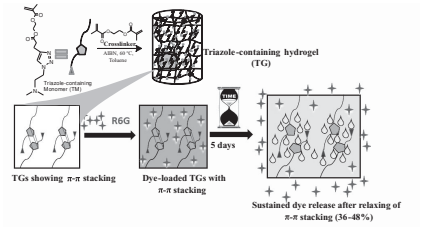
Triazole-Containing Hydrogels for Time-Dependent Sustained Drug Release

Vivek Mishra, Seo-Hyun Jung, Jong Mok Park, Han Mo Jeong, Hyung-il Lee*

The purpose of this study is to develop novel triazole-containing hydrogels (TGs) as drug carrier and to investigate the sustained drug release accomplished by their time-dependent swelling behavior. The synthetic pathway of TGs includes: (1) DCC-coupling on hydroxyethyl methacrylate (HEMA) to prepare HEMA-alkyne (HA), (2) click-coupling to prepare a triazole-ring-containing monomer (TM), and (3) the synthesis of a series of TGs. The aggregation between triazole rings is found to be responsible for drug release controllability. Rhodamine 6G is studied as a model anticancer drug for release experiments. The effects of pH and temperature on the properties of sustained drug release are also studied.



1. Introduction

Drug vehicles[1–4] have a significant impact on the treatment and potential cure of many chronic diseases, including cancer,[5] diabetes,[6] and drug addiction.[7,8] However, the poor controllability and fast release of drugs make it difficult to achieve the essential requirements and can damage the therapeutic expression of drugs.[9] Normally, drugs are managed at a high dose at a given time. This is not economical and results in side effects. Sustained release technology allows patients to take certain types of medication less frequently. Increasing attention has been focused on methods to render drug release for a prolonged period of time in a controlled fashion.

There are different types of polymeric drug delivery vehicles, including hydrogels,[10–13] homopolymers,[14,15] copolymers,[16,17] polymeric micelles,[18–21] liposomes,[22] nanoparticles,[23] and dendrimers.[24] During the last few decades, much effort has been focused on designing hydrogels for controlled drug release.[25] Hydrogels are a topic of interest for biomaterial researchers due to their biocompatibility, biodegradability, and hydrophilicity.[26–29] Hydrogels can swell up to many times their dry weight depending on the nature of their functional moieties, cross-linking density, and pH environment. To achieve better chemotherapeutic outcomes, hydrogels should be able to release drugs to a specific site while maintaining concentration at the optimal level required for effectiveness over a specific period of time.[30]

In this study, we report the preparation of triazole-containing hydrogels (TGs) that swell gradually in water. The time-dependent increase in swelling was attributed to hydrophobic aggregations caused mainly by π–π stacking[12,31] between triazole rings. This structure was gradually disrupted by water molecules penetrating into the hydrophobic domain, which allowed hydrogen bond formation between one of the nitrogen atoms on the triazole ring and the surrounding water molecules.[12] To the best of our knowledge, this is the first observation of gradual hydrogel swelling in water due to time elapsing and without chemical modifications such as hydrolysis. One of our ultimate goals is to develop a universal drug carrier with time-dependent drug release properties. This triazole-ring-containing hydrogel and its environmental responsive behavior can provide a route for time-dependent sustained drug release.

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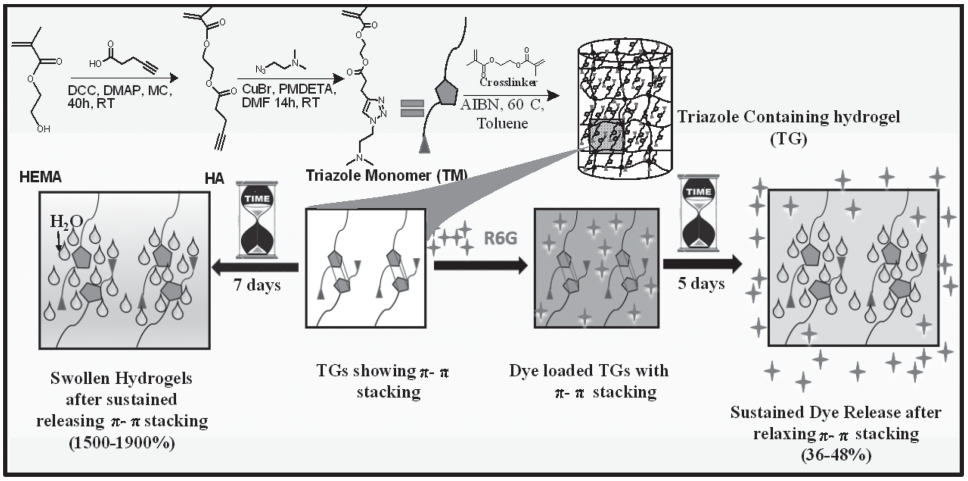

Scheme 1. Synthetic route for triazole-containing hydrogels (TGs) and their sustained swelling and drug release behaviors.

