JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

# *In situ* Crosslinkable Thiol-ene Hydrogels Based on PEGylated Chitosan and β-Cyclodextrin

**Mehmet Arslan*, Tolga Yirmibesoglu, Mithat Celebi**

[1]University of Yalova, Faculty of Engineering, Department of Polymer Engineering, 77200, Yalova, Turkey.

**Abstract:** Novel β-Cyclodextrin incorporated injectable hydrogels employing PEGylated chitosan as bio-based hydrophilic matrix have been fabricated via thiol-ene reaction. As thiol bearing polymer counterpart of hydrogel precursors, native chitosan was firstly modified with polyethylene glycol groups to increase its water solubility and bioinertness and then decorated with thiol groups to facilitate thiol-ene crosslinking with acryloyl-modified β-cyclodextrin. A series of hydrogels with varying amounts of acryloyl β-CD and PEGylated chitosan feed were synthesized with high efficiency under mild aqueous conditions. The resulting hydrogels were characterized by equilibrium swelling, structural morphology and rheology. These materials were investigated as controlled drug release platforms by employing a poorly water soluble anti-inflammatory drug diclofenac as model compound. Benefiting from the inclusion complex formation of the drug with β-CD groups in gel interior, prolonged release profiles were maintained. The total drug absorption and release of hydrogels were shown to be dependent on the amount of β-CD in gel matrix. These hydrogels combined efficient crosslinking and β-CD incorporation into clinically important chitosan scaffold and might have potential applications as injectable drug reservoirs such as in regenerative tissue engineering.

**Keywords:** Drug releasing hydrogels, β-cyclodextrin, thiol-ene crosslinking, injectable gels.

**Submitted:** September 15, 2018. **Accepted:** November 03, 2018.

**Cite this:** Arslan M, Yirmibesoglu T, Celebi M. In situ Crosslinkable Thiol-ene Hydrogels Based on PEGylated Chitosan and β-Cyclodextrin. JOTCSA. 2018;5(3):1327–36.

**DOI:** http://dx.doi.org/10.18596/jotcsa.460275.

***Corresponding author.** E-mail: mehmet.arslan@yalova.edu.tr, Tel: +90-226-811-5959.

## INTRODUCTION

Injectable hydrogel formulations that possess *in situ* formation of crosslinking process at target contour have been of interest, especially in regenerative medicine, tissue engineering, and drug delivery (1–8). Contrary to the conventional pre-formed hydrogels or scaffolds that require proper surgical implantation to the defect site, *in situ* gel forming systems procure to reach the formulation into deep tissues with maximum invasiveness by overcoming the high risk of infections, pain, and scarring (9,10). Pre-gelation preparation of gel formulation allows straightforward inclusion of several bioagents such as, growth factors (11), drugs (12), and genes (13), into the precursor solution which are primarily responsible for supporting, healing, or rejuvenating the damaged tissues. Injectable hydrogels can take complex shapes in applied region and bind to surrounding tissues during gelation which allows advanced cell/tissue encapsulation (14).

Chitosan is a biopolymer in polysaccharide structure obtained from chitin by partial deacetylation. The molecular structure of chitosan is composed of glucosamine and N-acetyl glucosamine units in which in body, glucosamine is converted into glycosaminoglycans which are parts of extracellular matrix and cartilage tissue (15). Due to unique characteristics, such as bioavailability, biodegradability and biocompatibility, chitosan derivatives have found numerous applications in various fields of biomedicine (16). Hydrogels, especially injectable hydrogel formulations employ chitosan-based polymer precursors as building blocks that construct matrix structure of the network while imparting eximious attributes. (17, 18–20), However, low aqueous solubility of

native chitosan is often a drawback in applications and entails proper modification of the polymer structure by conjugating various small molecules or polymers (21,22).

The virtual utilization of hydrogels in drug delivery demands efficient drug loading and sustained drug release. Though covalent incorporation of drug molecules into hydrogels possess several advantages (23), a much sought and operationally simple strategy involves the physical entrapment of drug molecules into the matrix. However, several limitations are often encountered during drug loading and release, primarily due to the chemical dissimilarity of drug molecules and gel structure. Hydrophobic or poorly water-soluble drug molecules usually have low interactions with hydrophilic matrix causing weak drug loading efficiencies during formulation. On the other part, controlled or sustained release in aquatic medium is hardly maintained with hydrophilic drugs. A general strategy to overcome these limitations is the incorporation of hydrophobic pockets into the gel network to maintain increased drug-carrier interactions. In this regard, hydrogels including cyclodextrins (CDs) in gelous matrix have been investigated as promising drug delivery platforms (24–29). The unique molecular structures of CDs enable inclusion complexation of hydrophobic molecules into molecular cavities present on CD structure, thus enabling increased drug loading and prolonged release.

The addition of thiols to alkenes to form a thioether bond is referred to as thiol-ene reaction and constitutes an important reaction in polymer chemistry. Highly efficient nature of thiol-ene reactions have been exemplified in design and synthesis of diverse macromolecular platforms and polymeric material functionalization (30–37). The addition reaction of thiols over electron deficient alkene groups is a special type of thiol-ene reaction known as the nucleophilic thiol-ene reaction and is a highly valuable tool in click chemistry toolbox for fabrication and functionalization of hydrogels (26,27,38). Due to the presence or straightforward installation of thiol groups on many biomolecules or biopolymers, synthesis and modification of thiol-ene biomaterials offer ease formulation and implementation in various applications.

In this study, design, fabrication and applications of hydrogels based on PEGylated chitosan and β-cyclodextrin was reported (Figure 1). Hydrogels were synthesized using nucleophilic thiol-ene reactions between thiol-modified PEGylated chitosan and acryloyl modified β-cyclodextrin. The methodology encompasses novel O-hydrophilic modification and N-thiolation of native chitosan which provided the fabrication of hydrogels without the need of any inorganic or metal catalyst or thermal and photo-activation. The simplicity and rapid gelation kinetics suggest that this approach can be utilized to obtain injectable hydrogels. These materials uniquely combine three biomedically-relevant polymers i.e. chitosan, polyethylene glycol and cyclodextrin in fabrication of functional soft materials and can endow potential applications in various areas of biomedical sciences such as controlled drug release.

