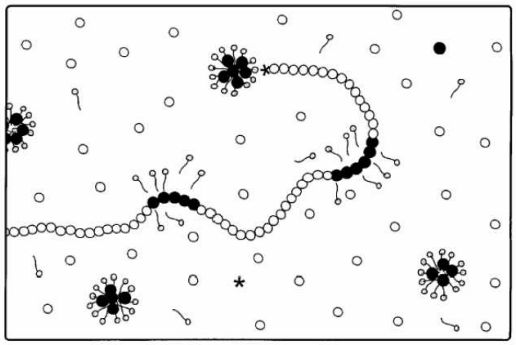

Figure 5 Micellar polymerization process. ○ represents hydrophilic monomers; ● represents hydrophobic monomers; ○⌒ represents surfactants (Excerpt from [20]).

The process is depicted in Figure 5. The hydrophobic monomer is soluble inside the surfactant micelles, while the hydrophilic monomer is soluble in the aqueous continuous medium. The reaction is a microheterogeneous copolymerization system in which micelles act as microdomains where the hydrophobes concentrate. This system differs from solution polymerization where the hydrophobic and hydrophilic monomers are polymerized in a random order, whereas the micellar polymerization results in addition of hydrophobes on the hydrophilic backbone in a block-like structure [20, 21].

As a result, an important parameter used to characterize the polymers is the number of hydroprobes per micelle (referred to as N_H number). It is determined from the following equation [20, 23].

$$N_H = \frac{[Hydrophobes]}{[Micelles]} = \frac{[Hydrophobes]}{\left(\frac{[surfactant]-cmc}{N_{agg}}\right)} \quad (1)$$

Where cmc is a critical micellar concentration of surfactant and N_{agg} is a surfactant aggregation number.

A higher N_H value means more hydrophobes are incorporated, which results in a higher degree of blocking whereas a lower number results in more evenly distribution of hydroprobes along the backbone [20].

By employing the micellar polymerization technique, it is possible to synthesize hydrophobically-modified polymer with the same degree of hydrophobic substitution but different degree of blocking. These polymers exhibited unique rheological characteristics [19, 20, 24, 25] due to interaction among the hydrophobic side chains. Hydrophobically-modified polymer with the higher degree of blocking (or higher N_H value) exhibited stronger interaction even if the degree of hydrophobic substitution remained the same. The result suggested that the hydrophobic interaction can also be moderated by having other hydrophobic chains in the vicinity. As a result, the hydrophobic interaction can be moderated by changing the N_H values [21, 23, 26, 27].

We will use a similar technique to moderate the interaction among the NIAM

chains in the PAM hydrogels. Incorporating the micellar polymerization technique with the temperature-sensitive properties of the NIAM, we proposed a method of preparing a modified PAM hydrogel by mimicking micellar polymerization technique. As such, we can produce modified PAM hydrogels with different properties, depending on different N_H values of PNIAM.

We will examine the effects of the arrangement of *N*-isopropyl acrylamide (NIAM), through the use of micellar polymerization technique [6], on the temperature-sensitive properties of the modified PAM hydrogel.

3. Experimental

3.1 Raw Material

The acrylamide monomer (Fluka, 01200) and Sodium Dodecyl Sulfate, SDS (Fluka, 75370) were used as received. *N*-isopropylacrylamide (NIAM, Acros Organics, 412780250) was precipitated in hexane. Methylene-bis-acrylamide (MBAM, Fluka, 66670) was used as a cross-linking agent, and N,N,N,N-Tetramethylenediamine (TEDEM, Sigma-Aldrich, T22500) was used as an accelerator. Sodium persulfate (NaPS, Fluka, 71890) was used as an initiator.

3.2 Sample Preparation

3.2.1 Hydrogel Synthesis

The modified PAM hydrogel synthesis was carried out following the example of Guilherme *et al.* [11] with the addition of a surfactant, SDS during polymerization. To synthesize hydrogels, two aqueous solutions were prepared. Solution A was prepared with 2500 μM/mL of acrylamide and degassed for 1 hour. Later, 200 μM/mL (1.3 wt %) of NIAM and an appropriate amount of SDS were added to solution A (see Table 2). Degassing was continued for the next 30 minutes [20]. Subsequently, 50 μmol/mL MBAM and 3.2 μmol/ml of TEDEM were added and continued to degas for another 15 minutes. Solution B was prepared with 42 μmol/ml NaPS degassed for 20 minutes. After degassing, 90 mL of solution A was mixed with 10 mL of solution B. The mixture was quickly injected between two glass plates with circular spacers [11]. The concentrations of PNIAM and SDS for the synthesis are described in Table 2. The mixture was allowed to set for 24 hours at room temperature. After the gel was formed, it was removed from the mold, and washed with excess methanol 3 times to remove excess SDS. The gel was rinsed with excess water in the final step before using in the partition coefficient measurements.