2. Experimental Section

2.1. Materials and Methods

Details are described in the Supporting Information.

2.2. Synthesis of Triazole-Containing Monomer (TM)

The synthetic pathway of TM included: (1) DCC-coupling of hydroxyethyl methacrylate (HEMA) using 4-pentynoic acid to prepare HEMA-alkyne (HA), (2) synthesis of 2-azido-1-ethyldimethylamine, and (3) click-coupling of HA using 2-azido-1-ethyldimethylamine to prepare a triazole-ring-containing monomer (TM) (Scheme 1). A detailed procedure is given below.

2.2.1. Synthesis of HEMA-Alkyne

HEMA (5 mL, 0.038 mol), N,N'-Dicyclohexylcarbodiimide (DCC) (9.5 g, 0.046 mol), and 4-pentynoic acid (4.5 g, 0.046 mol) were added sequentially into a 100 mL round-bottomed flask containing 45 mL of methylene chloride (MC). The flask was immersed into an ice bath. 4-Dimethylaminopyridine (DMAP) (0.234 g; 0.002 mol) in 5 mL of MC was added to the mixture within 5 min.The reaction mixture was stirred for 40 h at room temperature. The product was extracted with water to remove excess pentynoic acid. After removing pentynoic acid, MC was evaporated. The resulting product was dried under nitrogen (yield: 88%, liquid product). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 6.06 (s, H_c); 5.60–5.42 (m, H_b); 4.42–4.19 (m, $H_{d'}$); 2.516–2.474 (m, J = 2.5, 2.7 Hz, H_g); 2.467–2.445 (d, J = 1.1 Hz, H_j); 1.93–1.89 (m, H_h); 1.86–1.83 (m, H_a).

2.2.2. Synthesis of HEMA-Alkyne-Click-2-Azido-1-Dimethylamine (Triazole Monomer)

The ratio of reagent [HA]/[2-azido-1-ethyldimethylamine]/[CuBr]/[PMDETA] was 1:1:0.01:0.01. The click reaction between HA (2 g, 9.5 mmol), 2-azido-1-ethyldimethylamine (1.1 mL, 9.5 mmol), and PMDETA (20 μL, 0.095 mmol) was conducted in a dry Schlenk flask containing 3 mL of DMF. The reaction mixture was degassed by three freeze-pump-thaw cycles. CuBr (0.0137 g, 0.095 mmol) was added into the freeze-dried mixture and then left in argon and stirred at room temperature for 10 h. The resulting solution was exposed to air, diluted with MC, and passed through neutral alumina to remove the copper catalyst. MC was then evaporated, resulting in pure liquid monomer. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 7.43 (s, H_i); 6.07 (d, J = 0.8 Hz, H_c); 5.61–5.51 (m, H_b); 4.45–4.31 (m, H_j); 4.30 (s, $H_{d'}$); 3.00 (t, J = 7.3 Hz, H_k); 2.766–2.662 (m, $H_{h,g}$); 2.23 (s, H_l); 1.918–1.880 (d, J = 0.9 Hz, H_a).

2.3. Synthesis of Triazole-Based Hydrogels

Hydrogels were prepared by free radical polymerization using an azobisisobutyronitrile (AIBN) initiator. Four components were used to prepare the triazole-ring-containing hydrogel, namely TM, EGDMA, AIBN, and toluene solvent. To prepare monomer mixtures, TM was first mixed with EGDMA in three different ratios of 50:1 (TG-50), 100:1 (TG-100), and 200:1 (TG-200). These solutions were mixed with AIBN-containing toluene solvent. The amount of toluene in all final mixtures was 5% (w/w). Prepared monomer solutions were placed in long cylindrical tubes with 4 mm inside diameters. After sealing the tubes with rubber caps, the solution was purged with oxygen-free nitrogen gas for 30 min. The reaction mixture was placed in a preheated oil bath at 60 °C. Polymerization reactions were carried out for 4 h at 60 °C. In all experiments, AIBN and solvent concentrations were 1% (mole) and 5% (mole) of the total amount of monomers, respectively. Hydrogels obtained as long cylindrical shapes were cut into round pieces 4 mm in diameter for swelling, dye absorption, and release studies.

