Temperature-sensitive polyethyleneglycol / N-isopropylacrylamide hydrogels: Impact of material parameters on swelling and drug release

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Key words: Temperature sensitive hydrogels, polyethylene glycol/ N-isopropylacrylamide hydrogels, Insulin drug, Drug delivery.

http://dx.doi.org/10.12692/ijb/5.11.129-137 Article published on December 08, 2014

Abstract

In this study, we fabricated Temperature-sensitive polyethyleneglycol/N-isopropylacrylamide (PEG/NIPAm) hydrogels by a free-radical polymerisation method with variation in the content of monomer, polymer and cross-linking agent. Swelling was performed in USP phosphate buffer solutions of pH 7.4 for selected samples. It was observed that swelling and drug release from hydrogels can be modified by changing composition and degree of cross-linking of the hydrogels under investigation. As expected LCST increased from 34 to 39°C as PEG content in copolymers increased from 2 to 8% (w/w). The presence of polyethyleneglycol in the hydrogel formulation resulted in the higher mechanical strength and swelling. Network structure was evaluated by different parameters and FTIR confirmed the formation of cross-linked hydrogels.

The percent swelling, equilibrium swelling, diffusion constant values are evaluated for PEG/NIPAm hydrogels at 1% of Insulin solution at room temperature. Drug release increased by increasing PEG contents in hydrogels while increasing the concentration of cross-linking agent had the opposite effect. Based on the release kinetic of the Insulin drug, the hydrogels displayed a non-Fickian diffusion mechanism. According the diffusion kinetic data in hydrogels became clear that diffusion kinetic data were best described by Higuchi model. Permeation from PEG/NIPAm hydrogels followed a Super Case II transport mechanism, most likely driven by macro molecular chain relaxation and swelling of hydrophilic polymers.

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Introduction

Hydrogels are three-dimensional polymeric networks capable of sequential adsorption and desorption of water and physiological fluids can be in a variety of applications as smart materials in engineering science and medicine (Wei and cai, 2009). This is due to the presence of hydrophilic chains in the gel structure as associated with the ionic group on the chains, can become super-absorbent hydrogels with the ability to change in many different situations large volumes different environmental conditions. So with changing pH, ionic strength and temperature and the electric field can be used in a variety of super absorbent. Among the applications of nano-gels are sensitive to environmental stimuli can pointed out to separated proteins, biomolecular detection, separation of heavy ions in water, and rheology control of pharmaceutical and health care products, and transport of drugs, scaffolds for tissue engineering applications, cell culture and tissue fillers (Ma et al., 2010). Smart or intelligent polymer networks show sharp changes in response to physical stimuli like pH, temperature, ionic strength, electric or magnetic field (Baumann et al., 2009). Among many intelligent polymers poly (N-isopropylacrylamide) and demonstrate a lower critical solution temperature (LCST) of about 32 °C in aqueous medium. It undergoes a sharp coil to globule transition in water above the lower critical solution temperature (LCST = 32 °C) i.e. changing from a hydrophilic state to a hydrophobic state above LCST. This unique property is widely used in drug delivery, tissue engineering and biotechnology (Manna and Patil, 2009; Qiu and Bae, 2007). These hydrogels are rendered Temperature-sensitive by copolymerizing NIPAAm with PEG or basic polymers, often at low degree of substitution (Alli et al., 2012). Changes in PEG constant and hydrophilic/hydrophobic balance in these hydrogels, shifting the LCT above or below ambient temperature. The controlled drug delivery devices can assure a sustained release and targeted effect (Qiu and Park, 2001). The great advantage of the drug-controlled release from the hydrogels is a possibility for improvement of patient compliance (Park, 1993). In recent years, polyethylene glycol (PEG) and its copolymers have often been used as carriers in drug release systems, because of their multifunctional nature, unique properties and good biocompatibility (Devine et al., 2006; Dittgen et al., 1997). N-isopropylacrylamide/ polyethylene glycol hydrogels were synthesized by redox-initiated free radical polymerization in water at room temperature for 24 hours by using N-isopropylacrylamide) particles and acrylic acid as monomer, polyethylene glycol as macronomer, N,N'-methylene bisacrylamide (BIS) as crosslinking agent, APS as free-radical initiator and TEMED as accelerator. The dependence of swelling and release properties on percent of PEG and BIS were examined. In this study, Insulin was chosen as a model peptide drug for the investigation of drug release behavior of the NIPAM/PEG hydrogels. Insulin is a peptide drugs and these kinds of drugs are usually used for chronic conditions, and injections on a daily basis during long-term treatment has obvious drawbacks. It would be highly advantageous if insulin could be administered orally, because the oral delivery of insulin can mimic the physiological fate of insulin and may provide better glucose homeostasis. Almost since the initial discovery of insulin alternative effective routes other than subcutaneous injection have been an elusive goal for many investigators. The oral route is considered to be the most convenient and comfortable means for administration of insulin for less invasive and painless diabetes management, leading to a higher patient compliance (Krauland, A. H et al., 2009; Khafagy, El-S, 2007). Oral administration of insulin has some limitations, including low oral bioavailability due to degradation in the stomach, inactivation, and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity. Consequently, various approaches have been examined to overcome the delivery problems of these peptides when orally administered (Komplella, U. B et al., 2001). In recent years, synthesis of hydrogels was studied based on poly (HEMA-MAA) Hydrogel AS Carrier for Oral Delivery of Insulin (Priyanka et al., 2011). In other works, synthesis of poly(N-isopropylacrylamide) hydrogels and their application was studied for the
control release of anticancer drugs (Castro et al., 2012). However, in this work, we have studied drug release behavior of NIPAAm/PEG hydrogels. The effect of Insulin solution on swelling characteristics NIPAAm/PEG hydrogels have also been investigated. Insulin was trapped in the gels by its inclusion in the polymerization mixture. To incorporate the Insulin to the feed mixture of polymerization, water solutions of Insulin were used. The controlled release profiles were followed by UV-Vis spectroscopy and the mechanisms of drug release by diffusion were modeled. These measurements are aim of this work and are made with the purpose of characterizing these hydrogels as drug delivery systems.