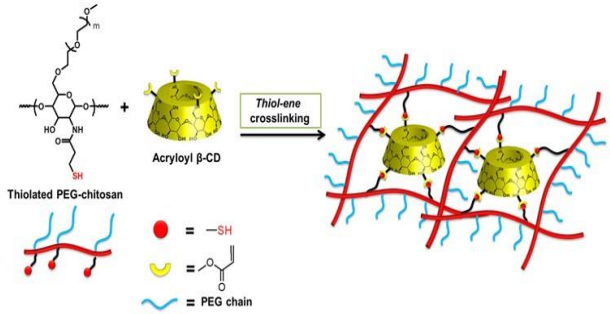


**Figure 1:** General representation of hydrogel formation via thiol-ene reaction of thiolated o-PEGylated chitosan and acryloyl-modified β-CD.

## EXPERIMENTAL SECTION

### Materials and characterization

Medium molecular weight chitosan (75-85% acetylation degree), β-cyclodextrin, poly(ethylene glycol) monomethyl ether (average Mn: 2000), phthalic anhydride, hydrazine hydrate, 3-mercaptopropionic acid, acryloyl chloride, 5,5′-dithiobis(2-nitrobenzoic acid) and diclofenac sodium salt were obtained from Aldrich Chemical Co. All solvents and inorganic materials were purchased from Merck Co. Syntheses of acryloyl-modified β-cyclodextrin (β-CD-Ac, average degree of acetylation ~5.4 per molecule) (39) and N-phthaloylated chitosan (40) were conducted according to reported procedures. p-Toluenesulfonate activation of poly(ethylene glycol) methyl ether (41) and N-hydroxysuccinimide (NHS) activation of 3-mercaptopropionic acid (42) was performed based on literature protocols. Characterization of materials was performed using $^{1}$H NMR spectroscopy (Varian 400 MHz) and attenuated total reflectance-Fourier transform infrared spectroscopy (Nicolet 380). UV studies were conducted with a Varian Cary 50 Scan UV/Vis spectrophotometer. The hydrogel surface morphologies were analyzed by using an ESEM-FEG/EDAX Philips XL-30 (Philips, Eindhoven, The Netherlands) instrument with 10 kV accelerating voltage. The dynamic frequency scan analyses were performed using an Anton Paar MCR 302 rheometer with a 0.5% strain between 0.05-100 rad/s (at 25 °C). A parallel plate of 8 mm diameter was set up and the plate gap was adjusted to 2.0 mm.

### Methods

*Synthesis of N-phthaloylated, O-PEGylated chitosan:* N-phthaloylated chitosan (1.0 g, 3.2 mmol repeating units) was charged in a two-

necked round bottom flask with magnetic stir bar and dissolved in dimethylformamide (DMF, 20 mL) by ultrasonication. To this mixture, p-toluene sulfonate-activated poly(ethylene glycol) methyl ether (8.2 g, 3.84 mmol) in 10 mL DMF and $K_2CO_3$ (0.53 g, 3.84 mmol) was added. The reaction mixture was stirred under nitrogen atmosphere at 80 °C for 24 h. After the reaction, the purification of the resulting polymer was conducted by dialysis against water using a dialysis membrane (MWCO 10K). The purified polymer was collected by evaporating the solvent and drying at 50 °C, overnight (Yield: 77%. FT-IR ($v_{max}/cm^{-1}$): 3600-3300 (broad O-H), 2882 (C-H stretching), 1771 (imide C=O), 1707 (imide C=O), 1647 (C=O acetyl), 1170-1015 (C-O stretching)).

*Hydrazinolysis of N-phthaloylated, O-PEGylated chitosan:* 5.4 g N-phthaloylated o-PEGylated chitosan and hydrazine hydrate (30 mL) were dissolved in distilled water (60 mL). The mixture was heated at 90 °C for 16 h. After the reaction, purification of the polymer was carried out by dialysis against water (MWCO 10K). The resulting polymer o-PEGylated chitosan was collected by removing solvent and drying the sample at 50 °C, overnight. (Yield: 86%. FT-IR ($v_{max}/cm^{-1}$): 3600-3300 (broad O-H), 2882 (C-H stretching), 1655 (C=O acetyl), 1170-1010 (C-O stretching)).

*Thiolation of o-PEGylated chitosan:* To the stirring solution of o-PEGylated chitosan (1.0 g in 10 mL DMF) was added 5 mL DMF solution of NHS-activated 3-mercaptopropionic acid (1.0 g, 5.0 mmol). The reaction was continued under argon atmosphere at 80 °C for 16 h. After the reaction, the mixture was dialyzed against water (MWCO 10K), evaporated and dried at 50 °C. (Yield: 94%. FT-IR ($v_{max}/cm^{-1}$): 3600-3300 (broad O-H), 2882 (C-H stretching), 1672 (C=O acetyl), 1655 (C=O acetyl), 1170-1010 (C-O stretching)).

*Determination of sulfhydryl content:* Total sulfhydryl content of thiolated o-PEGylated chitosan was determined using Ellman's method (43). Briefly, 4.0 mg of 5,5'-dithiobis(2-nitrobenzoic acid) was dissolved in 1 mL of reaction buffer (0.1 M sodium phosphate, pH 8.0 containing 1 mM EDTA) and to this solution, 5.0 mg of thiolated o-PEGylated chitosan in 1 mL reaction buffer was added. The resulting mixture was incubated at 37 °C for 2 h. The total sulfhydryl group content in the sample was obtained by measuring the maximum absorbance at 412 nm and using the molar extinction coefficient of 2-nitro-5-thiobenzoate ($TNB^{2-}$) ion (14,150 $M^{-1}cm^{-1}$) (44).

