THIOL REACTIVE HYDROGELS FROM SINGLE PRECURSOR

by

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ABSTRACT

THIOL REACTIVE HYDROGELS FROM SINGLE PRECURSOR

Hydrogels have become very popular materials in last few decades. They have found high potential of use in tissue engineering, drug delivery systems, biosensors and wound healing dressing. Recently, there is a great interest in fabricating well defined hydrogels with easier methods. In this study, functionalizable hydrogels from single precursor were synthesized by convenient way and functionalizations of the hydrogels were done. In the first part, ABA type triblock polymers were synthesized by Atom Transfer Radical Polymerization (ATRP). Linear polyethylene glycol (PEG), polymer was chosen as middle block and it was modified as ATRP initiator. Furan protected maleimide methacrylate (FPMMA), furfuryl methacrylate (FFMA) and oligoethylene methyl ether methacrylate (OEGMEMA) were polymerized using the PEG based macroinitiator using ATRP. Resulting triblock polymers were characterized by gel permeation chromatography (GPC), infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR). The triblock copolymers were activated by retro Diels-Alder (rDA) reaction and crosslinked by Diels-Alder (DA) in situ to obtain hydrogels. Thermogravimetric analysis and water uptake capacity of the hydrogels were done. The nature of crosslinking was examined in presence of anthracene to demonstrate that crosslinking is due to Diels-Alder reaction of maleimide and furan on the polymers. Presence of the scavanger diene anthracene, prohibits hydrogel formation and result in linear polymers containing anthracene-maleimide cycloadducts as side chains. In the second part of the study, by using thiolene chemistry fluorescent BODIPYC10SH molecules were attached to hydrogels to examine functionalization. It is shown that by changing block lengths of the polymers, the control over functional group density is achieved.

ÖZET

TEK PREKURSORDAN TİOL REAKTİF HİDROJELLERİN SENTEZİ

Hidrojeller son birkaç on yılda oldukça populer malzemeler olmuşlardır. Doku mühendisliğinde, ilaç salınım sistemlerinde, biyosensörlerde ve yara iyileştiren giysilerde yüksek kullanım potansiyellerinin olduğu görülmüştür. Son zamanlarda iyi yapılanmış ve yapımı kolay hidrojellere karşı bir ilgi başlamıştır. Bu çalışmada, fonksiyonelleştirebilinir hidrojellerin tek bir öncü bileşen tarafından kolay bir şekilde sentezi ve fonsiyonelitelerinin test edilmesi yapılmıştır. İlk kısımda, ABA tipi üçlü blok polimerler Atom Transfer Radikal Polimerizasyon (ATRP) tekniği ile sentezlenmiştir. Polietilen glikol (PEG) orta blok olarak düşünülmüş ve bu bağlamda ATRP başlatıcısı olarak modifiye edilmiştir. Furanla korunmuş maleimid metakrilat (FPMMA), furfuril metakrilat (FFMA) ve oligoetilen metil eter metakrilatın (OEGMEMA) PEG ATRP başlatıcısı kullanılarak polimerizasyonu gerçekleştirilmiştir. Elde edilen polimerler jel geçirgenlik kromatografisi (GPC), kızılötesi spektrskopisi ve nükleer manyetik rezonans spektroskopisi ile karakterize edilmiştir. Karekterizasyondan sonra bu polimerler ters Diels-Alder (rDA) reaksiyonu ile aktif hale getirilmiş ve Diels-Alder reaksiyonu ile çapraz bağlanarak hidrojelleri oluşturmuştur. Hidrojellerin termogravimetrik analizleri ve su alma kapasiteleri incelenmiştir. Çapraz bağlanmanın nedeninin polimer üzerindeki furan ve maleimidin Diels-Alder reaksiyonu sonucu olduğu antrasen testi ile incelenmiştir. Çalışmanın ikinci kısmında, floresans özellik gösteren BODIPYC10SH moleküller tiolen kimyası kullanılarak hidrojeller eklenmiş ve fonksiyonalite test edilmiştir. Üçlü blok polimerde blokların uzunluklarının değişimi ile fonksiyonalitenin kontrol edilebildiği gösterilmiştir. Ek olarak BODIPY-Br ile aynı şekilde yapılan fonksiyonalite testi floresans moleküllerinin hidrojellere kovalent bağla bağlandığını göstermiştir.

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LIST OF ACRONYMS/ABBREVIATIONS

ATRP	Atom Transfer Radical Polymerization.
DA	Diels-Alder
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
ECM	Extra Cellular Matrix
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
FFMA	Furfuryl Methacrylate
FPMMA	Furan Protected Maleimide Methacrylate
GPC	Gel Permeation Chromatography
MHz	Mega Hertz
Mn	The Number Average Molecular Weight
NMR	Nuclear Magnetic Resonance
OEGMEMA	Oligo Ethylene Glycol Methyl Ether Methacrylate
PEG	Polyethylene Glycol
PEGMA	Polyethylene Gylcol Methacrylate
PNIPAM	Poly-N-isopropylacrylamide
PVA	Polyvinyl Alcohol
rDA	Retro Diels-Alder reaction
SEM	Scanning Electron Microscopy
TEG	Tetraethylene Glycol
THF	Tetrahydrofuran

1. INTRODUCTION

1.1. Synthesis and Applications of Hydrogels

Hydrogels are crosslinked polymeric structures that can absorb water. The crosslinking of polymers to form hydrogels can be physical in nature arising due to supramolecular interaction, ionic force or it can be a covalent in nature due to interchain covalent linkages. Physically crosslinked hydrogels are reversible due to the nature of their crosslinking. Depending on temperature, ions or pH of the medium, polymers can crosslink or decrosslink. For example, addition of Ca^{2+} ions to sodium alginate results in crosslinking. Another example to physical crosslinking can be accomplished by local crystal formation as seen in Figure 1.1 [1]. An example to decrosslinking of a physically crosslinked hydrogel is heating of gelatin, which is a crosslinked polymer due to helix formation between polymer chains, results in non crosslinked polymer as shown in Figure 1.2 [2].

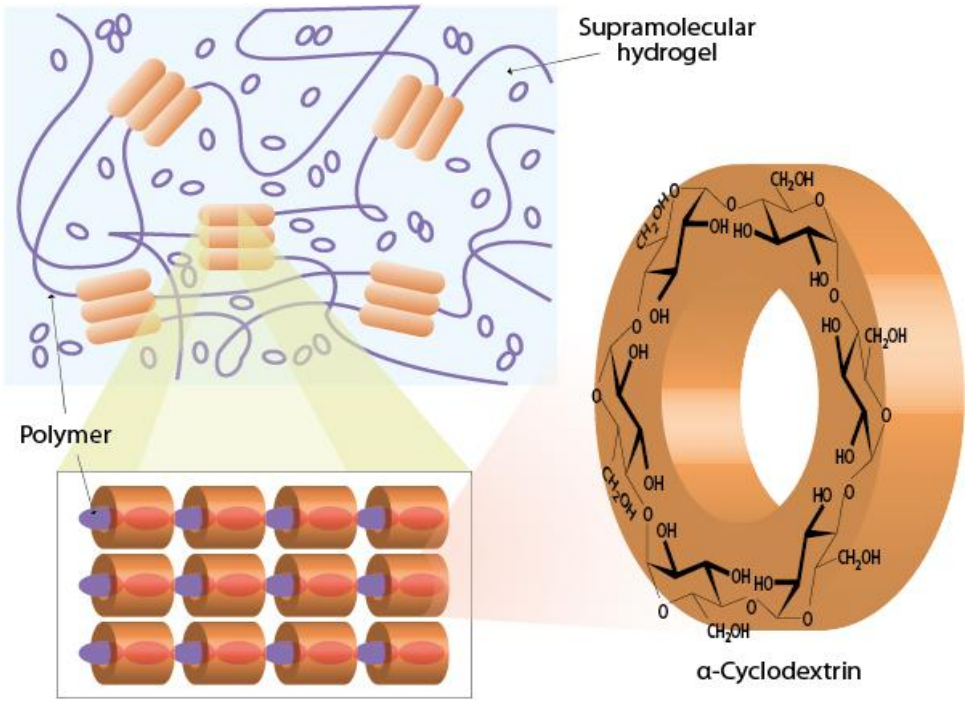


Figure 1.1. Physically crosslinked supramolecular hydrogel.

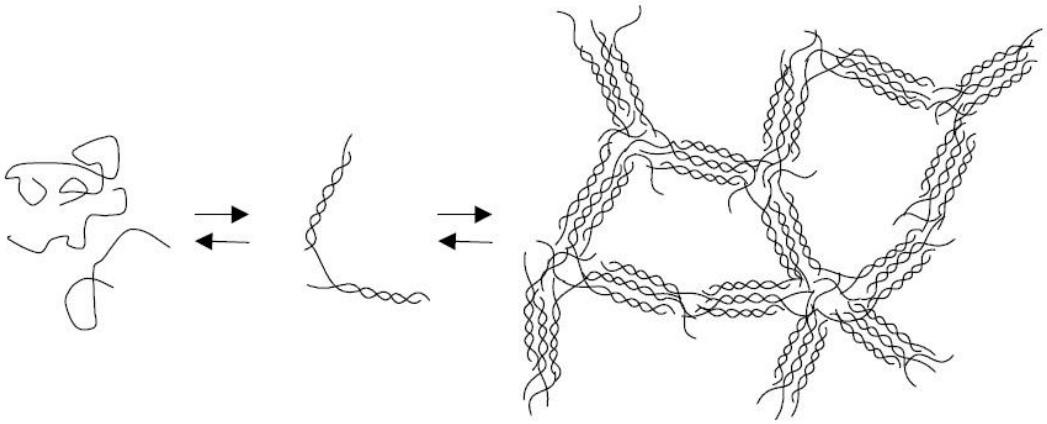


Figure 1.2. Crosslinking and decrosslinking of gelatin.

Crosslinking by a covalent bond is much stronger than physical crosslinking and mostly they are not reversible. Covalent crosslinking is advantageous for the applications which require strength and stability at the junctions of a hydrogel. An example to covalently crosslinked hydrogel is crosslinked polyurethane as shown in Figure 1.3 [3]. In this case the crosslinking is achieved by a C-O covalent bond and triol is responsible for crosslinking. By varying triol ratio crosslinking density can be controlled.

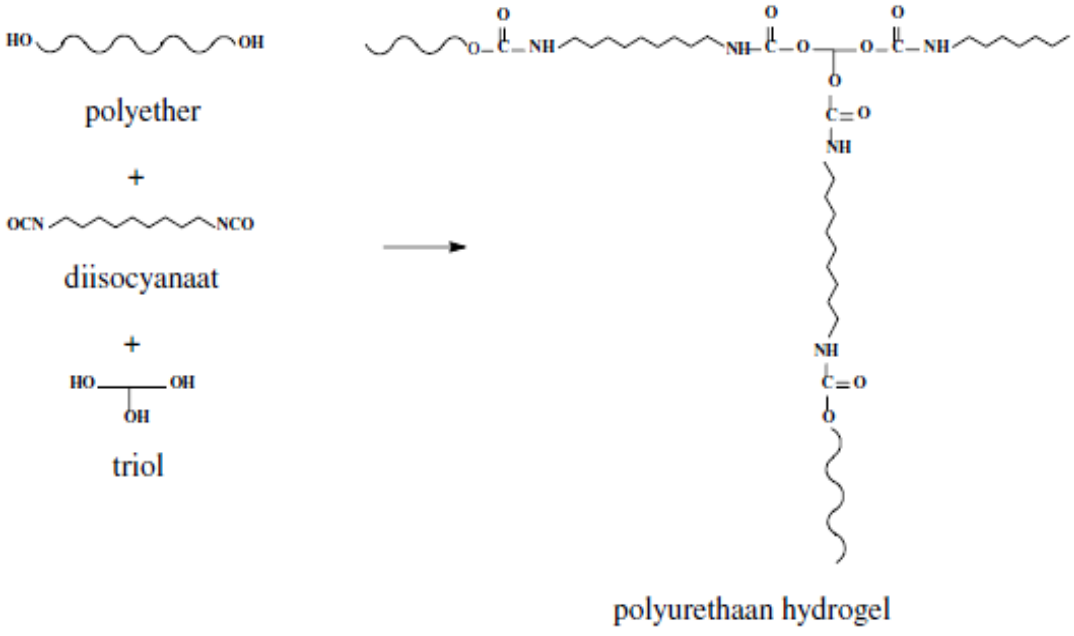


Figure 1.3. Covalently crosslinked polyurethane hydrogel.

Hydrogels have become very popular materials and they have been used and tried in many application areas in a few decades. They have been found to have high potential in the areas of tissue engineering, drug delivery, biosensors, contact lenses and wound healing dresses [4-7]. In tissue engineering they are used as scaffolds for cells to grow. They can be used as drug carriers and they can be tuned to release drug in different conditions. By suitable modification of hydrogels they can become responsive to biomolecules and used as biosensors. Hydrogels offers great advantage for wound healing since they can provide moisture and healing chemicals for long time [8]. Applications of hydrogels is not limited to these examples, they can be applied to new areas by creating novel structures.