Materials and methods

Materials and instruments

Specification of materials used in the construction of the hydrogel are presented in (Table 1). Raw materials used are purchased without any additional processing. Deionized water was used in all experiments. UV-visible absorption spectra were recorded on HP8251 spectrophotometer. Table 1.

Preparation of copolymeric hydrogels

We synthesised a series of cross-linked hydrogels of NIPAAm/PEG by a previously reported method at room temperature using BIS as cross-linker and APS as initiator by redox-initiated free radical polymerization (Adimi et al., 2014). The samples were prepared by varying amounts of PEG in the initial feed ranging from 2 to 8% w/w and 1.77 to 5% w/w for BIS. LCST of NIPAAm /PEG hydrogels increased with increasing PEG. In order to eliminate any unreacted monomer, oligomer and non-cross-linked polymer chains, each sample was washed in excess water for 3 days. Extracted gels were dried in freeze drier. Copolymer gels have been designated as NP by a previously reported method and then returned to the vials with 1 mL of fresh water or solution. The swelling percent (%ESR) was estimated by comparing the ratio of the wet hydrogel weight (M_wet), which was measured at the various time intervals, to the initial dry hydrogel weight (M_dry), which was measured before the swelling study began (Brahim et al., 2003):

\[
\text{%ESR} = \left( \frac{M_{\text{wet}} - M_{\text{dry}}}{M_{\text{dry}}} \right) \times 100 \% \quad (1)
\]