*Representative hydrogel formation:* Thiolated o-PEGylated chitosan (100 mg) was placed in a vial and dissolved in distilled water (200 µL). Desired amount of β-CD-Ac and catalytic amount of triethylamine (0.1 eq. of -SH) were dissolved in distilled water in another vial (200 µL) and then added to polymer solution. The gel solution was briefly sonicated to assist homogenous gelation. In approximately 10 min, there was no flow of sample and the gelation was continued for 6 h to ensure complete available crosslinking. Thereafter, unreacted species were removed by washing the gel sample with distilled water several times. The dried hydrogels were obtained by freeze-drying of water-swollen samples. Gel conversions: 66-87% (as obtained by proportioning the obtained mass of hydrogels after purification steps to amount of starting materials).

*Equilibrium swelling ratios (ESRs):* ESRs were determined by sampling 20 mg of hydrogel in distilled water and then following the increase in mass of the sample as a function of time until swollen hydrogels showed a constant weight. The percentage of swelling was determined using equation 1:

$$ESR\ (\%) = (W_{wet} - W_{dry}) / W_{dry} \times 100 \quad \text{(Eq. 1)}$$

The swelling studies were conducted in triplicate and average data was used for obtaining swelling curves.

*Drug loading and release studies:* A solution absorption method was employed to load diclofenac-Na into dry hydrogel samples. Hydrogel samples in disk shapes (~ 50 mg) were soaked in 10 mL 0.5 wt.% soaking solution at 37 °C and the solutions were incubated for 4 days, protected from light. The total amount of drug loading was calculated by subtracting the concentration in the soaking solution from the initial drug amount which was determined at 276 nm using a UV-Vis spectrophotometer.

For diclofenac-Na release, the drug-loaded hydrogels were rinsed with distilled water and then immersed in 3 mL distilled water medium at 37 °C incubation temperature. At pre-determined time intervals, 1.5 mL of release medium was collected and refreshed with same volume of fresh distilled water. The drug concentration in collected media was measured spectrophotometrically at 276 nm and the release profiles were expressed in terms of cumulative release.

## RESULTS AND DISCUSSION

### Synthesis and characterization of polymers

In order to obtain thiol modified chitosan-based hydrophilic polymer, a series of synthesis and post-polymerization modification steps were carried out (Figure 2). In the first step, native chitosan was phthaloylated to protect the free amine groups which were later utilized in conjugation of thiol-bearing molecule. In the next step, installation of PEG groups onto the chitosan hydroxyl functionalities was carried out

by following a 'grafting onto' approach. PEGylation is a common practice in modification of chitosan derivatives in order to increase their water solubility, as well as biocompatibility (22). Although, the common approaches of chitosan modification rely on the involvement of amino groups in chemical tailoring (22). In our study, PEG grafting was carried through hydroxyl functionalities in order to keep amine groups intact for later utilization. A nucleophilic substitution-based grafting of activated monomethoxy PEG polymer has resulted in the attachment of hydrophilic side chains onto chitosan backbone. Following the PEG grafting, protected phthaloyl groups were removed by hydrazinolysis to unveil the amino groups in their reactive form. In the last step, amino groups were conjugated with NHS-activated 3-mercaptopropionic acid to accomplish the installation of thiol-ene reactive mercapto groups onto side chains of PEGylated chitosan.

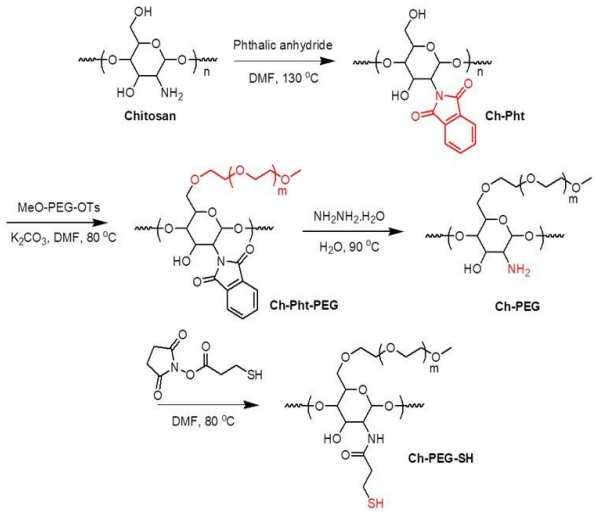


**Figure 2.** General reaction scheme for the synthesis of thiolated o-pegylated chitosan derivative (Ch-PEG-SH).

The synthetic steps of polymer synthesis were followed by FT-IR (Figure 3) and $^1$H NMR (Figure 4) spectroscopic techniques to confirm the transformations. In the PEG attachment onto phthaloylated chitosan (Ch-Pht), FT-IR studies confirmed the successful grafting as evidenced by the appearance or enhancement of characteristics bands at ($\nu_{max}$/cm$^{-1}$) 2882 (C-H stretching), 1069 and 959 (C-O stretchings). The $^1$H NMR analysis of resulting phthaloylated and PEGylated chitosan (Ch-Pht-PEG) revealed characteristics proton signals of chitosan pyranose groups and phthaloyl moieties, as well as signals of PEG repeating units and methoxy (-$OCH_3$) end groups. The degree of PEGylation, as determined by the integration of D-glucopyranose signal at 4.5 ppm with PEG methoxy signal at 3.4 ppm, was found as 43% (mole PEG/mole D-glucosamine). After the removal of phthaloyl groups via hydrazinolysis, FT-IR analysis revealed the disappearance of imide absorption bands at ($\nu_{max}$/cm$^{-1}$) 1771 and 1707. This transformation was also verified by the disappearance of phthaloyl aromatic signals at 7.3-8.0 ppm. The final step involves the reaction of free amino groups of chitosan polymer with 3-mercaptopropionic acid, pre-activated with NHS groups to give Ch-PEG-SH. Formation of a new amide band at 1672 cm$^{-1}$ of FT-IR spectrum and aliphatic protons signals of mercaptopropionamide units in $^1$H NMR spectrum establish the successful conjugation. In order to quantify the total number of reactive thiols groups at final polymer, a free sulfhydryl assay was accounted. According to the Ellman's analysis performed by employing 5,5'-dithiobis(2-nitrobenzoic acid), 8.4 $10^{-4}$ mmol/g thiol content was determined.