1.2. Functionalizable Hydrogels

Functionalization of hydrogels is crucially important to use them as desired material for specific purpose. It determines the characteristics of the hydrogel for the specific application. For example, different functionality may determine release kinetics of drugs in drug carriers. Similarly, it may determine the sensitivity of a hydrogel based biosensor.

In recent years, there has been a lot of effort to synthesize novel functionalizable hydrogels. Mostly, covalent functionalization has been used. It is because physical trapping of functional molecules to hydrogel or functionalization via diffusion is not a controllable method [9-12]. For covalent functionalization click chemistry is a convenient way because of its high efficiency and reliability. It can be used for both crosslinking and functionalization. Stereoselectivity, success in mild conditions and high yield of click reactions attracted scientist to benefit them during synthesis and functionalization of hydrogels [13-15]. Huisgen 1,3-dipolar addition, Diels-Alder reactions and Thiol-ene reactions are commonly used click reaction to synthesize hydrogel. Among them Diels-Alder reactions differ in terms of reversibility and reagents used during reaction. Depending on temperature Diels-Alder reactions can revert back to give the diene and dienophile. In addition to its reversible nature, Diels-Alder reactions are reagent free which is a great advantage in many cases. There is no need for additional effort to get rid of

residual metal impurities based catalyst such as copper in Huisgen type click reactions [16-23].

1.3. Hydrogels via Click Reactions

In 2006, Hawker *et al.* synthesized hydrogels using Huisgen type click chemistry. They first converted linear PEG polymers (with different Mn's) into PEG dialkyne. Then they synthesized a tetra-azide based crosslinker. At the end of the click chemistry between PEG's alkynes and tetraazide gel formation occurs as seen in Figure 1.4. By varying the alkyne/azide ratio they tuned hydrogel's chemical and physical properties. Since click chemistry is tolerant to many conditions and additives, it has been become a popular tool in recent years to synthesize hydrogels [14].

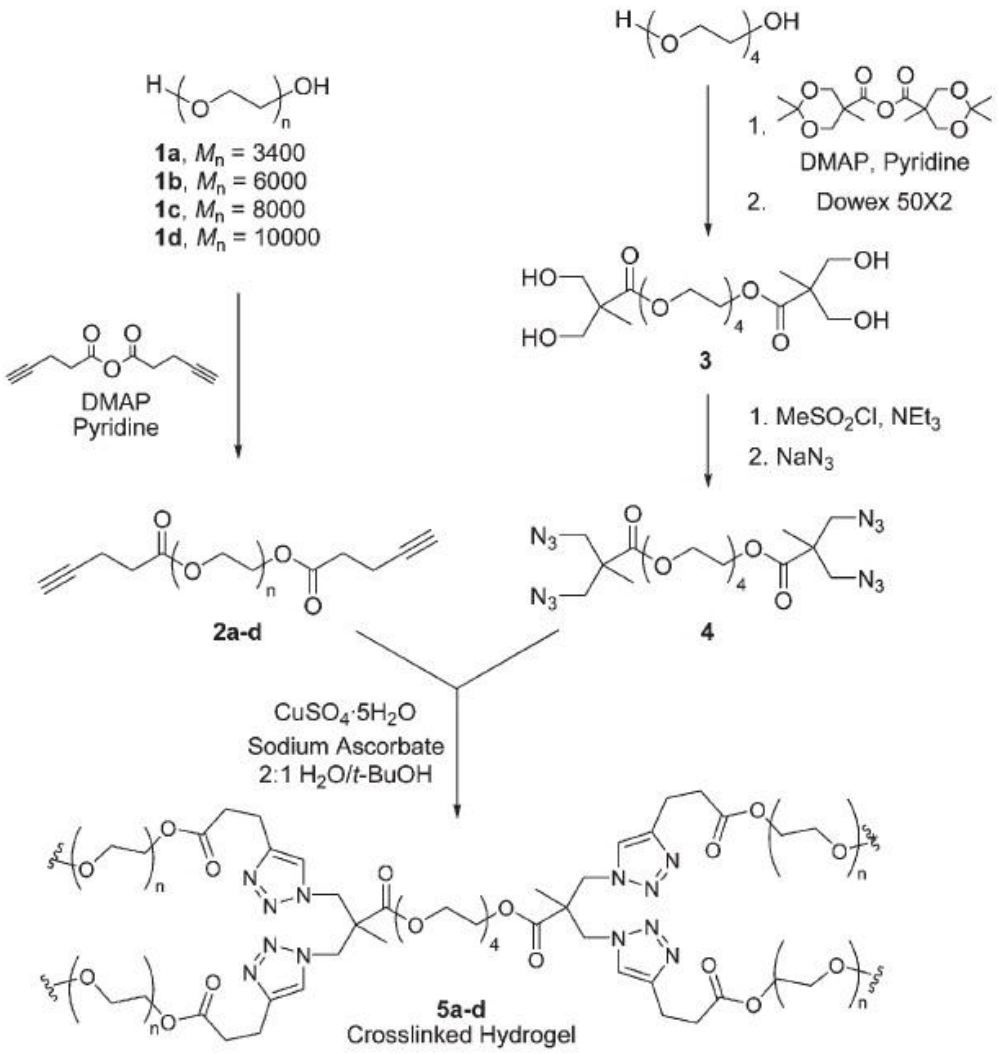


Figure 1.4. Hydrogel synthesized by using click chemistry.

Again in 2006 Hilborn *et al.* synthesized Poly(vinyl alcohol) based hydrogels using click chemistry. They modified PVA's to PVA azide and PVA alkyne. Then carrying out 1,3-cycloaddition between modified polymers resulted in hydrogel. They showed that by varying functional groups on polymers, the hydrogel's chemical and physical properties changes. They also compared the hydrogels formed by two polymeric components to polymer and a bifunctional crosslinker system. They conclude that mixing two polymeric components is advantageous then using bifunctional crosslinker because previous approach offers better physical properties and higher gel conversions.

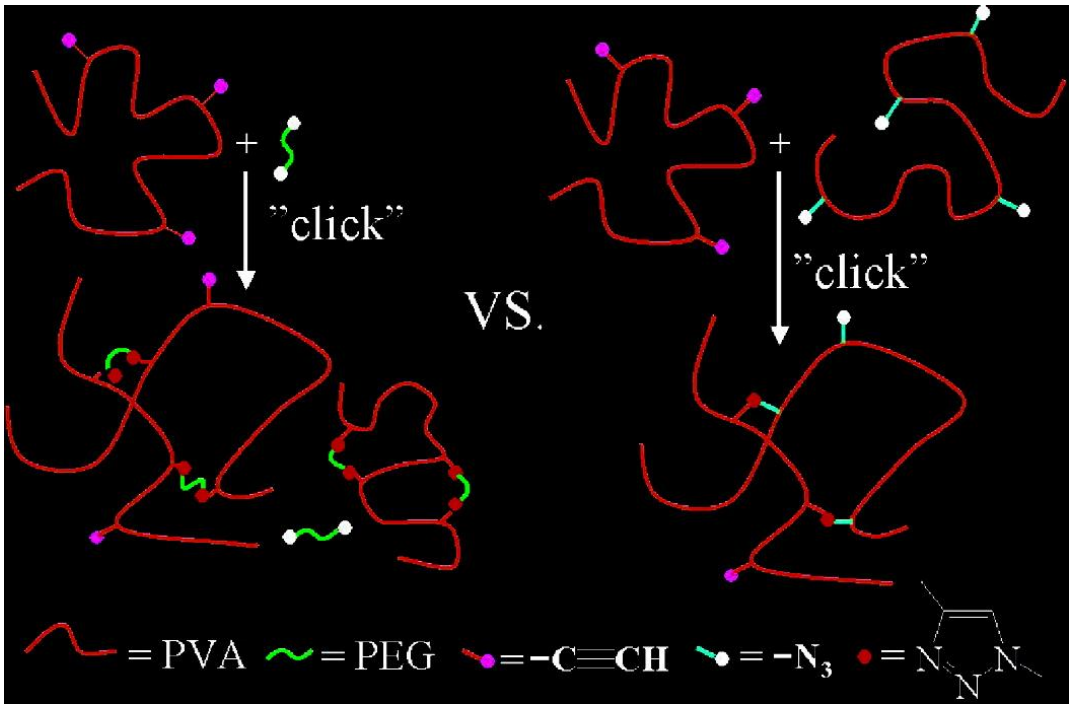


Figure 1.5. Different approaches to synthesize PVA based Hydrogel by click chemistry.

Crescenzi *et al.* synthesized two different hyaluronan based polymers, one having azide as side chains and other one having alkyne functionality as side chains. Click reaction between azide and alkyne functionality resulted in gelation shown in Figure 1.4. By addition of doxorubicin to gelation media they physically trapped the drug. They achieved control of release of the drug by changing azide and alkyne stoichiometry which resulted in changing of the crosslinking density [24].

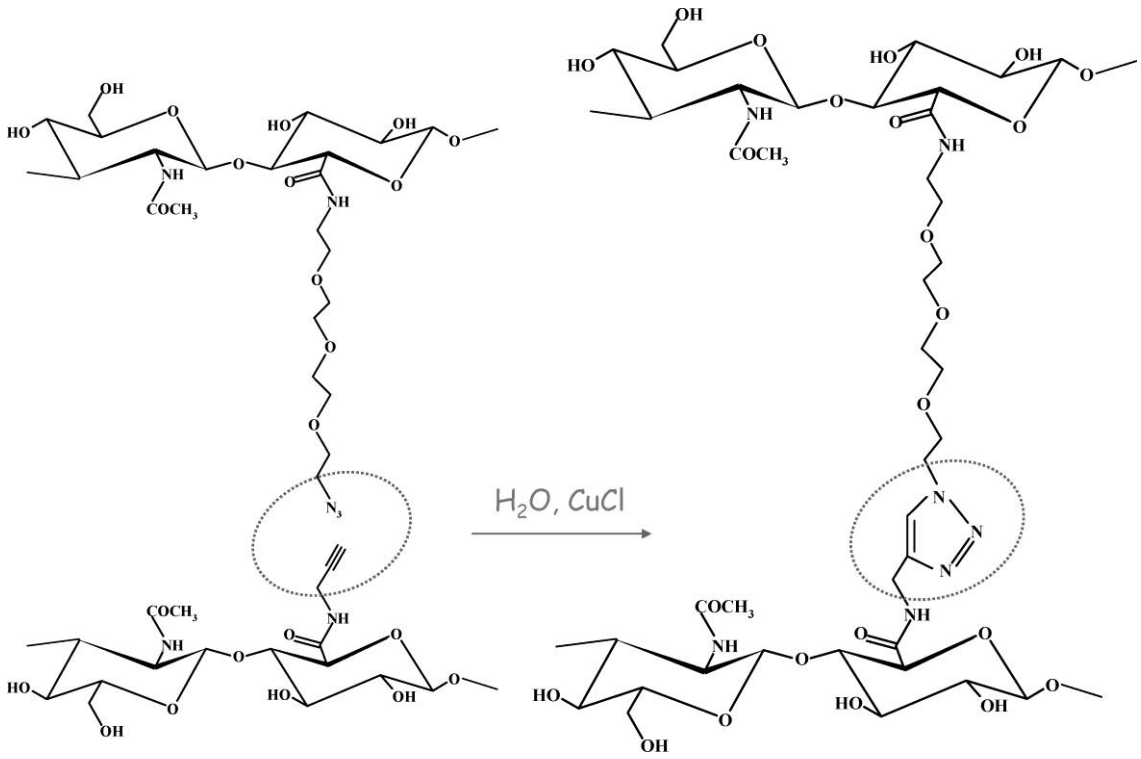


Figure 1.6. Gelation via Huisgen type click reaction.

The Huisgen [3+2] cycloaddition reaction often uses copper as catalyst. However, getting rid of copper will be a problem in highly crosslinked gels and this limits its potential to be used in biological systems. Diels-Alder reactions are reagent free and they will be advantageous to use to crosslink systems in cases where a residual reagent may cause problems for the applications. There are numerous examples of crosslinked polymeric materials obtained by the Diels-Alder reaction.

1.4. Gels and Hydrogels via Diels Alder Reactions

In 1990, Chujo *et al.* used retro Diels-Alder strategy to reverse the gelation of Polyoxazoline [22]. They showed that heating polymer at 80°C for 2 hours result in breaking of linkages in the network of the polymer.