Drug loading and release experiments

The dry hydrogels were equilibrated in a of Insulin solution (1 ml drug in 20ml water) at 4 °C for 24 hr in a dark environment (in order to avoid degradation). For drug release experiments, previously incubated drug gels were placed in a vessel containing 50 mL of water at a constant temperature (37 ± 0.1 °C) with a constant shaking rate. The amount of released drug was determined by UV-Vis spectrophotometer (HP8251) with a quartz cuvette at an absorbance wave length of 271 nm. In order to determine the concentration of Insulin, absorbance Insulin solutions were prepared in phosphate buffer with different concentrations and concentration (x, in 10^-3 g/mL) versus absorbance values (y) at 271nm curves were plotted. The amount of release drugs at any time was calculated from the Insulin standard calibration line

\[
y = 183.3x + 0.09 \quad \text{with a linear regression coefficient of} \quad R^2 = 0.9998.
\]

For examining the release of Insulin from NIPAAm/PEG hydrogels, percentage release of the drug was calculated from the following equation(Song et al., 2008; Zhang et al., 2004):

\[
\text{%Release} = \left( \frac{W_i}{W_{\text{total}}} \right) \times 100 \quad (2)
\]

Where Wi is the weight of released drug in water at any time and W_total is the initial total weight of the loaded drug by the gel system.

Results and discussion

Swelling properties

First, blank hydrogels (1cm length, 2.5mm diameter) were freshly made and then dried in freeze drier for 24 hr at -20°C. The swelling studies were carried out in triplicate by placing of blank gels in 50 mL water at room temperature. In time intervals, the gels were removed, gently dried with a kim-wipe and weighed, and then returned to the vials with 1 mL of fresh water or solution. The swelling percent (%ESR) was estimated by comparing the ratio of the wet hydrogel weight (M_wet), which was measured at the various time intervals, to the initial dry hydrogel weight (M_dry), which was measured before the swelling study began (Brahim et al., 2003):

\[
\text{%ESR} = \left( \frac{M_{\text{wet}} - M_{\text{dry}}}{M_{\text{dry}}} \right) \times 100 \% \quad (1)
\]
A fundamental relationship exists between the nature of the polymer and the solvent and swelling of a polymer in a solvent. Swelling curves in water with change of PEG content are shown in Fig. 1, swelling curves in water with change of BIS content are shown in Fig. 2. As can be seen from the figure, the swelling capabilities of the hydrogels are increased by time, reaching constant swelling (equilibrium swelling) after a certain period of time. It is indicate that the equilibrium percentage mass swelling of NIPAAm/PEG hydrogels in water increased from 800 to 1400 as PEG content increased from 2 to 8%(w/w). The equilibrium swelling percentages of NIPAAm/PEG hydrogels in deionized water, are given in Table 3. As expected, the swelling ratio of copolymer samples increased with increasing amount of PEG content (ranging from 2 to 8% w/w) in the copolymers. Table 3.

Table 1. The materials used in the preparation of the hydrogel and drug delivery.

<table>
<thead>
<tr>
<th>Material</th>
<th>Chemical Formula</th>
<th>Molecular Weight</th>
<th>Their role in the hydrogel</th>
<th>Appearance</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG 1000</td>
<td>HO(C₂H₄O)₄H</td>
<td>1000 gr/mol</td>
<td>Base Polymer</td>
<td>Clear liquid</td>
<td>Merk</td>
</tr>
<tr>
<td>N-isopropylacrylamide</td>
<td>C₆H₁₂NO</td>
<td>113.16 gr/mol</td>
<td>Base Monomer</td>
<td>White Solid</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>N,N-methylene bisacrylamide</td>
<td>C₁₀H₁₀N₂O₂</td>
<td>154.17 gr/mol</td>
<td>Cross linker</td>
<td>White powder</td>
<td>Merk</td>
</tr>
<tr>
<td>Ammonium persulfate</td>
<td>(NH₄)₂S₂O₅</td>
<td>228.18 gr/mol</td>
<td>Initiator</td>
<td>White Crystals</td>
<td>Yellowish Merk</td>
</tr>
<tr>
<td>Tetramethylenediamin</td>
<td>C₆H₁₆N₂</td>
<td>116.2 gr/mol</td>
<td>Accelerator</td>
<td>Colless liquid</td>
<td>Merk</td>
</tr>
<tr>
<td>Insulin</td>
<td>C₅₃H₇₇N₆O₇S₆S₆</td>
<td>5807/57 gr/mol</td>
<td>Drug</td>
<td>Milky liquid</td>
<td>Exir Pharmaceutical co</td>
</tr>
</tbody>
</table>