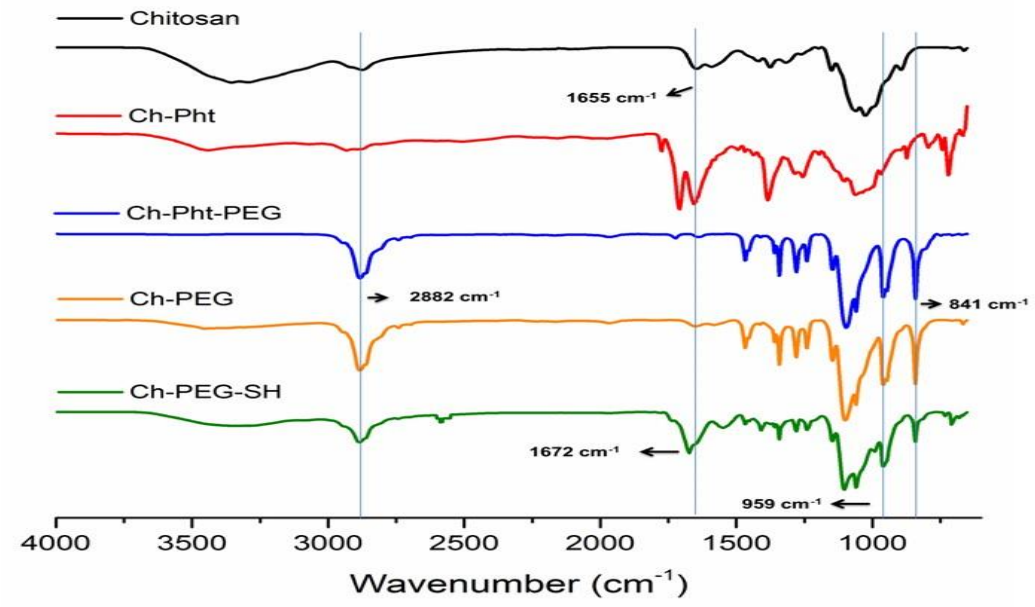


**Figure 3.** FT-IR structural analyses of polymers after o-PEGylation, hydrazinolysis, and thiolation steps.

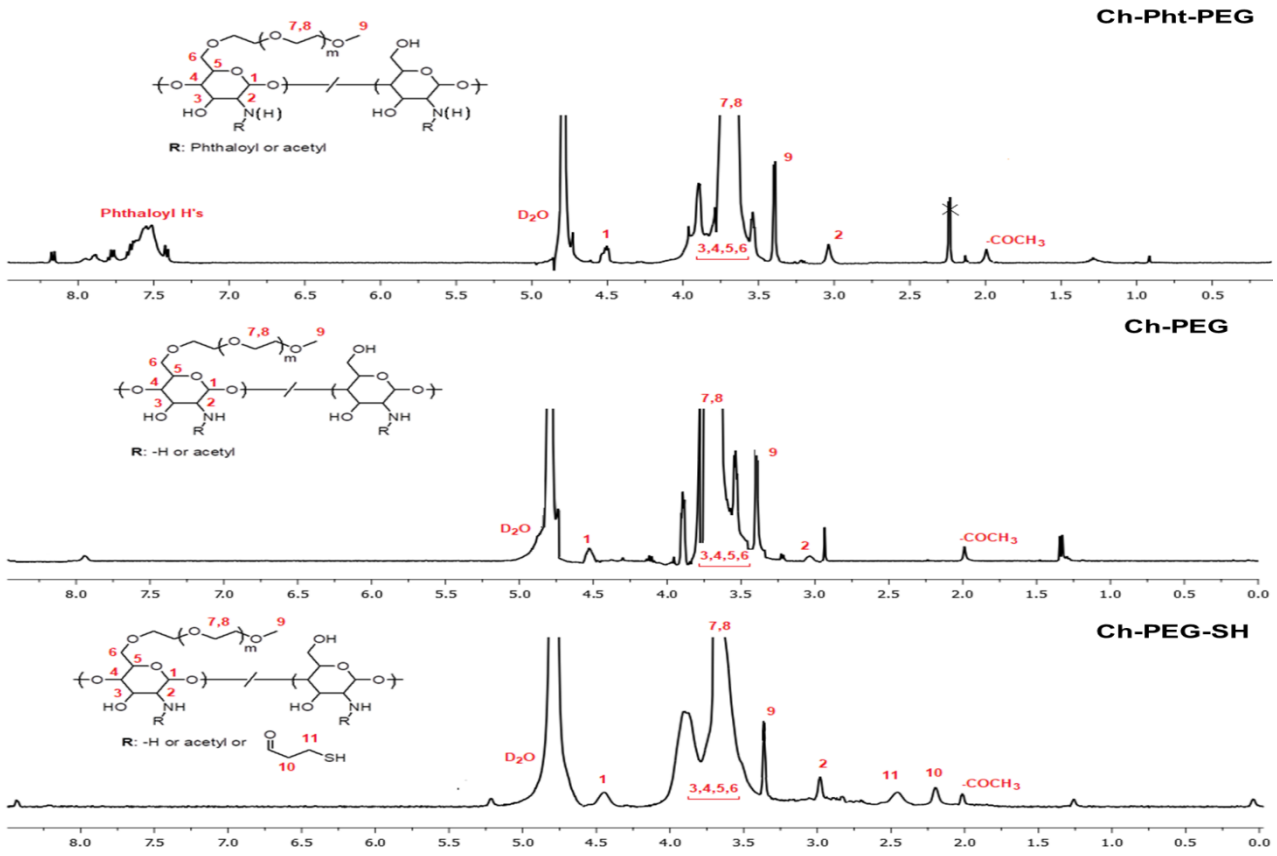


**Figure 4.** $^{1}$H NMR structural analyses of polymers after o-PEGylation, hydrazinolysis and thiolation steps.

**Synthesis and characterization of hydrogels**

Hydrogels were prepared via thiol-ene reactions of thiol functionalized PEGylated chitosan polymer (Ch-PEG-SH) with acryloyl-modified β-cyclodextrin crosslinker (β-CD-Ac) (Figure 1). Through multiple additions of thiols onto acryloyl groups, fast crosslinking network formation was established in approximately ten minutes and no flow of sample was observed. To ensure complete crosslinking process, gelation was continued for 6 h. Gel formation is promoted by using a catalytic amount of trimethylamine ($Et_3N$) as a non-nucleophilic organobase. In order to compare the effect of CD-based crosslinker ratio on physical and morphological properties of resulting gels, a library of hydrogels were prepared by using various Ch-PEG-SH/β-CD-Ac feeds (Table 1, hydrogels CCH-(1-4)). The properties of obtained hydrogels were summarized in Table 1.