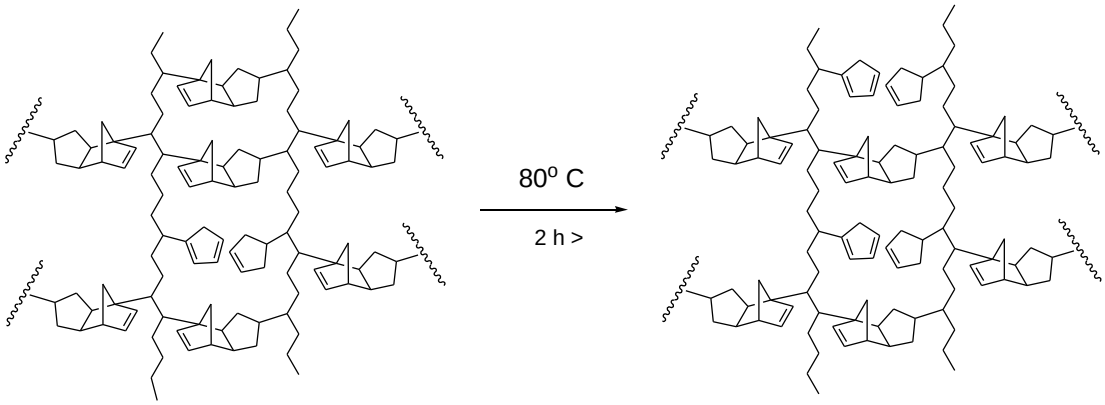


Figure 1.7. Reverse gelation via retro Diels-Alder reaction.

In 2002, Wudl *et al.* showed that a polymeric material crosslinked by Diels-Alder reaction can become a self healing material because of thermoreversible nature of the adduct (Figure 1.5). The novel and creative aspect of this work is that self healing mechanism does not require any additional monomer or catalyst and the self-healing process can be repeated many times [25].

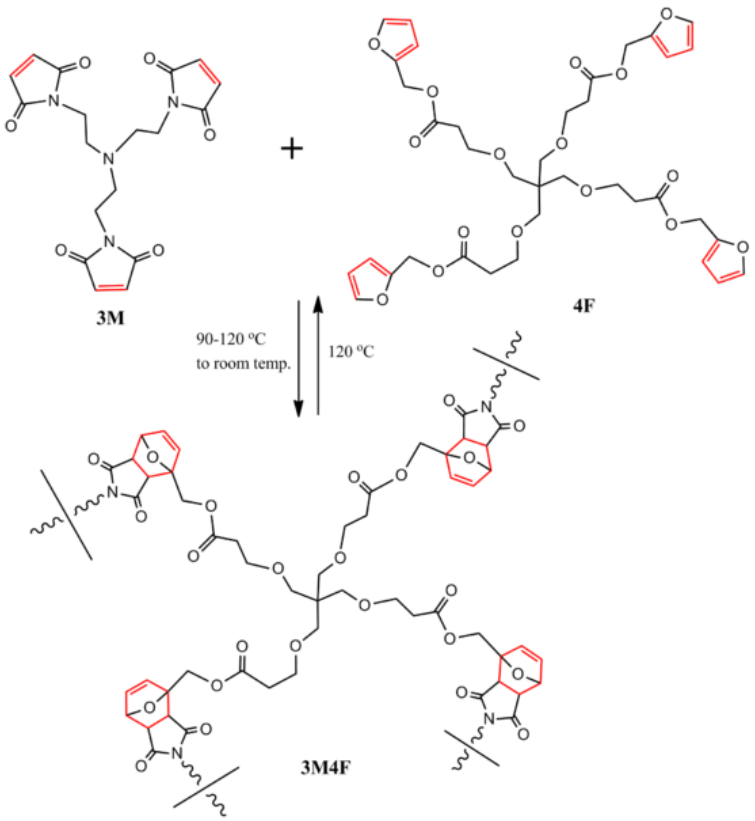


Figure 1.8. Gel crosslinked by DA reaction and decrosslinked by retro DA reaction.

Maleimide and furan gives the cycloadduct via Diels-Alder reaction over a wide range of temperatures. However, if the temperature becomes too high (over 120°C), this reaction goes back to give furan and maleimide. The reverse reaction is called retro Diels-Alder reaction. After the retro Diels-Alder reaction, letting the medium cools down results in Diels-Alder reaction of furan and maleimide. In other words, the material was first decrosslinked by retro Diels-Alder reaction and then crosslinked again by Diels-Alder reaction. As a result of this, cracks that have been produced due to stress on the material can repair slowly over time.

In 2007 Singha *et al.* synthesized furfuryl containing polymer via ATRP and using bismaleimide they showed crosslinking by Diels-Alder and decrosslinking by retro Diels-Alder [19].

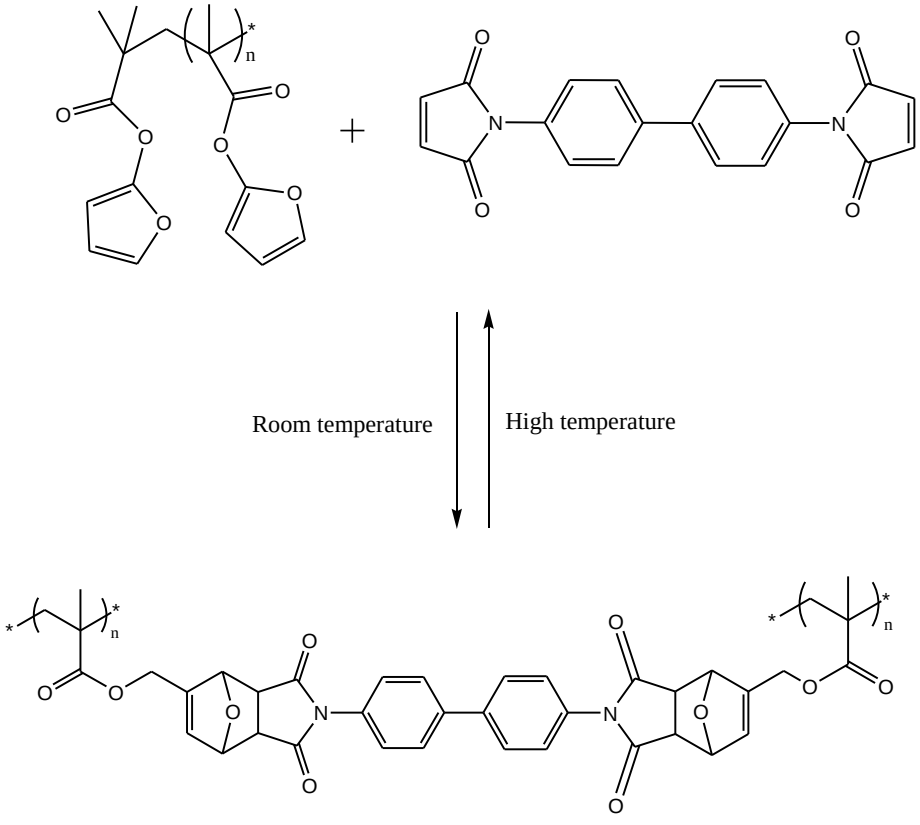


Figure 1.9. Polymers crosslinked by DA reaction and decrosslinked by retro DA reaction.

In 2010 Sanyal *et al.* used the Diels-Alder/retro Diels-Alder strategy for crosslinking of polymers in situ to produce. In their work they used Diels-Alder / retro Diels-Alder

strategy to protect and deprotect reactive maleimide group. After deprotection some of the double bound of the maleimide participate in polymerization to result in formation of crosslinked material [18]. The reactive double bonds of the maleimide can react with thiol containing molecules. By varying ratio of masked monomer to PEGMA, control over the amount of maleimide in the hydrogel can be achieved.

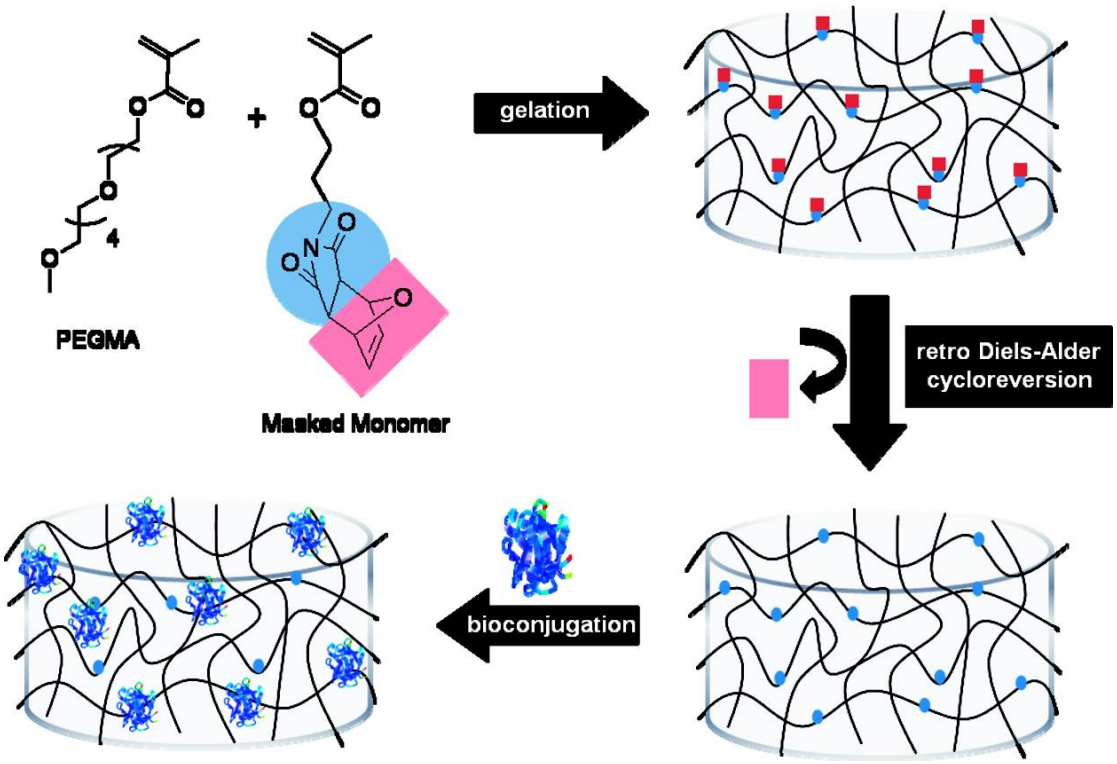


Figure 1.10. Activation of maleimide and crosslinking done by DA reaction.

In 2009 Wei *et al.* synthesized thermosensitive hydrogels by fast Diels-Alder reaction in water. They have demonstrated gelation of polymers in water by fast Diels-Alder reaction and decrosslinking of the hydrogels in DMF (Figure 1.9). They also showed that retro Diels-Alder reaction in water does not take place even if the temperature increased to 100°C [26].

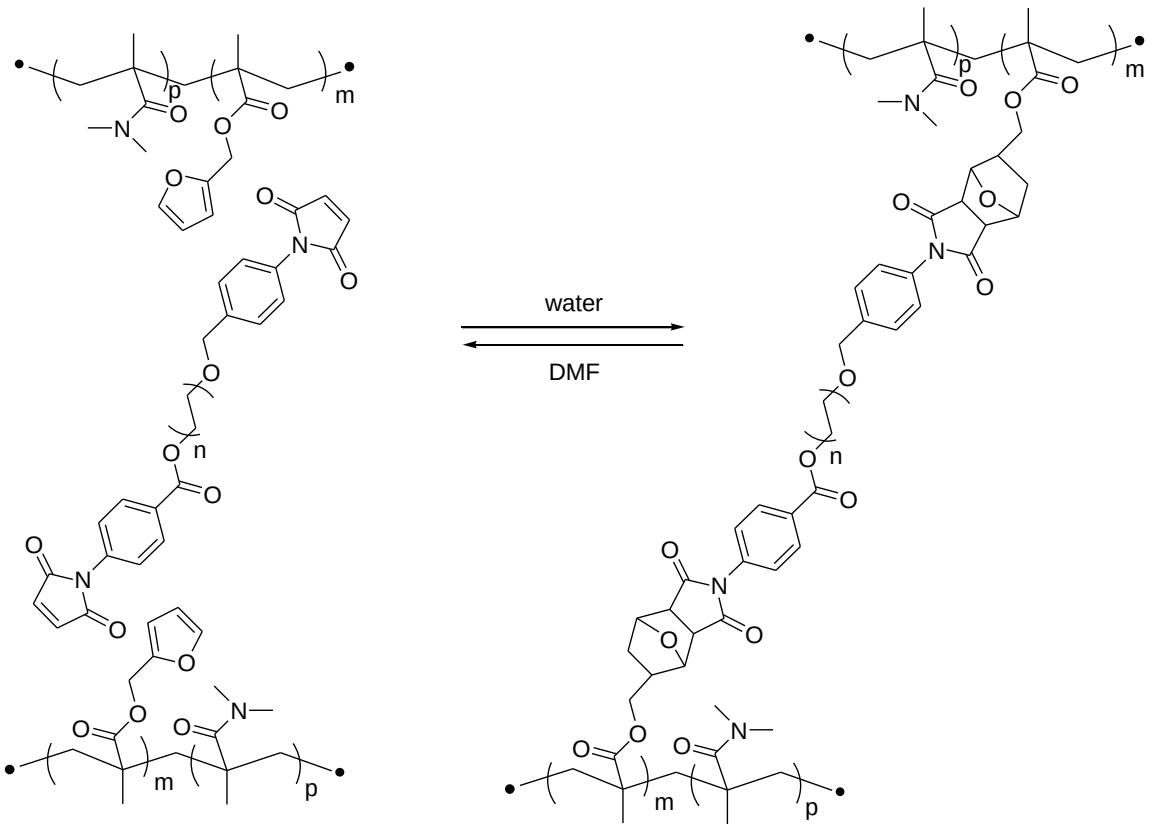


Figure 1.11. Crosslinking done by DA reaction between maleimide and furan.