A significant increase in swelling was observed as PEG concentration was increased from 2 to 4.1%. This could be due to the more hydrophilic nature of PEG as compared to PNIPAAm. Further increase in PEG content from 4.1 to 5.4% (w/w) resulted in a marginal increase in swelling. Here the maximum swelling capacity of hydrogels might be reached. On the other hand the temperature sensitivity of the system decreased with increasing amount of PEG (Fig. 3). Fig. 1, Fig. 2, Fig. 3.

Table 2. Details of feed composition and sample designation for hydrogels prepared using redox free-radical polymerization and water.

<table>
<thead>
<tr>
<th>Sample Designation</th>
<th>NIPAAm(wt%)</th>
<th>PEG(wt%)</th>
<th>BIS(wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1</td>
<td>8.56</td>
<td>2</td>
<td>1.71</td>
</tr>
<tr>
<td>NP-2</td>
<td>8.56</td>
<td>4.1</td>
<td>1.71</td>
</tr>
<tr>
<td>NP-3</td>
<td>8.56</td>
<td>5.4</td>
<td>1.71</td>
</tr>
<tr>
<td>NP-4</td>
<td>8.56</td>
<td>6.66</td>
<td>1.71</td>
</tr>
<tr>
<td>NP-5</td>
<td>8.56</td>
<td>8</td>
<td>1.71</td>
</tr>
<tr>
<td>NP-1(1)</td>
<td>8.56</td>
<td>4.1</td>
<td>2.54</td>
</tr>
<tr>
<td>NP-1(2)</td>
<td>8.56</td>
<td>4.1</td>
<td>3.04</td>
</tr>
<tr>
<td>NP-1(3)</td>
<td>8.56</td>
<td>4.1</td>
<td>4.01</td>
</tr>
<tr>
<td>NP-1(4)</td>
<td>8.56</td>
<td>4.1</td>
<td>5</td>
</tr>
<tr>
<td>NP-1(5)</td>
<td>8.56</td>
<td>4.1</td>
<td>1.71</td>
</tr>
</tbody>
</table>

The numerals within parenthesis represents the samples prepared using varying amounts of BIS.
Release mechanism

Hydrogel matrixes are considered swelling-controlled systems, because the drug release is controlled by the inward flux of solvent (Pepas et al., 2000). These swelling-controlled systems are often analyzed with Fickian and non-Fickian diffusional behavior kinetics.

Equation (3) displays the simplified expression for Fickian and non-Fickian diffusion that the Insulin Release data can be fitted against (Pepas et al., 2000; Pepas et al., 1980):
\[
\ln E = \ln \left( \frac{M_t}{M_\infty} \right) = n \ln t + C
\]

Table 3. The equilibrium swelling percentages of NIPAAm/PEG hydrogels in deionized water at room temperature with change of PEG wt% (BIS wt% = 1.71).

<table>
<thead>
<tr>
<th>Gel name</th>
<th>Equilibrium mass swelling (%) in distilled water</th>
<th>EWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1</td>
<td>800</td>
<td>0.875</td>
</tr>
<tr>
<td>NP-2</td>
<td>1000</td>
<td>0.9</td>
</tr>
<tr>
<td>NP-3</td>
<td>700</td>
<td>0.857</td>
</tr>
<tr>
<td>NP-4</td>
<td>1200</td>
<td>0.916</td>
</tr>
<tr>
<td>NP-5</td>
<td>1400</td>
<td>0.928</td>
</tr>
</tbody>
</table>