**Table 1.** Properties of hydrogels with varying Ch-PEG-SH / β-CD-Ac ratio.

| Entry | Hydrogel | -SH : acrylate | Gel Conv. (%) | ESR (×100%) | Drug Load (mg/g dry gel) |
|---|---|---|---|---|---|
| 1 | CCH-1 | 0.5 : 0.5 | 87 | 9.2 (± 1.4) | 88 (± 16) |
| 2 | CCH-2 | 0.6 : 0.4 | 76 | 13.3 (± 1.1) | 74 (± 12) |
| 3 | CCH-3 | 0.7 : 0.3 | 71 | 15.6 (± 2.8) | 66 (± 7) |
| 4 | CCH-4 | 0.8 : 0.2 | 66 | 19.5 (± 2.3) | 61 (± 9) |

Hydrogels were obtained with moderately good gel conversions via thiol-ene addition reactions of complementary functional precursors. As expected, higher gel conversions were obtained in case of using higher amount of β-CD-Ac crosslinker. The gels are clear transparent samples in their wet state appearance. The microstructure analyses based on scanning electron microscopy (SEM) revealed continuous non-porous structures (Figure 5). A very slight increase in porosity was accounted in case of employing lower crosslinker feed.

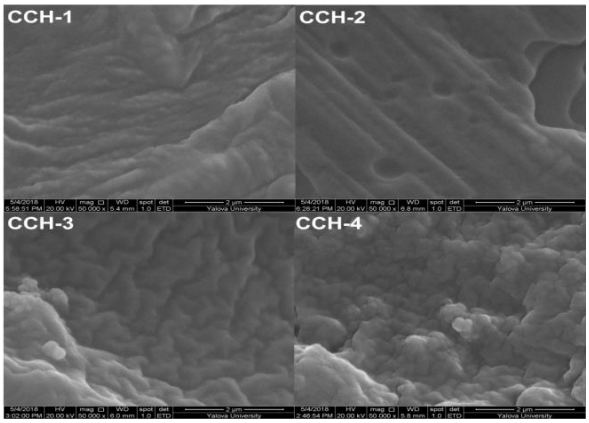


**Figure 5.** Representative SEM images of freeze-dried hydrogels (Scale bars: 2 μm).

Hydrogels were investigated in terms of swelling behaviors by recording the water uptake in pre-determined time intervals until a constant weight is attained. Hydrogels exhibited pronounced swelling degrees (~ 2000-1000 % with respect to initial gel amount) due to the presence of hydrophilic groups on polymer backbone and cyclodextrin crosslinker (Figure 6). Equilibrium swellings were attained in a relatively short period of time in pH-neutral hydration conditions. Although, the microstructures of obtained hydrogels possess non-porous morphology, it can be argued that the presence of hydrophilic PEG side chains have contributed to a relatively high degree of network hydration compared to the reported chitosan/cyclodextrin-based hydrogels (45-46). As expected, the swelling properties of hydrogels exhibit dependency on the feed of hydrophilic polymer and crosslinker ratios. Relatively higher swelling degrees were obtained by decreasing crosslinker ratio which can be attributed to the increase in free gel volumes of network.

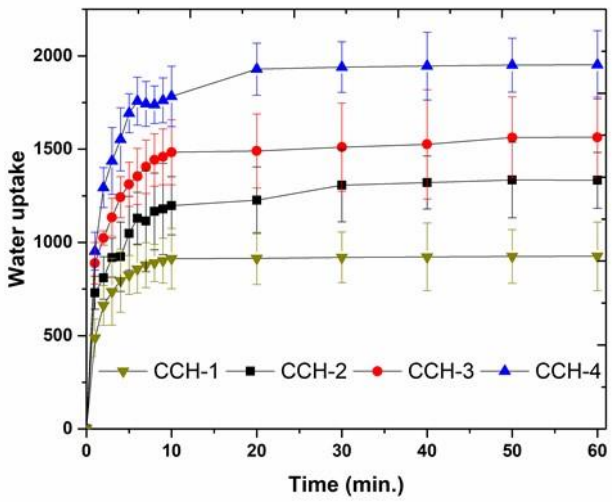


**Figure 6.** Equilibrium swelling profiles of hydrogels in water.

The visco-elastic gel properties of water-swollen hydrogels were examined via dynamic frequency scan analysis. The measurements revealed that the storage and loss moduli of networks show relatively low oscillation frequency dependency indicating the homogenous network formation (Figure 7).(47) The storage modulus (G') and loss modulus (G") values were ranging from $10^2$ to $10^5$ Pa and for the all samples tested, the storage moduli were found to be over ten times higher than those of loss moduli, indicating covalently crosslinked elastic network structure. (48) Higher modulus values were obtained for hydrogels prepared by using higher amount of β-CD-based crosslinker which could be attributed to the increased network structure. Moreover, in high oscillation frequencies, the damping factor (defined as G"/G) decreases with decreasing crosslinker amount. This indicates that the elastic properties of the hydrogels show a relative increase by lowering the crosslinking density which might yield more conformational freedom to side chain PEG units. (49)