In this work they successfully controlled the gelation by varying temperature. They also showed that with increasing temperature the swelling ratio of the hydrogels decreases. Therefore, by controlling temperature they achieve control over the water uptake capacity of the hydrogels.

In 2011, Nimmo *et al.* synthesized hyaluronic acid hydrogels by Diels Alder reaction. They choose hyaluronic acid as polymer because hyaluronic acid is natural polymer and it is promising for biological applications such as tissue engineering. They firstly modified hyaluronic acid with furan. To crosslinked HA polymers they choose bismaleimido PEG. In aqueous conditions they carry out Diels Alder reaction and get hydrogel [27].

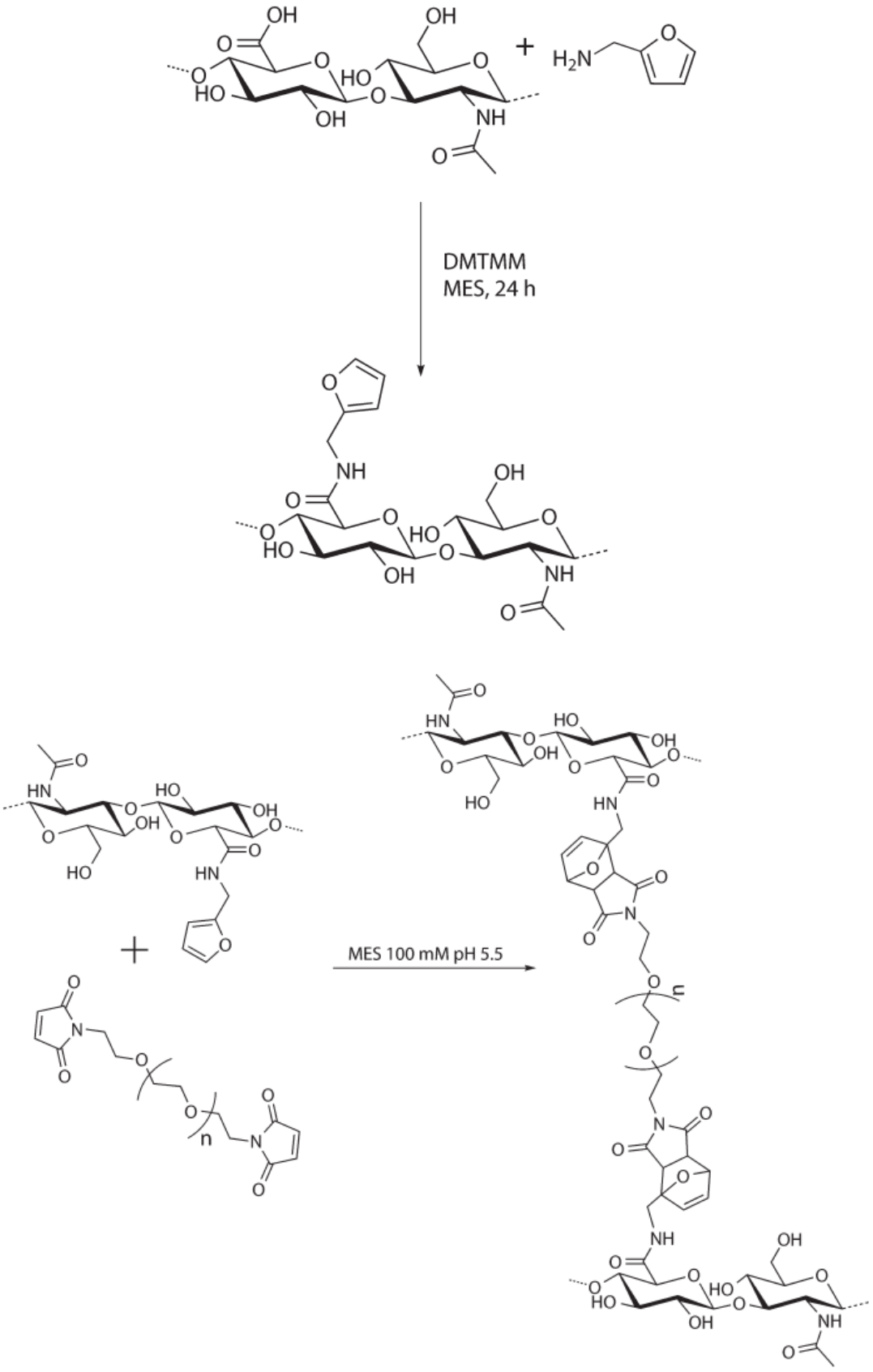


Figure 1.12. Crosslinking of HA polymers by Diels Alder reaction.

1.5. Functionalization of Materials via Diels Alder Reactions

Diels Alder reactions are very powerful tool not only for crosslinking materials but also functionalization of the materials such as polymers or nanotubes. Since it is single step, catalyst free and clean reaction it is beneficial to use it for functionalization and immobilization of biomolecules. In 2006 and 2007 Sun *et al.* have demonstrated carbohydrate and protein immobilization on a glass surface via Diels Alder reaction. They showed that Diels Alder reactions can be utilized to post functionalize or immobilize glass with variety of ligands [28].

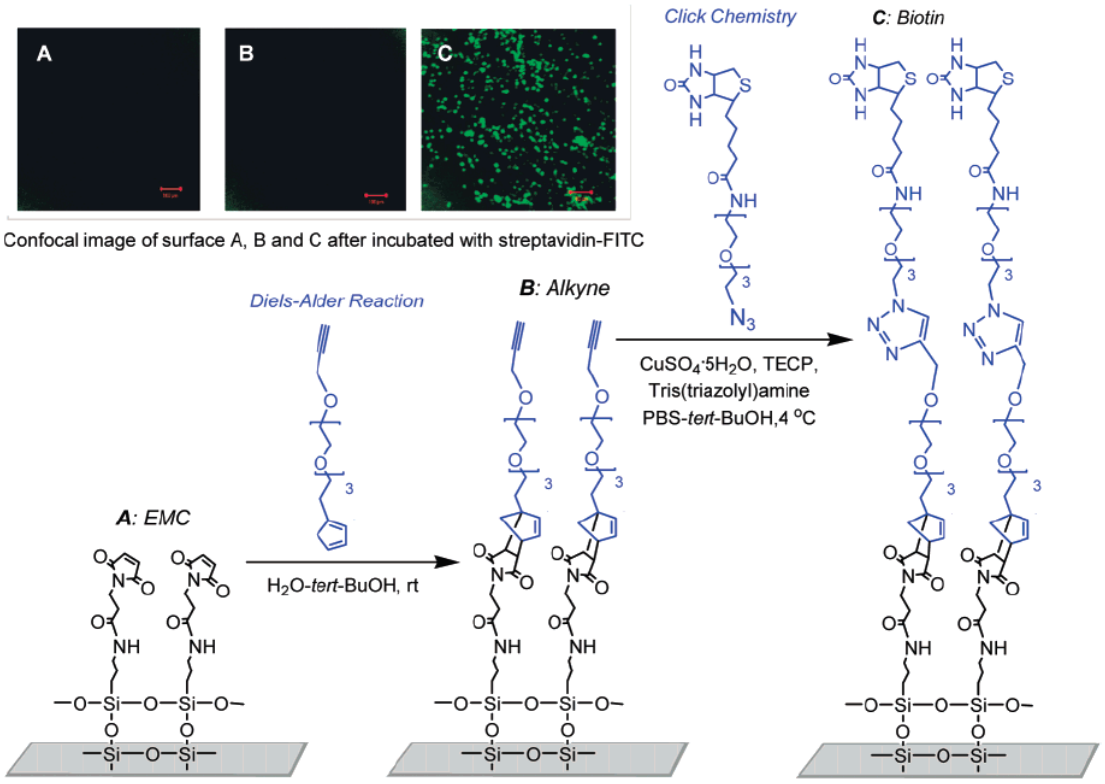


Figure 1.13. Sequential functionalization of glass surface by Diels Alder reaction.

Firstly, they introduced the functional group (alkyne, azide, cyclodiene etc.) onto glass surface by Diels Alder reaction. Then, with complementary functional group they immobilize the target molecule onto the glass surface.

In 2007, Shi *et al.* showed functionalization of nanoparticles by Diels Alder reaction. First, they synthesized amphiphilic furan modified polymer that can self assemble

to form micelle nanoparticles. Then they modified antibodies with maleimide to functionalize the micelles by antibodies via Diels Alder cycloaddition [29].

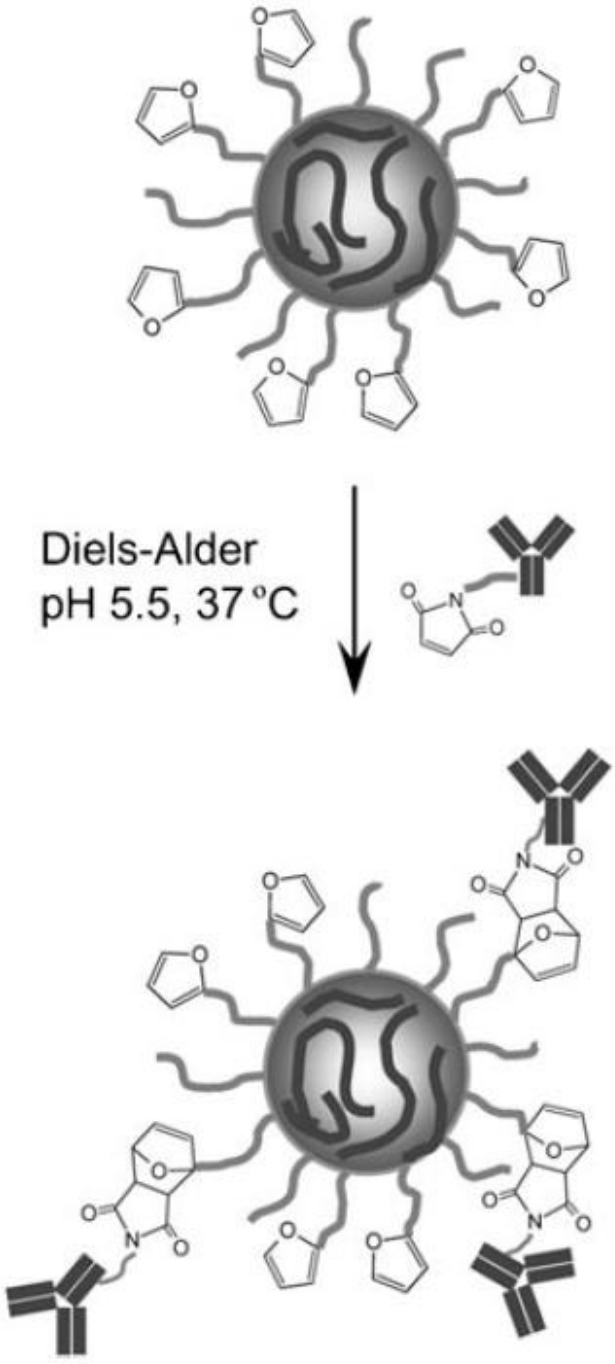


Figure 1.14. Functionalization of the surface of micelle nanoparticles by DA reaction.

1.6. Hydrogels for Biological Applications

Recent years have been shown a great interest in hydrogels to use in biological applications such as tissue engineering, drug delivery and biosensors. For all of these applications choice of polymers, synthetic methods, functionalization and design of the hydrogel network plays crucial role. Natural polymers such as collagen, hyaluronic acid, fibrin, aliginate and chitosan can be a good candidate for making hydrogel because they have the advantages of biocompatibility and low toxicity. Alternative to natural polymer synthetic polymers such as PEG, PVA and PHEMA can be used. Among them PEG is most widely used polymer because it is non toxic, non immugenic and approved by FDA as well [30].