Here E is the cumulative fraction of drug release, \( \frac{M_t}{M_\infty} \), where \( M_t \) is the amount of diffusional absorbed at time t, \( M_\infty \) is the maximum amount absorbed; C is the rate constant characteristic of the system, and n is the diffusional exponent (Brahim et al., 2003). Eq.(3) can only be applied to the first 60% of drug release. The diffusional exponent (n) is calculated as the slope and the rate constant (C) is calculated as the intercept of linear regression lines fitted to the \( \ln E \) versus \( \ln t \) plots. A calculated n equal to 0.5 represents Fickian diffusion, when the rate of diffusion is slower than the relaxation one, so we have a diffusion-controlled drug release, while a calculated n greater than 0.5 represents non-Fickian diffusion occurs, when the diffusion and relaxation rates are comparable. In this case, the drug release behaviour can be regarded as the superposition of both phenomena (Wei and Cai, 2009). Therefore, using the calculated n value, the diffusional behavior of the hydrogel release can be determined. The plots of \( E \) versus t for the series of NIPAAm/PEG hydrogels in Insulin solution are shown in Fig.4, respectively and the exponents n and C values were calculated from the slope and intercept of the lines, respectively. The data were collected in Table 4.

Table 4. Effect of PEG and BIS on the insulin release mechanism of NIPAAm/PEG hydrogels with peppas model.

<table>
<thead>
<tr>
<th>GEL SAMPLE</th>
<th>n</th>
<th>C</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-1</td>
<td>0.547</td>
<td>0.042</td>
<td>0.935</td>
</tr>
<tr>
<td>NP-2</td>
<td>0.589</td>
<td>0.023</td>
<td>0.965</td>
</tr>
<tr>
<td>NP-3</td>
<td>0.334</td>
<td>0.147</td>
<td>0.975</td>
</tr>
<tr>
<td>NP-4</td>
<td>0.29</td>
<td>0.193</td>
<td>0.97</td>
</tr>
<tr>
<td>NP-5</td>
<td>0.278</td>
<td>0.223</td>
<td>0.93</td>
</tr>
<tr>
<td>NP-1(1)</td>
<td>0.592</td>
<td>0.033</td>
<td>0.967</td>
</tr>
<tr>
<td>NP-1(2)</td>
<td>0.627</td>
<td>0.025</td>
<td>0.98</td>
</tr>
<tr>
<td>NP-1(3)</td>
<td>0.614</td>
<td>0.021</td>
<td>0.98</td>
</tr>
<tr>
<td>NP-1(4)</td>
<td>0.65</td>
<td>0.016</td>
<td>0.985</td>
</tr>
<tr>
<td>NP-1(5)</td>
<td>0.688</td>
<td>0.013</td>
<td>0.97</td>
</tr>
</tbody>
</table>

It is clear from the analysis that as the PEG content in the gel structure increases from 2%(w/w) to 8%(w/w), the diffusional release kinetic exponent n increases from 0.547 to 0.278 for NIPAAm/PEG hydrogels but if PEG content very increased n decreases because the hydrogel toughness increases...
with increasing acid and release rate decreases. \( n \) values are specified that diffusion of Insulin solutions into NIPAAm/PEG hydrogels was assumed to be non-Fickian character. Diffusion coefficients are important permeation parameters of some chemical species to polymeric systems. Using “\( n \)” and “\( C \)”, the diffusion coefficient (D) of solvent in the matrix could be calculated using the following equation (Brahim et al., 2003):

\[
e^{- C} = \frac{4 \left[ D / \pi r^2 \right]^n}{n}
\]

Equation (4)

Fig. 1. The equilibrium swelling percentages of NIPAAm/PEG hydrogels in deionized water at room temperature at different percent of PEG.

Where “D” is the diffusion coefficient and “\( r \)" is radius of gel disc. Diffusion coefficients of hydrogels in solutions of Insulin are also listed in Table 4. As expected, the diffusion coefficients increases with an increase in equilibrium mass swelling of the present hydrogel in the solutions.

Equilibrium water Content

Equation (5) displays the water absorbed by PNIPAAm and NIPAAm/PEG hydrogels is quantitatively represented by the equilibrium water content (EWC) (Wei and Cai, 2009):

\[
\text{EWC} = \frac{W_{eq} - W_0}{W_{eq}}
\]

Equation (5)

Where \( W_{eq} \) is the weight of the swollen gel at time \( t \) (equilibrium) and \( W_0 \) is the weight of the dry gel at time0. The EWC values of the hydrogels were calculated and tabulated in Table 3. All EWC values of the hydrogels (0.85–0.93) were greater than the percent values of body about 0.6. Thus, the NIPAAm/PEG hydrogels exhibited fluid contents similar to those of living tissues.