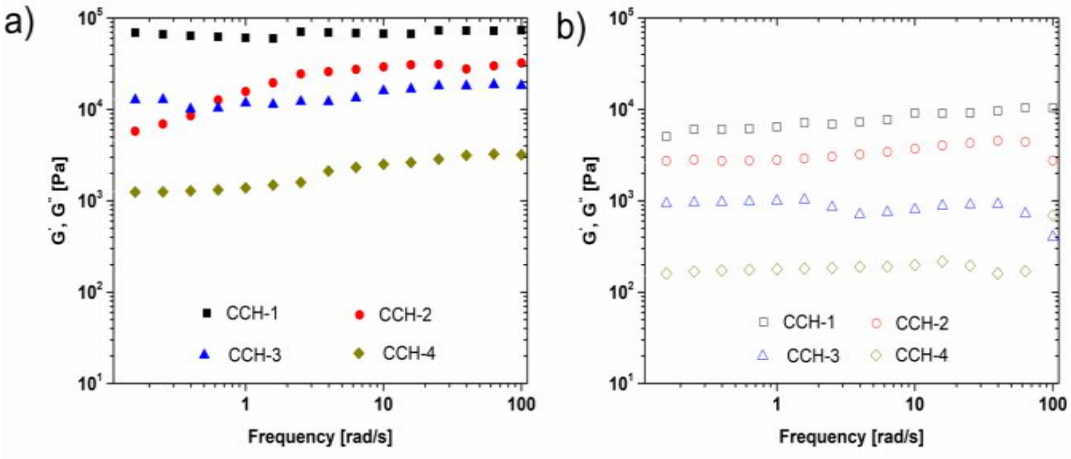


**Figure 7.** Dynamic frequency scan analysis of hydrogels: a) Storage and b) loss moduli of hydrogels.

**Drug loading and release studies**

The drug loading and release characteristics of hydrogels were examined using a model drug with low water solubility, diclofenac-Na, employed as a non-steroidal anti-inflammatory medication for treatment of several complaints and diseases. The drug was loaded to the hydrogel samples prepared as disks using solution absorption method. Pre-water swollen hydrogel disks were immersed in 0.5 wt.% soaking solution and the drug loading was monitored by UV-spectrophotometry until equilibrium was reached. The total drug amounts absorbed by the hydrogels, determined from the initial and final concentrations of soaking solutions were shown in Table 1. The loaded drug amounts were found to be affected by the β-CD-based crosslinker ratio. Highest drug loading was achieved with hydrogel CCH-1, containing the highest β-cyclodextrin ratio. In hydrogels, drugs are mainly diffused in aqueous phase or adsorbed to the polymeric backbones (50). In cyclodextrin-containing hydrogels, the ability of inclusion complexation between cyclodextrin and hydrophobic molecules provide another mean of drug loading. Since diclofenac-Na is able to form inclusion complexation with β-cyclodextrin (51), increased β-cyclodextrin ratio as going from CCH-4 to CCH-1 makes a notable contribution to drug loading.

The drug-loaded hydrogels were gently washed with distilled water before adding to the release medium. With regular time intervals, 5 mL release medium was replaced with a fresh solution and collected release solution was analyzed via UV spectrophotometer to monitor drug release. The release behavior of diclofenac-Na from hydrogels is shown in Figure 8. Initially, a burst release of the drug was observed for all hydrogels. This accelerated release is common in hydrogel based release systems and mainly attributed to the fast removal of free drug in aqueous phase and adsorbed drug on backbone of the hydrogel (52). The amount of burst release was dependent on the β-CD content in hydrogels as lowest burst release and highest sustained release was observed with hydrogel CCH-1 containing highest β-CD content. The slower release of hydrogels with higher β-CD content can be ascribed to the formation of inclusion complex of the drug with β-CD. As the relation between β-CD amounts that hydrogels bear and the drug loading capacities and sustained release profiles suggest, β-CD incorporation to hydrogel network maintains increased drug-carrier interactions while improving the versatility of fabricated biomaterials. Combining the efficient and easy fabrication with convenient attributes of hydrogel precursors, the demonstrated approach might find applications in design and synthesis of several hydrogel-based drug delivery systems.

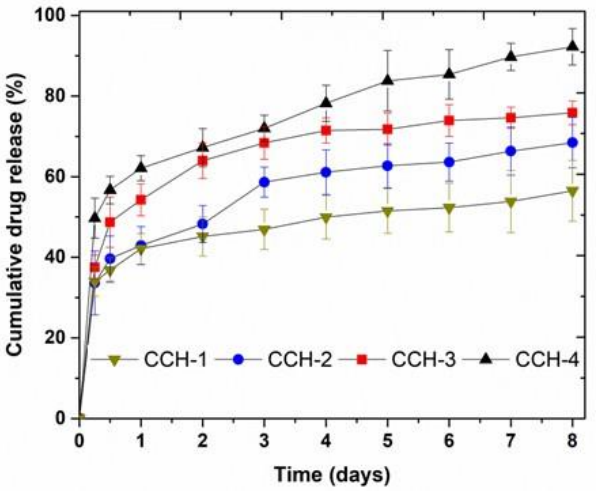


**Figure 8.** Cumulative release of diclofenac-Na from hydrogels.

**CONCLUSION**

Novel hydrogels employing PEG-modified chitosan as hydrophilic matrix and acrylated β-CD as crosslinker were synthesized using thiol-ene addition reaction. The method demonstrates the facile and efficient crosslinking process between complementary thiol and acrylate functional precursors which can be used as *in situ* injectable gel forming systems. It was shown that physical properties can be tuned by changing feed ratio between the hydrophilic polymer and crosslinker. A model drug was loaded to fabricated hydrogels and controlled release of the drug was monitored. Lower initial burst releases and more prolonged release profiles were obtained in case of increasing β-CD content in the hydrogels. The hydrogel synthesis methodology depicted here is believed to find potential application in design of macro and micro-scale controlled drug release systems especially in injectable formulations.

**ACKNOWLEDGEMENTS**

The authors thank Yalova University, Scientific Research Projects Coordination Unit for financial support with a grant number 2017/YL/0010. M. A. also expresses his gratitude to Bogazici University Center for Life Sciences and Technologies to access analytical equipments.