1.6.1. Tissue Engineering

Hydrogels have been used as scaffolds for cell growth in tissue engineering. Design of hydrogels for this specific purpose must mimic the natural extracellular matrix (ECM). A good scaffold hydrogel should be capable of good cell adhesion and migration. The more designed hydrogels mimic the natural tissue the more successful will be the hydrogel when used as a scaffold. Therefore, the parameters such as water content, mechanical properties and biocompatibility are crucial for these hydrogels. Another important aspect of hydrogels for tissue engineering is biodegradability. The successful candidate should degrade after cell growth. A popular and very important research in this area is 3D patterning of hydrogels. The aim in here is to make three dimensional networks of cell growths which is a must to successfully design desired neural cell (Figure 1.15) [31].

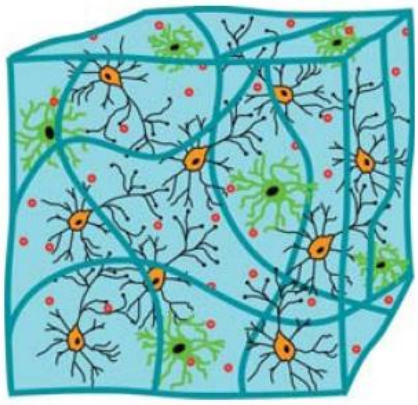


Figure 1.15. Three dimensional growth of cells in hydrogel.

1.6.2. Drug Delivery

For drug delivery applications hydrogels have been used as a carrier. Drug can be entrapped in hydrogel by chemically or physically**.** Design of the hydrogels is important for controlled release. Release of the drug can be controlled by a stimulus (temperature, pH, some enzymes or magnetic field) in the environment. For example PNIPAM hydrogel release drug in response to temperature changes [32]. Another example is in magnetic nanoparticles containing hydrogels release of the drug can be controlled by magnetic field (Figure 1.16) [33]. For drug delivery applications hydrogels having targeting groups and biodegradable structure are desired properties.

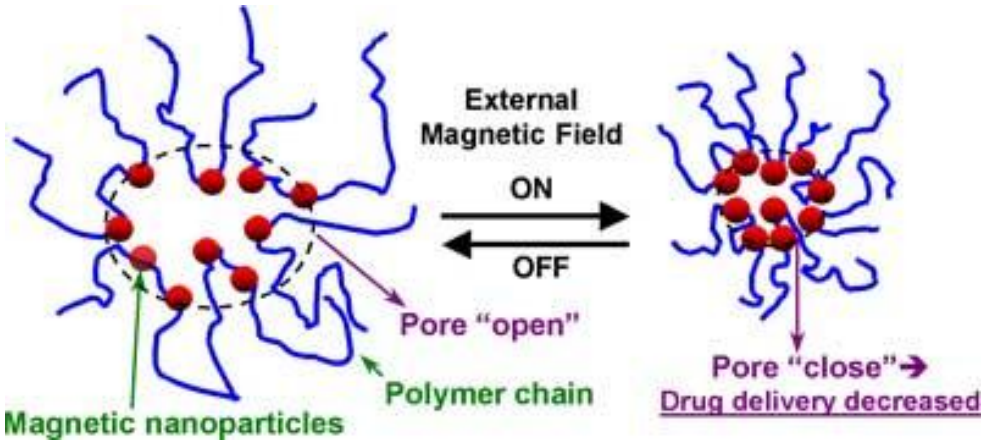


Figure 1.16. Drug release controlled by magnetic field.

1.6.3. Sensors and Biosensors

Hydrogels can be integrated to micro devices to use a biosensor. Like in drug delivery systems responsiveness of the hydrogel is important parameter for sensor applications. Han *et al.* use pH responsive hydrogels to make osmotic pressure sensor. Swelling of the pH responsive hydrogel changes during pH change and by measuring pH of the hydrogel the osmotic swelling pressure can be calculated [34]. For biosensor applications patterning of the hydrogels is important for better detection of analytes. Since patterning allows spatial location of various detection elements.

2. AIM OF THE STUDY

The aim of this research is to synthesize maleimide containing hydrogels that can find applications in biomolecular immobilization. To date there are only a few examples of hydrogels having maleimide functionality. The proposed methodology in here will allow a convenient, easy and catalyst free synthesis for maleimide containing thiol reactive of hydrogels. In addition to simplicity of the methodology, it will provide a homogenous hydrogels compared to other methods because a single precursor would be utilize to attain hydrogel.

The maleimide functionality on the polymers, which are going to form the hydrogel, can be achieved by Diels-Alder/retro Diels-Alder strategy. Using the same strategy the crosslinking of the polymers will be done. Having furan and masked maleimide on the same polymer will result in crosslinking after heating to the temperature at which retro Diels-Alder reaction taken place (Figure 2.1).

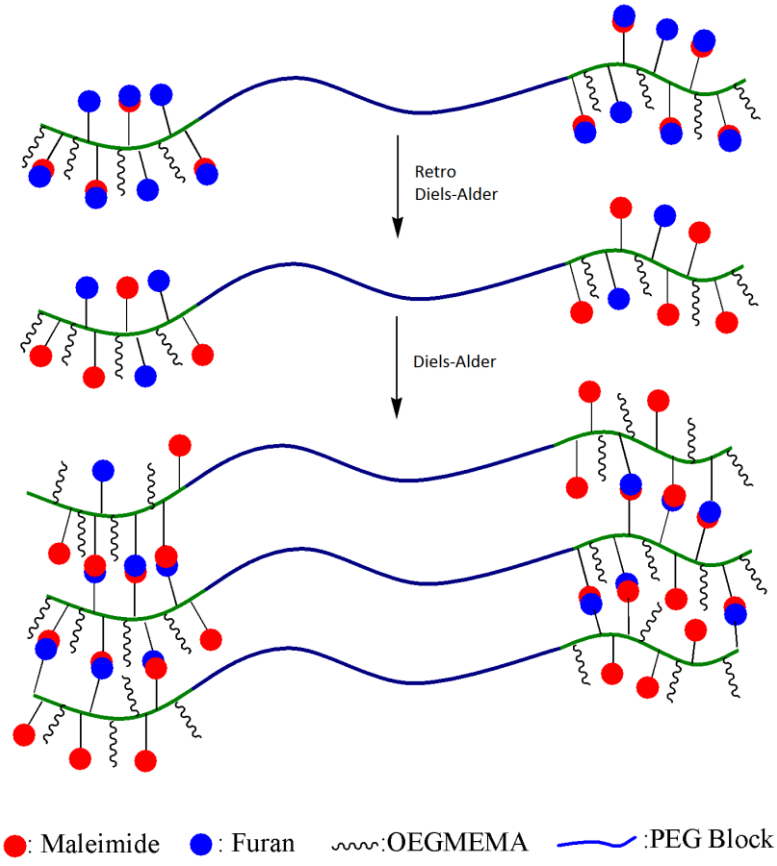


Figure 2.1. General scheme to illusturate the synthesis of hydrogels from a single polymeric precursor using DA/retro DA strategy.

In this research we designed a triblock ABA type polymer in which linear PEG polymer is the middle block. Since PEG is non-toxic, non-immugenic and biocompatible, is chosen as middle block. It will be shown that designing a triblock polymer will provide control over reactive chemical functionality within the hydrogel and also allow us to vary physical property of the hydrogel such as its swelling capacity.

3. EXPERIMENTAL

3.1. Methods and Metarials

Methacrylic anhydride was purchased from Sigma Aldrich. Polyethylene Glycols with a quoted molecular weights of *M*n = 6000, *M*n = 8000, *M*n = 10000 was purchased from Fluka. CuBr, 2,2'-Bipyridyl and triethylamine (TEA) were purchased from Sigma Aldrich and used without further purification. Furfuryl methacrylate was obtained from Sigma Aldrich. 2-Bromo-2-methylpropionyl bromide was purchased from Acros Organics. Other chemical reagents were obtained from commercial resources and were used as received. The dry solvents such as dichloromethane (DCM), tetrahydrofuran (THF) and toluene were obtained from SciMatCo purification system. Column chromatography was performed using silica gel 60 (43-60 nm). Thin layer chromatography was performed using silica gel plates (Kieselgel 60 F254, 0.2 mm, Merck). The plates were viewed under 254 nm UV lamp otherwise plates were developed either by $KMnO_4$ stain.

3.2. Measurements and Characterization

The monomer and polymer characterizations involved ^{1}H NMR spectroscopy (Varian Mercury-MX 400 Hz). Removing water from hydrogels was accomplished with LabConco lyophilizer. Thermogravimetric analysis (TGA) was performed on a TGA Q50 from TA instruments with a heating the polymer from ambient temperature to 600°C at a rate of 10°C/min under N_2 with a flow rate of 100 mL/min. The molecular weight and distribution of polymers were estimated by gel permeation chromatography (GPC) analysis (Viscotek GPCmax VE-2001 analysis system) and PLgel (length/ID 300 mm × 7.5 mm, 5 μm particle size) Mixed-C column was calibrated with polystyrene standards, using refractive index detector. THF was used as eluent at a flow rate of 1 mL/min at 30°C.

3.3. Synthesis of Furan Protected Maleimide Methacrylate Monomer (FPMMA)

In our previous study, we have reported synthesis and characterization of furan protected maleimide monomer functionalized with methacrylate end group as a polymerizable group, namely FPMMA [31]. In this study same monomer was synthesized by using methacrylic anhydride instead of methacryloyl chloride. In a typical synthesis, the alcohol 1 (2.35 g 0.010 mol), *exo*-3a,4,7,7a-tetrahydro-2-(3-hydroxypropyl)-4,7-epoxy-1*H*-isoindole-1,3(2*H*)-dione was dissolved in DCM (50 mL), TEA(1.11 g 0.011 mol) and 4-(Dimethylamino)-pyridine (0.37 g 0.003 mol) was added to solution. Methacrylic anhydride (1.7 g 0.011 mmol) was added to reaction mixture slowly at room temperature and reaction stirred for 10 hours. The mixture washed with water (50 mL), saturated $NaHCO_3$ solution (50 mL) and water (50 mL) respectively. Organic layer were dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified by column chromatography and characterized by NMR.

^{1}H NMR ($CDCl_3$): 6.49 (s, 2H, CH=CH), 6.11 (s,1H, CH_2=C), 5.55 (m, 1H, CH_2=C), 5.24 (s, 2H, CH bridgehead protons), 4.09 (t, 2H, J= 6.2 Hz, OCH_2), 3.59 (t, 2H, J = 7.0 Hz, NCH_2), 2.82 (s, 2H, CH-CH, bridge protons), 1.98–1.91 (m, 5H,$CH_2CH_2CH_2$ and CH_3); ^{13}C NMR ($CDCl_3$): 176.0, 167.1, 136.4, 136.1, 125.4, 80.8, 61.4, 47.3, 35.7, 26.6, 18.2; IR (KBr): υ = 1705.8 cm^{-1}.

3.4. Synthesis of Br-PEG-Br Initiator

In this study, three PEG macroinitiators with different molecular weights (6 K, 10 K, 20 K) were synthesized. In a typical experiment, PEG (0.25 mmol) was dissolved in toluene and dried by removal of water-toluene by azeotropic rotary evaporation. Then it kept under to high vacuum for 24 hours. Dried PEG was dissolved in 30 mL DCM. TEA (0.15 mL, 1.06 mmol) was added to the PEG solution under nitrogen. The temperature of the solution was cooled to 0°C and 2-bromo-2-methylpropionyl bromide (0.62 mL, 5.02 mmol) was added dropwise and the reaction was left stirring overnight. The mixture was washed with saturated $NaHCO_3$-solution. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was

dissolved in minimum anount of DCM and precipitated in diethyl ether. Product was obtained as white powder.

3.5. Polymerization of Br-PEG-Br initiated PEG-poly(FFMA-FPMMA-OEGMEMA)$_2$ triblock polymer

PEG macroinitiator (Br-PEG-Br) was used as a initiator to initiate the polymerization of PEG- poly(FFMA-FPMMA-OEGMEMA)$_2$ triblock copolymer in the presence of the complex of copper(I) bromide (CuBr) with 2,2-Bipyridyl. To a 10 mL round bottomed flask connected with the Schlenk line the FPMMA monomer (223 mg, 0.76 mmol), 2,2'-Bipyridyl (55 mg, 0.39 mmol) and CuBr (17 mg, 0.12 mmol) were charged and degassed with N_2. Degassed FFMA (0.058 mL, 0.38 mmol) and OEGMEMA (0.24 mL, 0.86 mmol) solution in MeOH (5 mL) and H_2O (1 mL) was added to the mixture and stirred. To the reaction mixture Br-PEG-Br macroinitiators (0.02 mmol) was added and stirred at room temperature for various time periods. The polymerization was monitoring by GPC periodically. After polymerization the reaction mixture was diluted with methanol and passed over a column of neutral alumina to remove the catalyst. The solution was concentrated and precipitated into excess amount of diethyl ether. The resulting polymer was dried under vacuum at room temperature for 24 hours and characterized by NMR.