2.4. Synthesis of DG Hydrogels

The same procedure as above was followed to prepare different ratios of *N,N*-dimethylaminoethyl methacrylate (DMAEMA) hydrogels, DGs (namely DG-50, DG-100, DG-200) with 50:1; 100:1, and 200:1 ratios with respect to an EGDMA cross-linker.

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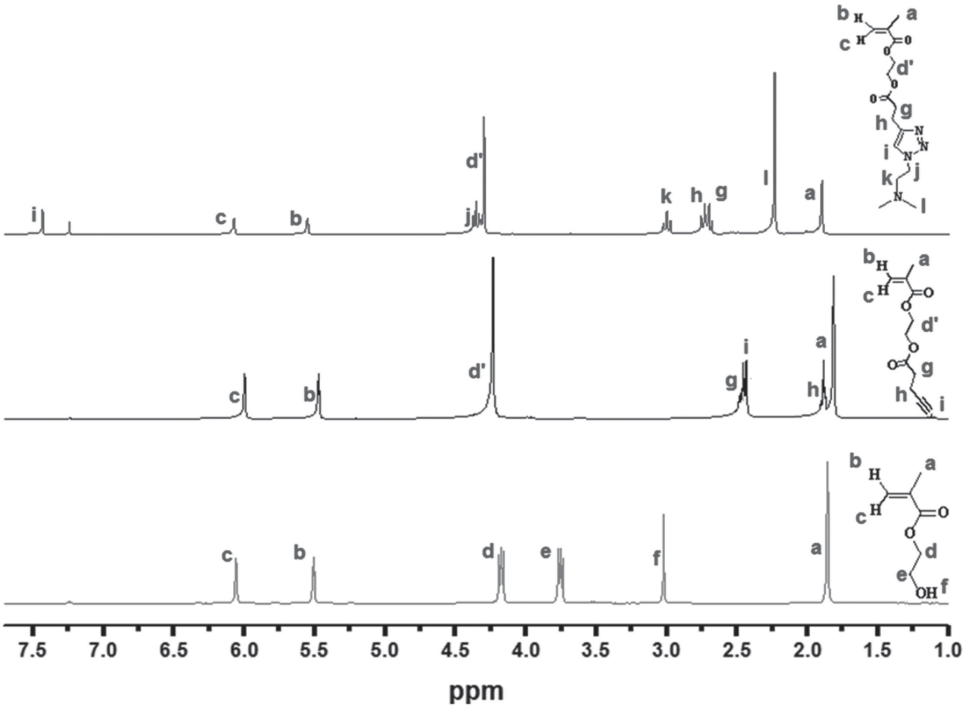

Figure 1. ^{1}H NMR spectra of HEMA, HA, and TM.

3. Results and Discussion

The synthesis of 2-azidoethyl dimethylamine involved reacting NaN_3 with 2-chloroethylamine hydrochloride in water. The ^{1}H NMR spectrum confirmed the successful synthesis of azido product. HA was synthesized using a DCC-coupling reaction between HEMA and 4-pentynoic acid. The presence of H_g, H_h, and H_i proton peaks at 2.516–2.474 (H_g), 2.467–2.445 (H_i), and 1.93–1.89 ppm (H_h), respectively, confirmed successful HA synthesis.

TM was synthesized by reacting HA with 2-azidoethyl-dimetylamine in the presence of CuBr/PMDETA in DMF. The presence of a triazole ring proton at 7.43 ppm (H_i) and a dimethyl proton at 2.23 ppm (H_l) confirmed the formation of TM (Figure 1). Free radical polymerization in toluene was employed at 60 °C to prepare DG and TG hydrogels with different cross-linking density. The obtained gels were washed several times with acetone and then immersed in acetone for several days. They were then washed in water to remove all reactants and dried for further analyses.