**REFERENCES**

1. Yang JA, Yeom J, Hwang BW, Hoffman AS, Hahn SK. In situ-forming injectable hydrogels for regenerative medicine. Progress in Polymer Science. 2014; 39(12): 1973-86.

2. Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials. 2003; 24(24): 4337-51.

3. Hunt JA, Chen R, Van Veen T, Bryan N. Hydrogels for tissue engineering and regenerative medicine. Journal of Materials Chemistry B. 2014; 2: 5319-38.

4. Vermonden T, Censi R, Hennink WE. Hydrogels for protein delivery. Chemical Reviews. 2012; 112 (5): 2853–88.

5. Yu F, Cao X, Li Y, Zeng L, Yuanab B, Chen X. An injectable hyaluronic acid/PEG hydrogel for cartilage tissue engineering formed by integrating enzymatic crosslinking and Diels–Alder "click chemistry". Polymer Chemistry. 2014; 5: 1082-90.

6. Takahashi A, Suzuki Y, Suhara T, Omichi K, Shimizu A, Hasegawa K, Kokudo N, Ohta S, Ito T. In situ cross-linkable hydrogel of hyaluronan produced via copper-free click chemistry. Biomacromolecules. 2013; 14(10): 3581-8.

7. Ahadian S, Sadeghian RB, Salehi S, Ostrovidov S, Bae H, Ramalingam M, Khademhosseini A. Bioconjugated hydrogels for tissue engineering and regenerative medicine. Bioconjugate Chemistry. 2015; 26(10): 1984-01.

8. Sivashanmugam A, Arun Kumar R, Vishnu Priya M, Nair S V., Jayakumar R. An overview of injectable polymeric hydrogels for tissue engineering. European Polymer Journal. 2015; 72: 543-65.

9. Yan S, Wang T, Feng L, Zhu J, Zhang K, Chen X, Cui L, Yin J. Injectable in situ self-cross-linking hydrogels based on poly(l-glutamic acid) and alginate for cartilage tissue engineering. Biomacromolecules. 2014; 15(12): 4495-08.

10. Xiao ZS, Ahmad S, Liu Y, Prestwich GD. Synthesis and evaluation of injectable, in situ crosslinkable synthetic extracellular matrices for tissue engineering. Journal of Biomedical Materials Research Part A. 2006; 79(4): 902-12.

11. Cai S, Liu Y, Zheng Shu X, Prestwich GD. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. Biomaterials. 2005; 26(30): 6054-67.

12. Tiller JC. Increasing the local concentration of drugs by hydrogel formation. Angewandte Chemie-International Edition. 2003; 42(27): 3072-75.

13. Paul A, Hasan A, Kindi H Al, Gaharwar AK, Rao VTS, Nikkhah M, Shin SR, Krafft D, Dokmeci MR, Shum-Tim D, Khademhosseini A. Injectable graphene oxide/hydrogel-based angiogenic gene delivery system for vasculogenesis and cardiac repair. ACS Nano. 2014; 8(8): 8050-62.

14. Seliktar D. Designing cell-compatible hydrogels for biomedical applications. Science. 2012; 336(6085): 1124-8.

15. Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan- A versatile semi-synthetic polymer in biomedical applications. Progress in Polymer Science. 2011; 36(8): 981-14.

16. Rinaudo M. Chitin and chitosan: Properties and applications. Progress in Polymer Science. 2006; 31(7): 603-32.

17. Bhattarai N, Gunn J, Zhang M. Chitosan-based hydrogels for controlled, localized drug delivery. Advanced Drug Delivery Reviews. 2010; 62(1): 83-99.

18. Ta HT, Dass CR, Dunstan DE. Injectable chitosan hydrogels for localised cancer therapy. Journal of Controlled Release. 2008;126(3): 205-16.

19. Bhattarai N, Ramay HR, Gunn J, Matsen FA, Zhang M. PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release. Journal of Controlled Release. 2005; 103(3): 609-24.

20. Jin R, Moreira Teixeira LS, Dijkstra PJ, Karperien M, van Blitterswijk CA, Zhong ZY, Feijen J. Injectable chitosan-based hydrogels for cartilage tissue engineering. Biomaterials. 2009; 30(13): 2544-51.

21. Sashiwa H, Aiba SI. Chemically modified chitin and chitosan as biomaterials. Progress in Polymer Science. 2004; 29(9): 887-08.

22. Casettari L, Vllasaliu D, Castagnino E, Stolnik S, Howdle S, Illum L. PEGylated chitosan derivatives: Synthesis, characterizations and pharmaceutical applications. Progress in Polymer Science. 2012; 37(5): 659-85.

23. Aydin D, Arslan M, Sanyal A, Sanyal R. Hooked on cryogels: A carbamate linker based depot for slow drug release. Bioconjugate Chemistry. 2017; 28(5): 1443-51.

24. Van De Manakker F, Vermonden T, Van Nostrum CF, Hennink WE. Cyclodextrin-based polymeric materials: Synthesis, properties, and pharmaceutical/biomedical applications. Biomacromolecules. 2009; 10(12): 3157-75.

25. Li J, Loh XJ. Cyclodextrin-based supramolecular architectures: Syntheses, structures, and applications for drug and gene delivery. Advanced Drug Delivery Reviews. 2008; 60(9): 1000-17.

26. Arslan M, Gevrek TN, Sanyal A, Sanyal R. Cyclodextrin mediated polymer coupling via thiol-maleimide conjugation: Facile access to

functionalizable hydrogels. RSC Advances. 2014; 4: 57834-41.

27. Arslan M, Aydin D, Degirmenci A, Sanyal A, Sanyal R. Embedding well-defined responsive hydrogels with nanocontainers: Tunable materials from telechelic polymers and cyclodextrins. ACS Omega. 2017; 2(10): 6658-67.

28. Arslan M, Gevrek TN, Sanyal R, Sanyal A. Fabrication of poly(ethylene glycol)-based cyclodextrin containing hydrogels via thiol-ene click reaction. European Polymer Journal. 2015; 62: 426-34.