3.6. Representative Hydrogel Synthesis

For the gelation of PEG-poly(FFMA-FPMMA-OEGMEMA)$_2$ triblock copolymer, 30.0 mg of polymer was dissolved in 0.15 mL dry toluene in a vial and heated at 110°C for 3 hours. Then sample was moved to an oil bath at 60 °C for 2 hours. The resulting gel was purified by washing with methanol under sonication to remove non-crosslinked polymers.

3.7. Swelling Studies

Dried hydrogel sample was transferred to a flask containing distilled/deionized water at room temperature. In definite time intervals the hydrogel was taken out from water and weighed after drying the surface with a filter paper.

3.8. Scanning Electron Microscopy

Swollen hydrogels were freezed and put into lyophilizer overnight. Lyophilized samples were immersed in liquid nitrogen and broken, and given to scanning electron microscopy. Morphology characterization of hydrogels was done by using ESEM-FEG/EDAX Philips XL-30(Philips, Eindhoven, The Netherlands) instrument with an accelerating voltage of 10 kV.

3.9. Functionalization with BODIPY-Thiol

Dried hydrogels were put into solution of BODIPY-Thiol in THF for 12 hours at room temperature. To get rid of unbound BODIPY thiol, the hydrogels washed with THF and kept in THF for 12 hours. Then, fluorescence images were taken.

3.10. Crosslinking Test with Anthracene

30 mg of P10-5K and 10 mg of anthracene were put in a 10 mL round bottom flask. 0.15 mL of toluene was added and the reaction mixture heated up to 115°C for 3 hours. Then, mixture was taken to an oil bath at 60°C and kept for 2 hours. Finally, the obtained polymer is precipitated in diethyl ether to get rid of excess anthracene.

4. RESULTS AND DISCUSSION

4.1. Synthesis of furan protected monomer

The synthetic scheme of the synthesis of masked monomer is shown in Figure 4.1 . The monomer previously synthesized by using methacryloyl chloride with a yield of around 80 percent. However, the toxicity, availability and price of the methacryloyl chloride were problems to use it easily. In this study, as an alternative to methacryloyl chloride we used methacrylic anhydride to get the masked monomer. The yield of this reaction was little lower (around 70 %) when compared to previous synthesis using methacryloyl chloride but it is more advantageous to use this route because of the above mentioned reasons.

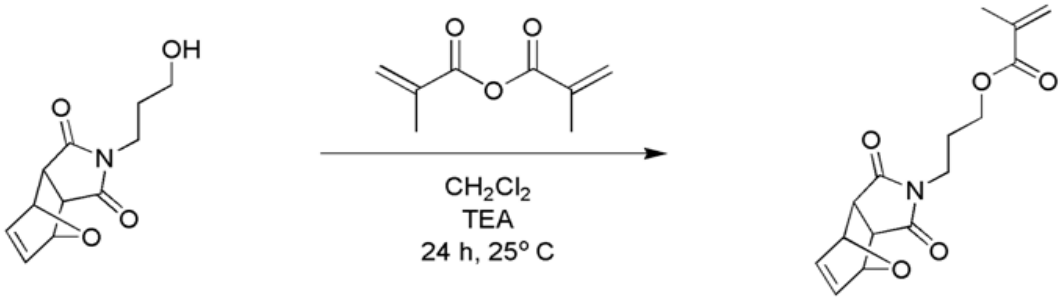
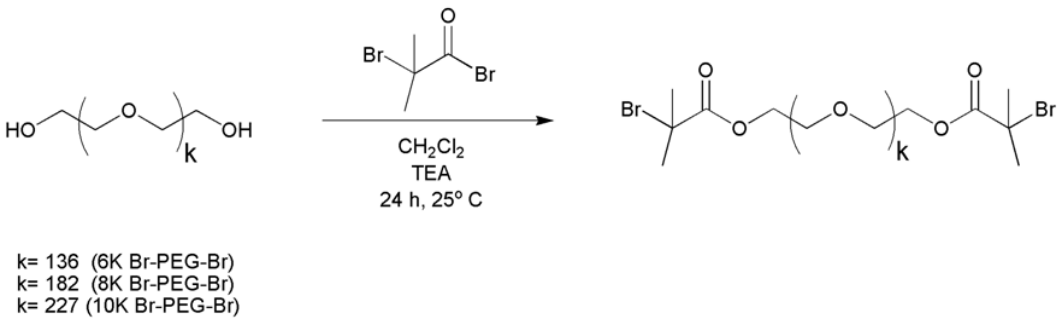


Figure 4.1. Synthesis of the masked monomer.

4.2. Synthesis of Macroinitiators

The synthetic scheme of PEG macroinitators is shown in Figure 4.2. It is a simple reaction between and alcohol and acyl bromide in the presence of triethylamine. Excess 2-bromo-2-methylpropionyl bromide is used to ensure conversion of both hydroxyl groups on the PEG. The macroinitiators were obtained in high yields (>90%) and high purity as deduced from ^{1}H-NMR spectroscopy and GPC (Figure 4.3 and Figure 4.4).



k= 136 (6K Br-PEG-Br)
k= 182 (8K Br-PEG-Br)
k= 227 (10K Br-PEG-Br)

Figure 4.2. Synthesis of Br-PEG-Br macroinitiator.

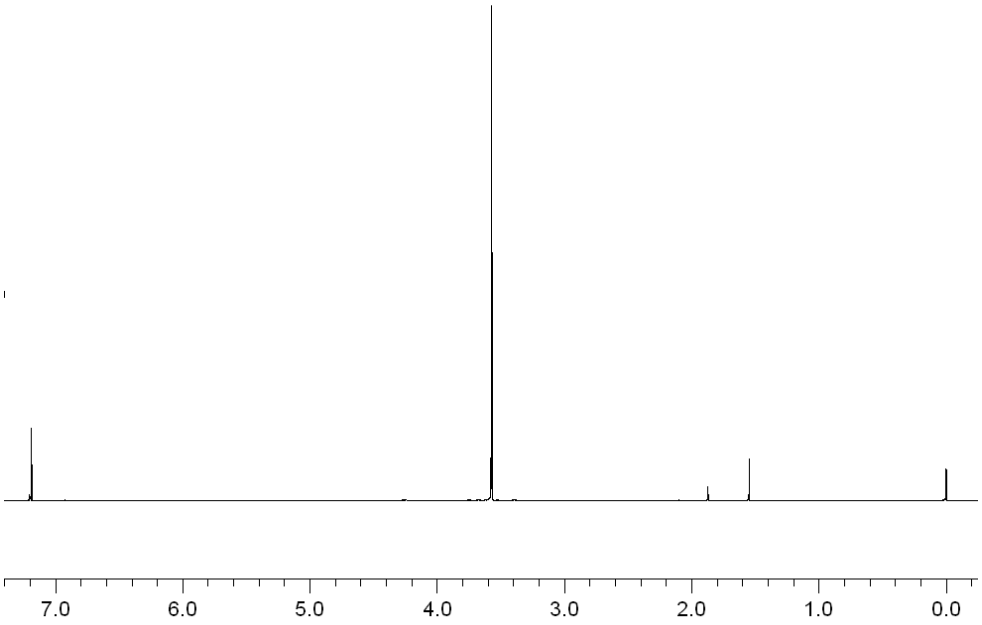


Figure 4.3. NMR spectrum of Br-PEG-Br macroinitiator.

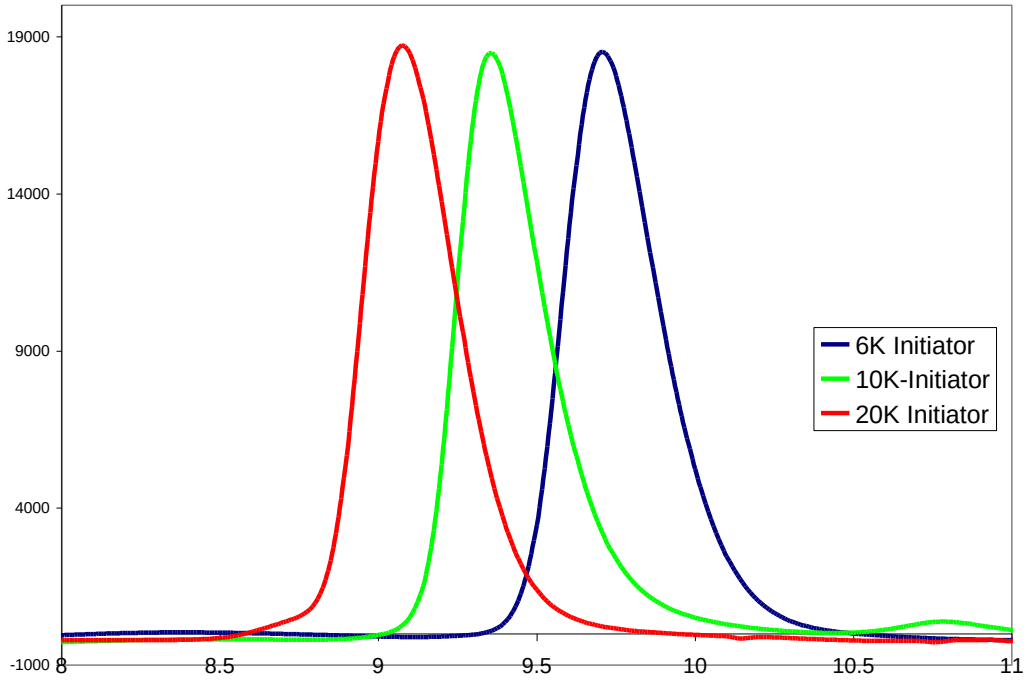


Figure 4.4. GPC chromatogram of Br-PEG-Br macroinitiators.

4.3. Synthesis and characterization of PEG-poly(FFMA-FPMMA-OEGMEMA)$_2$ triblock copolymer

The synthetic route of PEG-poly(FFMA-FPMMA-OEGMEMA)$_2$ triblock copolymer is shown in Figure 4.5. We used ATRP method for polymerization because it gives narrow PDIs and it is easy to carry out. Water/methanol system was appropriate for our purpose. The polymerization is fast in H_2O/MeOH at room temperature, hence we do not need to carry out reaction in high temperatures which may cause a partial retro DA of FPMMA monomers. Polymers were characterized by NMR, GPC and TGA.

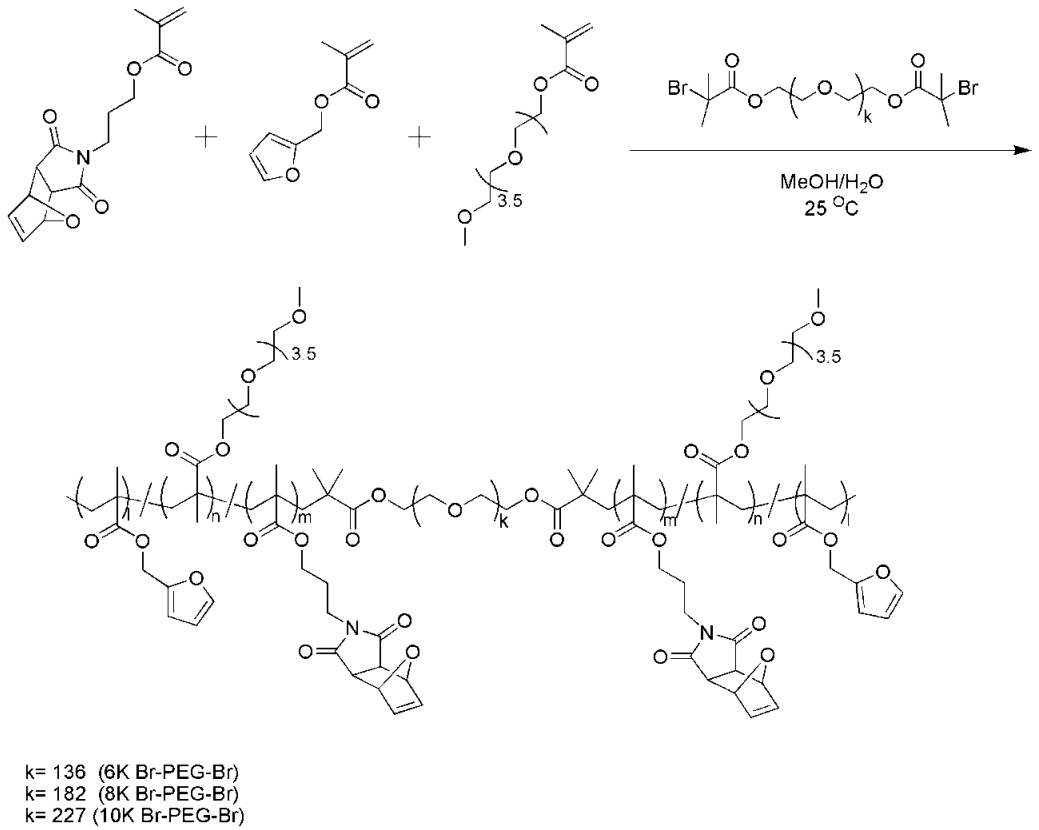


Figure 4.5. Synthesis of PEG-poly(FFMA-FPMMA-OEGMEMA)$_2$ triblock copolymer by ATRP.