Fig. 3. Effect of PEG content on the swelling ratio in NIPAAm/PEG hydrogels with BIS(1.71% wt).

Release behavior of hydrogels

Fig. 4. In vitro release profiles of Insulin in NIPAAm/PEG hydrogels with different percentages of PEG in deionized water at 37°C.

Fig. 5. In vitro release profiles of Insulin in NIPAAm/PEG hydrogels with different percentages of BIS in deionized water at 37°C.
The release profiles of Insulin in NIPAAm/PEG hydrogels in water at 37°C with different content PEG and BIS are shown in Fig. 4 and Fig. 5. The fractional Insulin release, expressed as $M_t / M_\infty$, where $M_t$ and $M_\infty$ are the amounts of drug released at the times $t$ and infinite, respectively, as a function of time for the hydrogels. In this figure, the drug release during the first stage could be influenced for the relaxation of polymer chains. Thus, the values for $n$, or the slope of the linear regression lines fitted to the ln $E$ versus ln $t$ plots, all resulted in values greater than 0.5 (Table 4), suggesting non-Fickian diffusion. Non-Fickian diffusion is desirable as it indicates that the media penetration rate is in the same range as drug diffusion (Tasdelen et al., 2005).

One of the most attractive features of PNIPAAm based hydrogels as drug carriers is their intelligent property to external temperature changes. Thus, the effect of temperature is important factor to the hydrogel’s drug release. Optimum release should occur around 37 °C for the hydrogel to be effective inside the body’s conditions. It is important and practical to examine the drug release data from those NIPAAm/PEG hydrogels at a temperature LCST like the body temperature (37 °C) (Aoshima and Kanaoka, 2008). Fig. 4, Fig. 5.

**Conclusion**

This research is a first step towards finding an efficient drug delivery system for peptid drugs. The experiments explored the use of hydrogels for Insulin as a model peptid drugs release by analyzing the synthesis and temperature-responsive characteristics of the NIPAAm/PEG hydrogels the ability to promote Insulin release into the surrounding environment, and the significant factors involved in optimal drug release. The results from these experiments support the following conclusions:

1) The equilibrium percentage swelling of the NIPAAm/PEG hydrogels in Insulin solutions increased from 800 to 1000 as PEG content increased from 2 to 4% (W/W). This has been explained due to the incorporation of more specific acidic groups into the Network and consequent higher swelling capacity of the gels.

2) In the diffusion transport mechanism study, Using the Insulin release data at various BIS and PEG ratios and fitting it to the Fickian and non-Fickian diffusion Eq.(3) The values for $n$ is found to be over 0.5 for the hydrogels. This implies that the swelling transport mechanism is a non-Fickian transport. The fractional cumulative release of the drug from the hydrogels have showed an initial non-Fiction behavior, probably indicating a comparable rates of Fiction diffusion and polymer relaxation. This finding is confirmed by similarities between our swelling ratio and Insulin release profiles.

3) When loading and releasing the drugs, pore size of the hydrogel decreased and increased, respectively, without reaching the initial pore size of the hydrogel. The result show that the greater concentration of drug loaded into the hydrogel, the greater reduction in pore size.

4) NIPAAm/PEG hydrogels easily absorbed and released Insulin, and its release was temperature-responsive.

5) Phase transition temperature increased with increasing amount of PEG and attained a temperature of 37 °C (which is ideal temperature for drug release) after copolymerization with 4.1% (w/w) of PEG. Copolymer composition had a large effect on release of Insulin. Copolymers of NIPAAm prepared using 4.1% (w/w) of PEG as polymer. sample NP-1(5) showed an optimum combination of physical properties and performance.

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http://dx.doi.org/10.12692/ijb/5.2.183-191


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