Table 2 Concentration of Acrylamide (AM), *N*-Isopropyl Acrylamide (PNIAM), the initiator (MBAM), and Sodium Dodecyl Sulfate (SDS) used for synthesis of hydrogel. Concentrations are expressed as μmol/ mL

No.	Hydrogel	[PNIAM]	[SDS]	N_H
1	[2.5-0-L]	0	0	0
2	[2.5-0-H]	0	0	0
3	[2.5-1-L]	20	1.21	1
4	[2.5-1-H]	20	1.21	1
5	[2.5-5-L]	20	0.24	5
6	[2.5-5-H]	20	0.24	5

3.2.2 Partition coefficient measurements

The partition coefficient measurements were done following the procedure detailed by *Guilherme et al.* [11]. Aqueous solution of Methylene Blue was prepared with a concentration of 140 μmol/mL. The hydrogels prepared in part A, were cut into equal size and immersed in 25 mL of the dye solution. After 24 hours of immersion, the concentration of the remaining dye in the aqueous solution was determined by UV-Vis spectroscopy at wavelength 664

nm. The partition coefficient, K, was determined from the ratio of the dye concentration in the hydrogel vs. that in the solution according to the following equation.

$$K = \frac{[\text{dyes in hydrogel}]}{[\text{dyes in water}]} \qquad (2)$$

The partition coefficients were determined at both 25 °C and 45 °C.

4. Results and Discussions

Methylene Blue exhibited hydrophobic characteristic [11]. This character influenced the solubility of the dyes in the modified hydrogels, which were observed in terms of the partition coefficients, K.

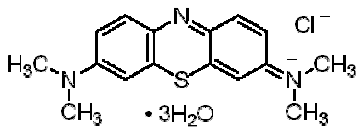

Figure 6 Molecular Structure Methylene Blue.

The partition coefficients, K, of Methylene Blue in the modified hydrogels were measured at two different temperatures (25 °C to 45 °C), and the results are plotted in Figure 7 and Figure 8. The K values of the dye in the PAM hydrogel before the addition of the hydrophobic NIAM remained relatively constant (K = 2.7) as the temperature increased from 25 °C to 45 °C. The same trend is seen in the modified PAM hydrogels with the addition of NIAM for N_H = 1. However, when the N_H value increased to N_H = 5, the K value of Methylene Blue in the modified PAM hydrogel increased from 2.7 to 9.6 as the temperature increased from 25 °C to 45 °C.

Because Methylene Blue is hydrophobic, the increase in the K values demonstrates the hydrophilic to hydrophobic transition as the temperature increases above the LCST for PNIAM, which is approximately 31 – 33 °C [4, 12-14]. As the modified hydrogel is warmed and temperature increased above the LCST of PNIAM, the hydrophobic interaction among the NIAM side chains becomes stronger, and the hydrophobic chains collapse creating a more hydrophobic environment , allowing more Methylene Blue to partition into the hydogel [11, 28].

Figure 7 also shows that for the modified PAM hydrogel with higher N_H values (N_H = 5), the transition from lower K value at lower temperature to higher K value at high temperature, is more dramatic than the unmodified hydrogel or the modified hydrogel with lower N_H values (N_H = 1). The result suggests that by having the NIAM side chains arranged in block-like manner enhances the hydrophobicity of the modified PAM hydrogels. The results are similar to the thickening properties of hydrophobically modified polymers seen by Candau *et al* [20, 23, 29].

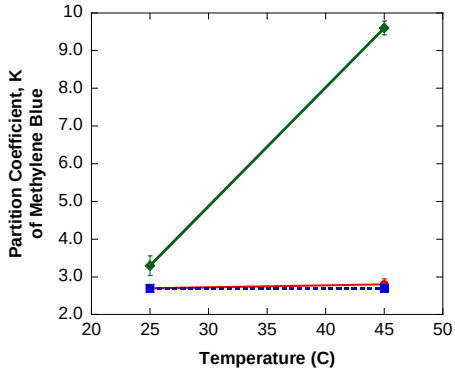

Figure 7 The partition coefficient, K, of Methylene Blue in hydrogels as a function of temperature. ● represents K values for PAM hydrogel, ■ represents K values for modified PAM hydrogel with N_H = 1, and ◆ represents K values for modified PAM hydrogel with N_H = 5.

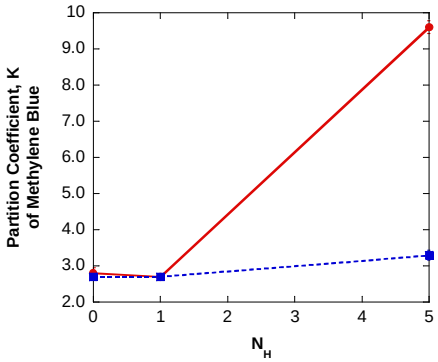

Figure 8 The partition coefficient, K. of Methylene Blue in hydrogels as a function of N_H at two different temperatures. ● represents K values at 45 °C and ■ represents K values at 25 °C.