To study swelling behavior, dehydrated hydrogels were immersed in distilled water at 25 °C. Swollen gels were taken out of the medium at regular intervals, dried superficially with filter paper, and weighed. These measurements were continued until a constant weight was reached for all samples. DG-50, DG-100, and DG-200 hydrogels completely swelled during initial swelling measurements up to 4 h. The swelling ratios of a series of DGs ranged from 130% to 160% depending on cross-linking density. As the cross-linking density decreased from DG-50 to DG-200, the polymer swelling capability increased. Conversely, TGs showed slow swelling due to hydrophobic aggregation caused by strong π–π stacking between triazole ring side chains. This hydrophobic aggregation might have decreased water absorption. As time progressed, water molecules slowly penetrated the hydrophobic portion of hydrogels, leading to controlled relaxation of hydrophobic aggregation. During this 4 h period of aggregation, the swelling ratios (%) of TGs were smaller than those of DGs. After 4 h, a series of TGs with different cross-linking densities swelled continuously for up to 7–8 days. Their maximum swelling ratios ranged from 1500% to 1900% (Figure 2).

A highly water-soluble dye, Rhodamine 6G (R6G), was chosen as a model drug for loading and in vitro release studies. It is regarded as a model drug for the most commonly used anti-cancer drug, doxorubicin, since it has similar molecular weight and charge characteristics. The loading efficiency of R6G in hydrogel was determined by measuring the absorbance maximum of R6G at 525 nm. Equal amounts (0.01 g) of each DG (DG-50, DG-100, and DG-200) and TG were used (TG-50, TG-100, and TG-200) for the dye loading and release analysis. Fixed amounts of 0.01 g DGs and TGs were immersed in 10 mL aqueous R6G dye solution with an initial concentration of 110 mg L^{-1}. Dye

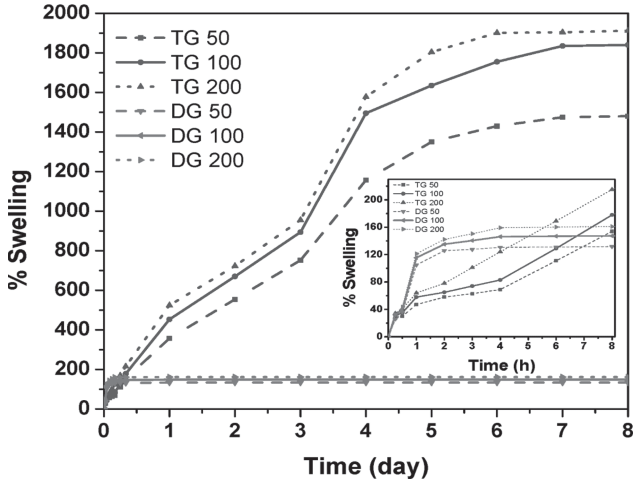

Figure 2. Swelling (%) as a function of time for DGs and TGs; (inset) initial % swelling up to 8 h.

absorption was monitored by the steady decrease in absorbance over time due to R6G physically absorbing into gels.

It was observed that 3%–5% of the dye was loaded in DGs, while 3%–6% was loaded in TGs. The results showed similar drug-loading levels for both series of gels. DGs absorbed a maximum amount of R6G of 3.6–5.3 mg g^{-1}, while TGs absorbed 3.7–5.9 mg g^{-1} of R6G with respect to increasing cross-linking density (Figure S2, Supporting Information).

The release of R6G from DGs and TGs was investigated under different conditions, with (i) variable DG and TG cross-linking density at 25 °C and (ii) variable stimuli changes related to pH (pH 5–7) and temperature (25–37 °C).

Figure 3 shows the different dye release behaviors of all six hydrogels (three DGs and three TGs) at 25 °C and pH 7. Dye release is facilitated by the swelling behavior of gels. As DGs and TGs cross-linking density decreased, swelling efficiency increased, which in turn caused the dye release efficiency to increase.

In the initial 4–8 h, the percent of dye released from DGs (≈3%–5%) was higher than that of TGs. The dye release of TGs was slower than that of DGs due to slow water penetration. This is because of the π–π stacking of triazole rings and hydrophobic aggregation into polymer matrices. Time-dependent TG swelling was mainly due to the relaxation of π–π stacking in triazole rings and sustained water absorption, which caused the polymers to slowly straighten. Once water was absorbed in the matrix, the triazole rings were free to bind with more water molecules. Subsequently, the percent of dye released gradually increased in a sustained manner. After 8–12 h, the dye released from the DGs stopped, whereas dye release from TGs was continuous for up to 4 d with an efficiency of 36%–48%.