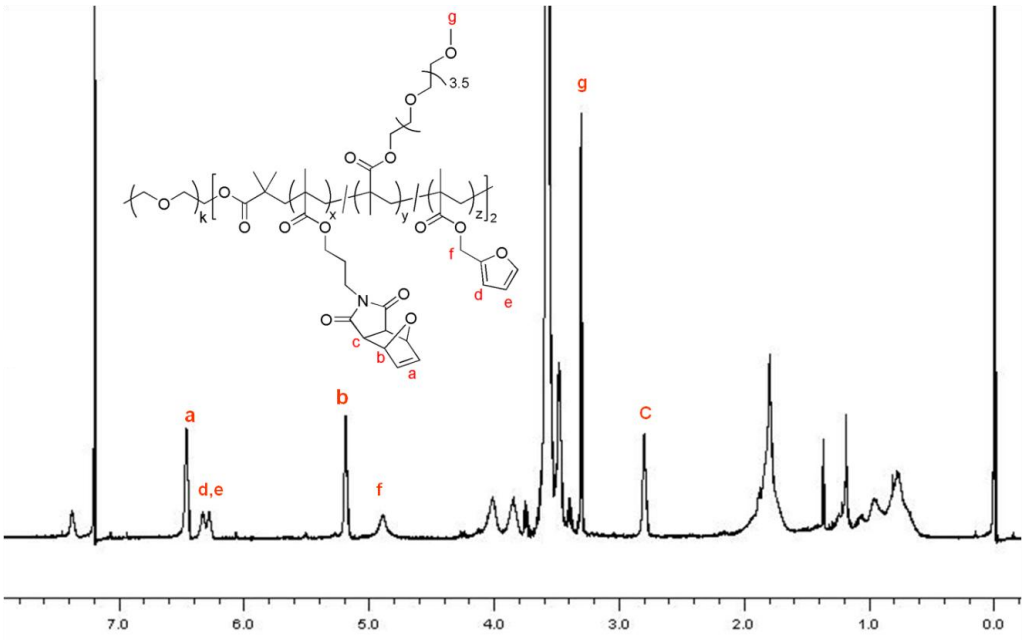


Figure 4.6. NMR of PEG-poly(FFMA-FPMMA-OEGMEMA)$_2$ triblock copolymer by ATRP.

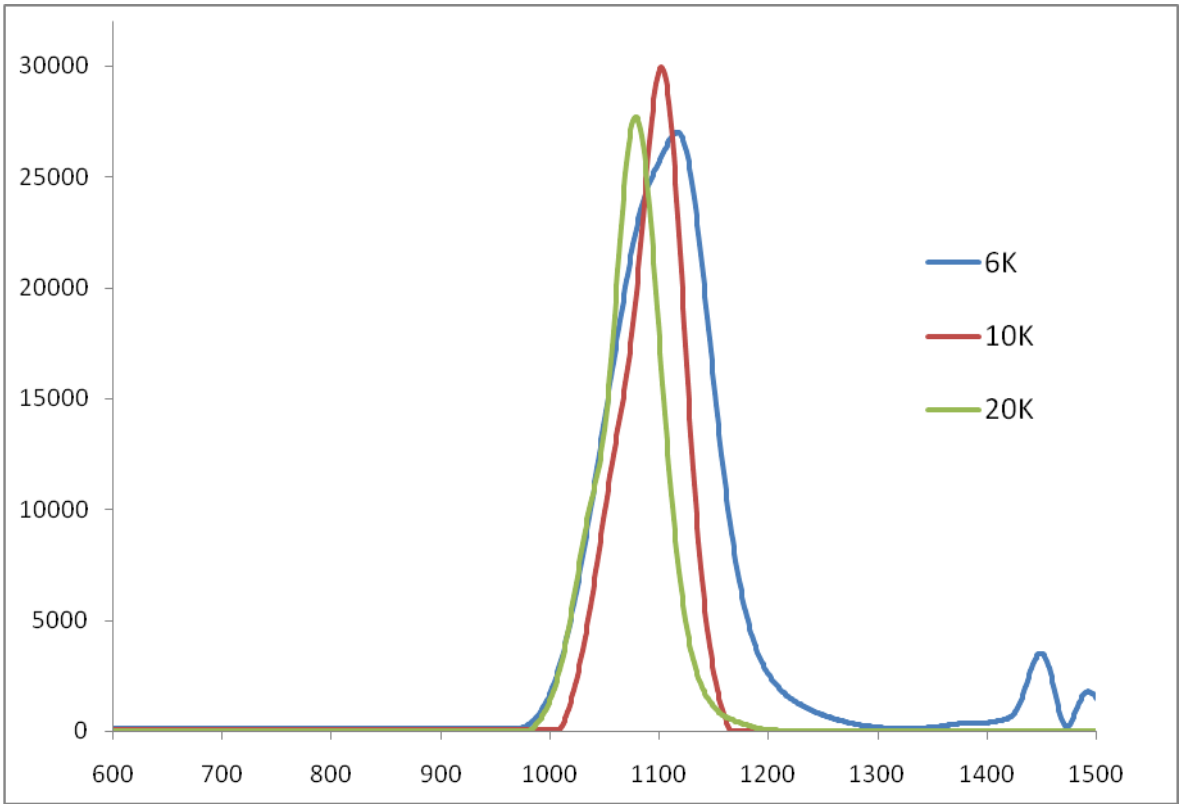


Figure 4.7. GPC chromatogram of polymers initiated with 10K macroinitiator.

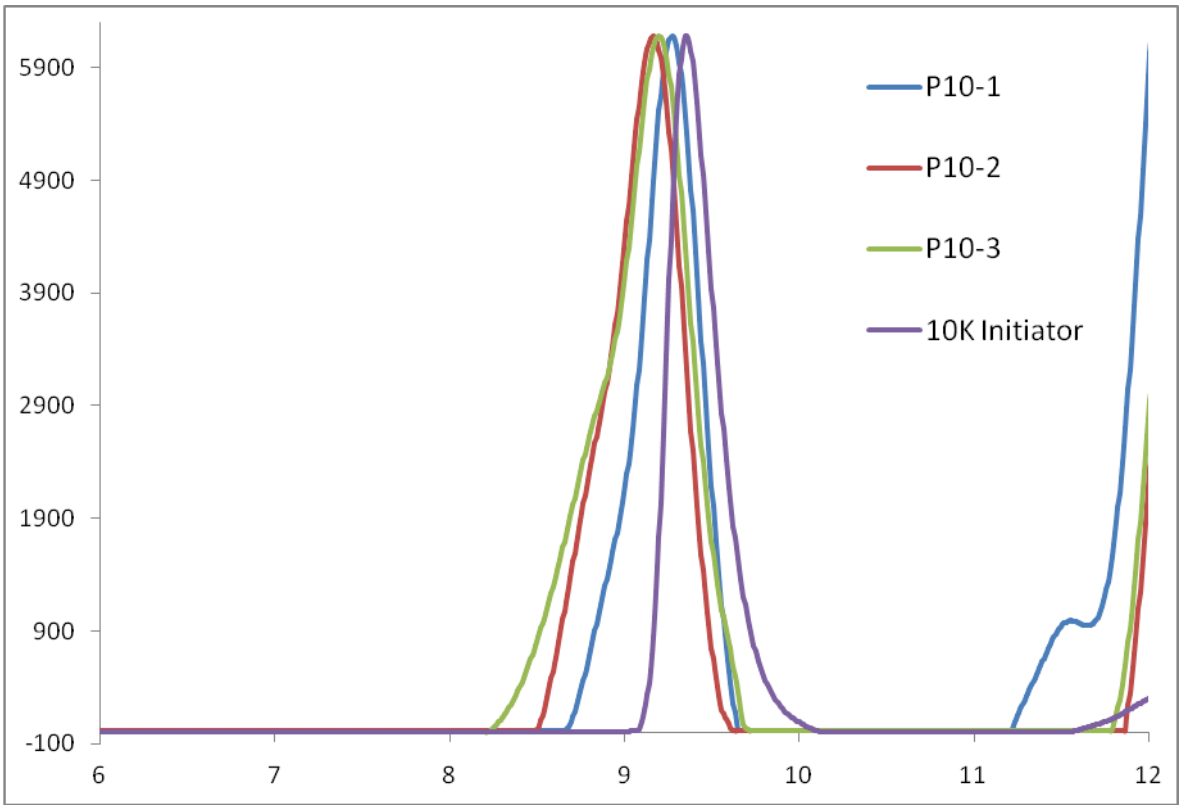


Figure 4.8. GPC chromatogram of polymers initiated with different macroinitiators.

Summary is synthesized polymers are shown in Table 4.1.

Table 4.1. Summary of the synthesized polymers and their properties.

Polymer	[A]/[B]/[C]		Molecular Weight Analysis				Calc. Furan Loss[b] %	Obs. Loss[c] %
	Feed Ratio	NMR Ratio	Mn Initiator[a]	Mn Polymer[a]	Mn Polymer[b]	PDI		
P-6	1:2:2	1:2:2	8K	18K	12K	1.39	5.26	5.28
P10-1	1:2:2	1: 2.2: 2.1	13K	19K	16K	1.15	1.91	3.28
P10-2	1:2:2	1: 2.3 : 2.4	13K	23K	18K	1.15	3.90	4.22
P10-3	1:2:2	1: 2.5 : 2.6	13K	27K	21K	1.32	4.14	4.20
P20	1:2:2	1 : 2.2 : 2.1	22K	29K	27K	1.16	1.18	2.19

[A]: FFMA [B]: PFMMA [C]: OEGMEMA [a]GPC analysis [b]Calculated from NMR [c] TGA analysis

4.4. Synthesis and Characterization of Hydrogels

Hydrogels were synthesized from polymers by crosslinking via Diels-Alder reaction of furan and maleimide group (Figure 4.10). After retro Diels-Alder reaction the furan of masked monomer escaped from medium because of its low boiling point (30°C). However, furan of the furfuryl methacrylate on the polymer can react with deprotected maleimide groups. Since the ratio of masked monomer to furan on the polymer is 2:1 after crosslinking there is free maleimide groups left on the hydrogel (Figure 4.11). TGA results showed that activation of protected maleimide was complete in 3h and there was no protected maleimide on the hydrogels were left. Furan loss from the polymers were seen on thermogram starting at 110°C whereas for the hydrogels there was no significant weight loss until 200°C as shown in Figure 4.12.

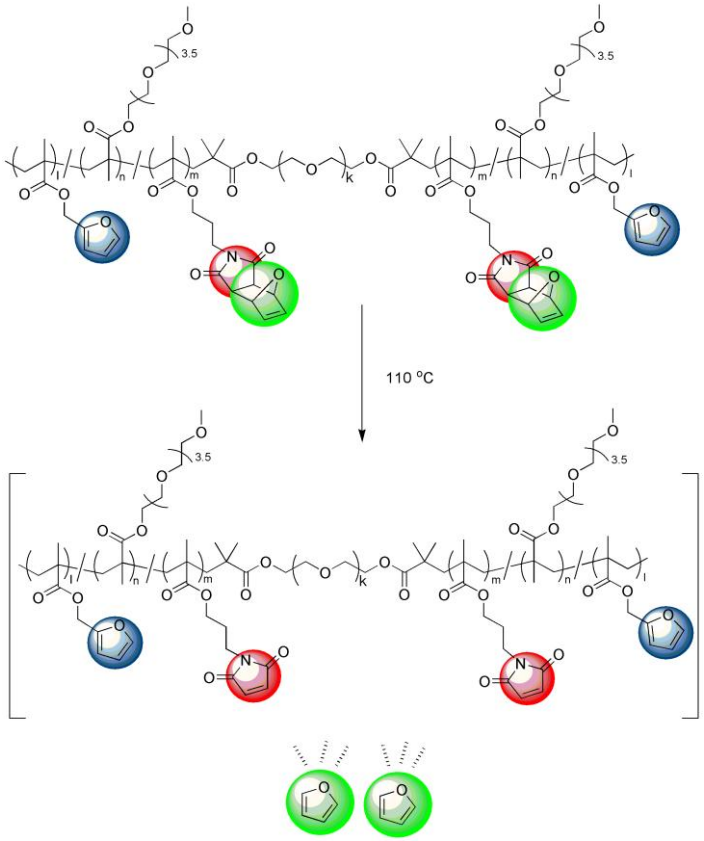


Figure 4.9. Activation of the polymer by retro Diels-Alder reaction.