5. Conclusion

The study showed that the modified PAM hydrogel prepared by the proposed method retained the temperature sensitivity of Poly(*N*-isopropylacrylamide). This behavior was observed through the increase in K as the temperature increased. In addition, transition was more dramatic with the modified PAM hydrogel with higher N_H values.

The result illustrates that the arrangement of the hydrophobic molecules in a 'block-like' structure contributes to more hydrophobic interaction. As a result, more grouping (high N_H value) of the hydrophobic *N*-isopropylacrylamide on the polyacrylamide gel network shows higher hydrophobic characteristics, which result in higher solubility of Methylene Blue. The high solubility of the dye manifests itself in terms of high partition coefficient (K values) observed in the experiment.

This work is the first step in showing that the hydrophobic interaction in hydrogel network can be modified through the organization of the hydrophobic chains in a block-like structure, while the temperature-sensitive property of Poly(*N*-isopropyl acrylamide) can still be retained.

In future studies, we plan to investigate the temperature range of 30 - 35 ºC in order to observe the transition at the LCST of PNIAM. In addition, we will develop a procedure to synthesize these hydrogels in micron and submicron particles, and study their release behaviors. These steps are essential to further develop these hydrogels for their use in pharmaceutical applications.

6. Acknowledgement

I would like to thank Sirindhorn International Institute of Technology for its financial support.

7. References

[1] N. A. Peppas *et al.*, Advanced Materials, Vol. 18, p. 1345, 2006.

[2] N. A. Peppas, Chemical Engineering Progress, Vol. 103, p. 14 , 2007.

[3] N. A. Peppas, in *Biomaterials Science*, Edited by B. D. Ratner *et al.* (Elsevier Academic Press), p. 100, 2004.

[4] J. T. Zhang *et al.*, Colloid and Polymer Science, Vol. 283, p. 461, 2005.

[5] L. Serra, J. Domenech, and N. A. Peppas, Biomaterials, Vol. 27, p. 5440, 2006.

[6] C. C. Lin, and A. T. Metters, Advanced Drug Delivery Reviews, Vol. 58, p. 1379, 2006.

[7] L. Brannon-Peppas, and N. A. Peppas, Chemical Engineering Science, Vol. 46, p. 715, 1991.

[8] J. Ricka, and T. Tanaka, Macromolecules, Vol. 17, p. 2916, 1984.

[9] M. Mahkam, Journal of Bioactive and Compatible Polymers, Vol. 19, p. 209, 2004.

[10] A. Afrassiabi, A. S. Hoffman, and L. A. Cadwell, Journal of Membrane Science, Vol. 33, p. 191, 1987.

[11] M. R. Guilherme *et al.*, Polymer, Vol. 44, p. 4213, 2003.

[12] H. G. Schild, Progress in Polymer Science, Vol. 17, p. 163, 1992.
[13] W. Xue, and I. W. Hamley, Polymer Vol. 43, p. 3069, 2002.
[14] L. C. Dong, and A. S. Hoffman, Journal of Controlled Release, Vol. 4, p. 223, 1986.
[15] B. Jeong, S. W. Kim, and Y. H. Bae, Advanced Drug Delivery Reviews, Vol. 54, p. 37, 2002.
[16] N. A. Peppas *et al.*, European Journal of Pharmaceutical Formulations, Vol. 50, p. 27, 2000.
[17] W. Xue *et al.*, European Polymer Journal, Vol. 40, p. 47, 2004.
[18] G. Chen, and A. S. Hoffman, Nature Vol. 373, p. 49, 1995.
[19] S. Biggs, J. Selb, and F. Candau, Langmuir, Vol. 8, p. 838, 1992.
[20] F. Candau *et al.*, Prog. Org. Coat. Vol. 24, p. 11, 1994.
[21] W. Siriwatwechakul, in *Chemical Engineering Department* (Princeton University, Princeton NJ), p. 173, 2005.
[22] S. R. Turner, D. B. Siano, and J. Bock, (Exxon Research & Engineering Company, United States), 1985.
[23] F. Candau, and J. Selb, Advances in Colloid and Interface Science, Vol. 79, p. 149, 1999.
[24] S. Biggs *et al.*, J. Phys. Chem., Vol. 96, p. 1505, 1992.
[25] E. Volpert, J. Selb, and F. Candau, Polymer, Vol. 39, p. 1025, 1998.
[26] J. Bock *et al.*, Abstracts of Papers of the American Chemical Society, Vol. 192, p. 77, 1986.
[27] J. Bock *et al.*, Advances in Chemistry Series, Vol. 411, 1989.
[28] C. Tanford, *The Effect of Temperature* (Joh Wiley & Sons, Inc., New York), p. 21, 1980.
[29] F. Candau, E. J. Regalado, and J. Selb, Macromolecules, Vol. 31, p. 5550, 1998.