29. Arslan M, Sanyal R, Sanyal A. Cyclodextrin-containing hydrogel networks. In: Mishra M, editor. Encyclopedia of Biomedical Polymers and Polymeric Biomaterials. Taylor and Francis: New York; 2015. p. 2243-58. Available from: https://www.taylorfrancis.com/books/e/9781466501799/chapters/10.1081%2FE-EBPP-120050543

30. Gevrek, TN, Arslan, M, Sanyal, A. Design and synthesis of maleimide group containing polymeric materials via the Diels-Alder/Retro Diels-Alder strategy. In: Theato P and Klok H, editors. Functional Polymers by Post-Polymerization Modification. Wiley-VCH Verlag GmbH & Co.; 2013. p. 123-55. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/9783527655427.ch5

31. Arslan, M, Gevrek, TN, Sanyal, A. Maleimide containing thiol-reactive polymers: Synthesis and functionalization. In. Shunmugam R, editor. Functional Polymers. Apple Academic Press: New York; 2017. Ch. 7. Available from: https://www.taylorfrancis.com/books/e/9781771882972/chapters/10.1201%2F9781315366524-17

32. Arslan M, Tasdelen MA. Polymer nanocomposites via click chemistry reactions. Polymers. 2017; 9(10): 499.

33. Arslan M, Gok O, Sanyal R, Sanyal A. Clickable poly(ethylene glycol)-based copolymers using azide-alkyne click cycloaddition-mediated step-growth polymerization. Macromolecular Chemistry and Physics. 2014; 215(22): 2237-47.

34. Oz Y, Arslan M, Gevrek TN, Sanyal R, Sanyal A. Modular fabrication of polymer brush coated magnetic nanoparticles: Engineering the interface for targeted cellular imaging. ACS Applied Materials ans Interfaces. 2016; 8(30): 19813-26.

35. Arslan M, Gevrek TN, Lyskawa J, Szunerits S, Boukherroub R, Sanyal R, Woisel P, Sanyal A. Bioinspired anchorable thiol-reactive polymers: Synthesis and applications toward surface functionalization of magnetic nanoparticles. Macromolecules. 2014; 47(15): 5124-34.

36. Yilmaz II, Arslan M, Sanyal A. Design and synthesis of novel "orthogonally" functionalizable maleimide-based styrenic copolymers. Macromolecular Rapid Communications. 2012; 33(9): 856-62.

37. Hoyle CE, Bowman CN. Thiol-ene click chemistry. Angewandte Chemie-International Edition. 2010; 49(9): 1540-73.

38. Kharkar PM, Rehmann MS, Skeens KM, Maverakis E, Kloxin AM. Thiol-ene click hydrogels for therapeutic delivery. ACS Biomaterials Science and Engineering. 2016; 2(2): 165-79.

39. Wang FP, Li, GF, Zhou QQ, Yang CX, Wang QZ. Removal of metal ions from aqueous solution with cyclodextrin-based hydrogels. 2016; 6(5): 394-02.

40. Nishimura SI, Kohgo O, Kurita K, Kuzuhara H. Chemospecific manipulations of a rigid polysaccharide: Syntheses of novel chitosan derivatives with excellent solubility in common organic solvents by regioselective chemical modifications. Macromolecules. 1991; 24(17): 4745-48.

41. Bentzen EL, Tomlinson ID, Mason J, Gresch P, Warnement MR, Wright D, Sanders-Bush E, Blakely R, Rosenthal SJ. Surface modification to reduce nonspecific binding of quantum dots in live cell assays. Bioconjugate Chemistry. 2005; 16(6): 1488-94.

42. Sharpe JC, Mitchell JS, Lin L, Sedoglavich N, Blaikie RJ. Gold nanohole array substrates as immunobiosensors. Analiytcal Chemistry. 2008; 80(6): 2244–49.

43. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959; 82(1): 70-77.

44. Riddles PW, Blakeley RL, Zerner B. Reassessment of Ellman's reagent. Methods in Enzymology. 1983; 91: 49-60.

45. Kono H, Teshirogi T. Cyclodextrin-grafted chitosan hydrogels for controlled drug delivery. Int. J. Biol. Macromol. 72, 299-308 (2015).

46. Dong ZQ, Cao Y, Yuan QJ, Wang YF, Li JH, Li BJ, Zhang S. Redox- and glucose-induced shape-memory polymers. Macromol. Rapid Commun. 34, 867–872 (2013).

47. Siemoneit U, Schmitt C, Alvarez-Lorenzo C, Luzardo A, Otero-Espinar F, Concheiro A, Blanco-Méndez J. Acrylic/cyclodextrin hydrogels with enhanced drug loading and sustained

release capability. International Journal of Pharmaceutics. 2006; 312(1-2): 66-74.

48. Li R, Zhang X, Zhang Q, Liu H, Rong J, Tu M, Zeng R, Zhao J. b-cyclodextrin-conjugated hyaluronan hydrogel as a potential drug sustained delivery carrier for wound healing. Inc. J. Appl. Polym. Sci. 133, 43072 (2016).

49. Jin R, Moreira Teixeira LS, Dijkstra PJ, Karperien M, van Blitterswijk CA, Zhong ZY, vd. Injectable chitosan-based hydrogels for cartilage tissue engineering. Biomaterials. May 2009;30(13):2544-51.

50. Andrade-Vivero P, Fernandez-Gabriel E, Alvarez-Lorenzo C, Concheiro A. Improving the loading and release of NSAIDs from pHEMA hydrogels by copolymerization with functionalized monomers. Journal of Pharmaceutical Sciences. 2007; 96(4): 802-13.

51. Das S, Subuddhi U. Studies on the complexation of diclofenac sodium with β-cyclodextrin: Influence of method of preparation. Journal of Molecular Structure. 2015; 1099: 482-89.

52. Xu J, Li X, Sun F. Cyclodextrin-containing hydrogels for contact lenses as a platform for drug incorporation and release. Acta Biomaterialia. 2010; 6(2): 486-93.