TG-100 and DG-100 were chosen for a pH and temperature-triggered release study. The release of R6G from hydrogels was monitored at different time periods using a UV–vis spectroscopy. Figure 4 shows the pH-sensitive dye release behavior of DGs and TGs in pH 7 and pH 5 solutions at 25 °C. For a DG-100 solution at pH 7, ≈5% of the dye was released in 1 day. Conversely, ≈15% of dye was released in 1 day for a TG-100 solution at pH 7. For up to 5 days, the amount of dye released from TG-100 increased continuously in a sustained manner up to 39%. There was no further noticeable increase in DG-100 dye release over this time period. In acidic medium (pH 5), free dimethyl amino groups in the polymeric chain were quarternized, increasing the hydrophilicity of polymer chains. This increase in hydrophilicity facilitated dye escape from gels.

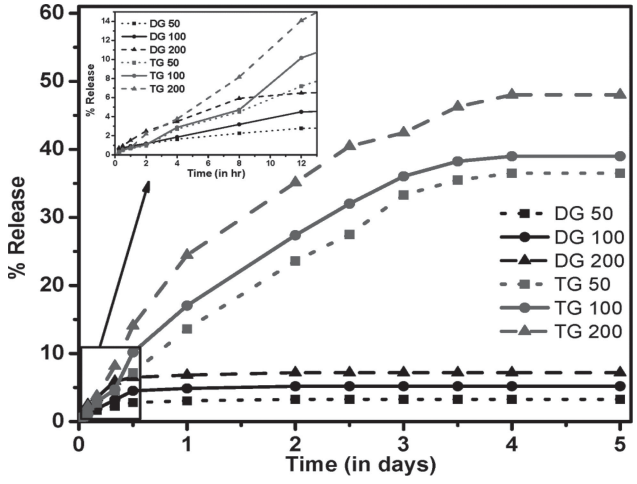

Figure 3. Plot of time versus percent dye release showing cumulative R6G release from DGs and TGs of variable cross-linking density at room temperature (25 °C) and pH 7 for 5 days; Inset: dye release from DGs and TGs for initial 12 h.

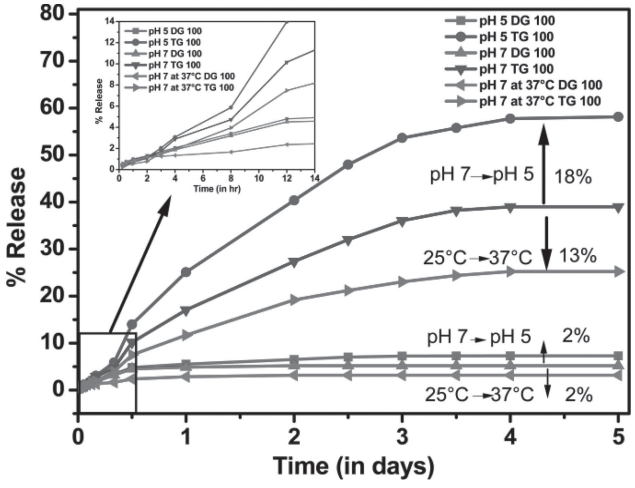

Figure 4. Cumulative R6G release from DGs and TGs at variable pH and temperatures plotted as time versus percent dye release. (Inset) Cumulative percent dye release from DGs and TGs up to 14 h at variable pH and temperatures.

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Auto-adjustable efficiency with temperature change is a very attractive feature of thermoresponsive hydrogels as drug carriers. For this reason, it was practical to observe TG-100 and DG-100 drug release behaviors at body temperature (37 °C) and room temperature (25 °C). As temperature increased from 25 to 37 °C, the polymer network shrunk due to lower critical solution temperature (LCST) properties. The decrease in percent dye release from TGs and DGs was 13% and 2%, respectively. Overall, the results show that these hydrogels respond to both temperature and pH stimuli to control drug release.

4. Conclusions

We prepared a series of hydrogels on the basis of the polymerization of a triazole-ring-containing monomer. The triazole-ring-containing hydrogels showed continuous swelling up to 7 days and sustained model drug release behavior. These properties are due to the gradual disruption of hydrophobic aggregation caused by π–π stacking between triazole ring side chains. As time elapses, hydrophobic aggregation relaxed due to interactions with slowly penetrating water molecules. Dye release studies gave valuable insights into delivering drugs in a controlled manner. These hydrogels exhibit obvious thermal and pH sensitivity, which allows for stimuli-triggered drug release. The overall results suggest that this triazole-ring-containing hydrogel is a good candidate as a drug carrier for maintaining therapeutic drug levels through slow and prolonged release.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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