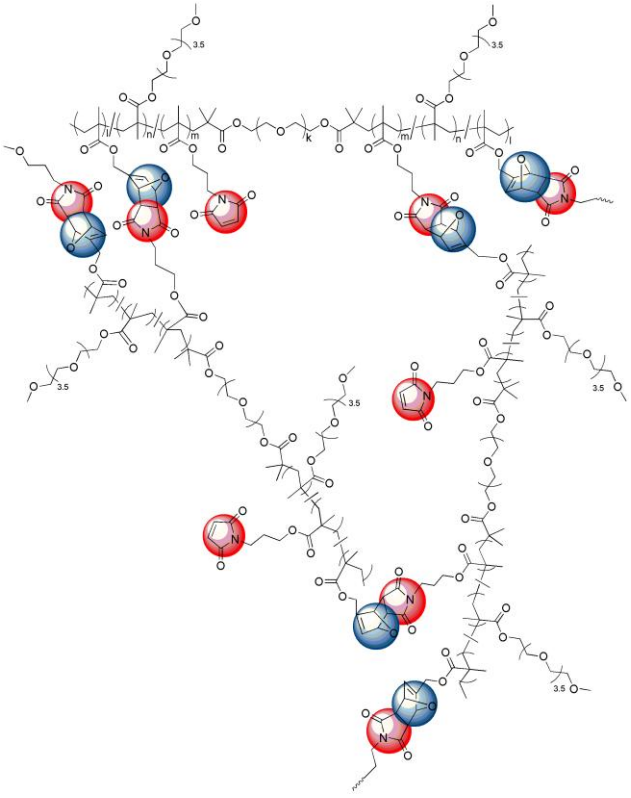


Figure 4.10. Hydrogel formation after activation and Diels-Alder reaction.

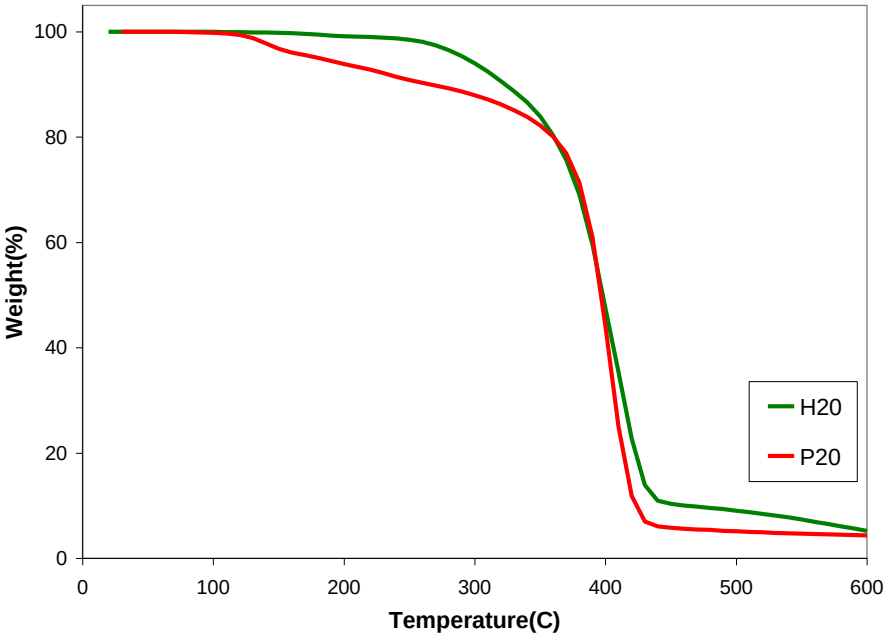


Figure 4.11. TGA thermogram of H-20 and P-20.



Figure 4.12. The hydrogel synthesized using Diels-Alder/retro Diels-Alder strategy.

Scanning electron microscopy (SEM) images showed that the microstructures of the hydrogels are rubber like instead of porous structure (Figure 4.13). It was the expected result because their swelling capacities were not much like porous hydrogels (Figure 4.14). The rubbers like structures are not surprising because each of the polymer's side blocks are crosslinker and this resulted in closely crosslinked microstructures.

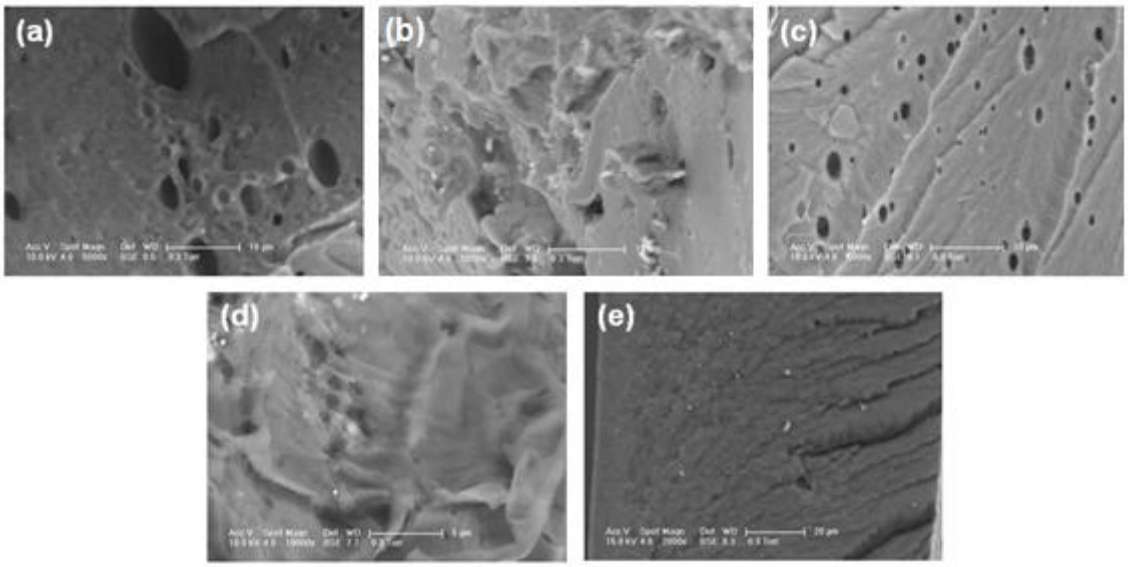


Figure 4.13. SEM images of the hydrogels. (a) P10-1, (b) P10-2, (c) P10-3, (d) P6, and (e) P-20.

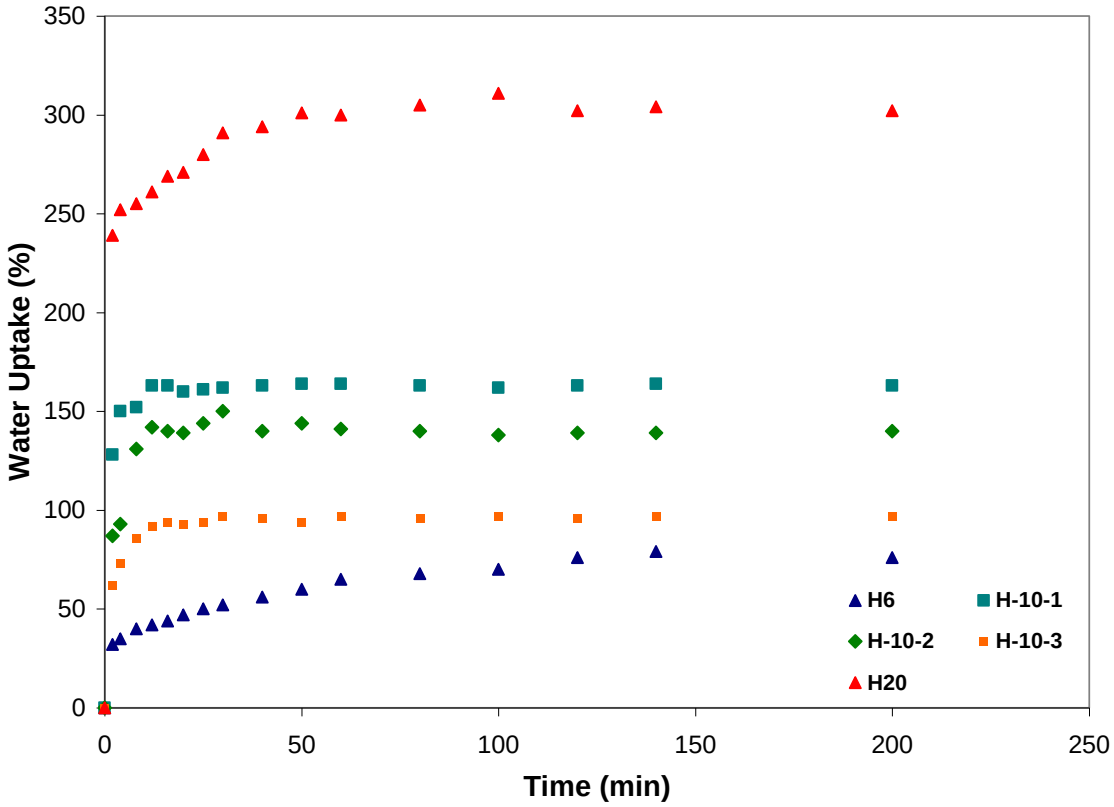


Figure 4.14. Swelling graph of the hydrogels.

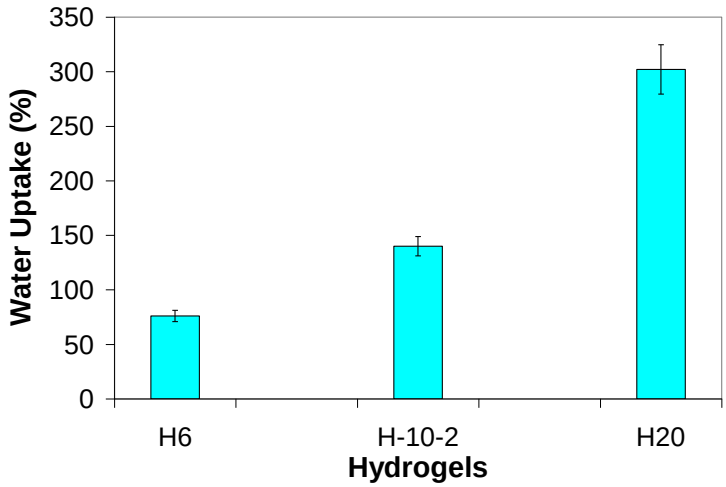


Figure 4.15. Swelling graph of the hydrogels with different hydrophilic PEG block length.

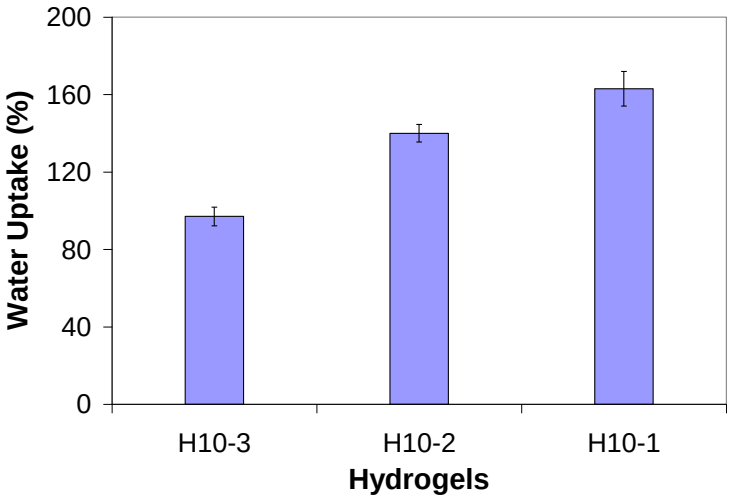


Figure 4.16. Swelling graph of the hydrogels with different hydrophobic poly(FFMA-FPMMA-OEGMEMA) block length.

Water uptake capacities of the hydrogels were checked in definite time intervals. Until equilibrium point every datum recorded and swelling behavior examined. It was observed that with increasing chain length of PEG middle block the swelling capacity of hydrogel increased as expected. It is also observed that with increasing hydrophobic poly(FFMA-FPMMA-OEGMEMA) block length, the water uptake capacity of hydrogels decreased. This trend is due to increase in crosslinking density and hydrophobicity of these hydrogels.

4.5. Functionalization of Hydrogels via Thiol-Ene Addition

After crosslinking there are free maleimides left in the hydrogel. Double bond of the maleimide can react with thiols via Michael Type thiol-ene addition. To examine the funtionalizable maleimide groups a fluorescent dye BODIPYC10SH was used. Hydrogels were kept in THF containing BODIPYC10SH for 12 hours and washed with THF excessively to remove unreacted fluorescent dyes. Fluorescent microscope images showed that with increasing maleimide content of the polymer the fluorescence intensity of the hydrogel increased as seen in Figure 4.18. It is as expected because longer side chains contain more maleimide and therefore the ratio of maleimide increases with increasing side length. Hydrogels also kept in THF containing BODIPY-Br as a control experiment. After treating with same procedure no fluorescence intensity was detected with BODIPY-Br. This result is expected since no reaction is expected between maleimide and Br group. This also proved that the dye is bound to the hydrogel via chemical bonding instead of physical entrapment.

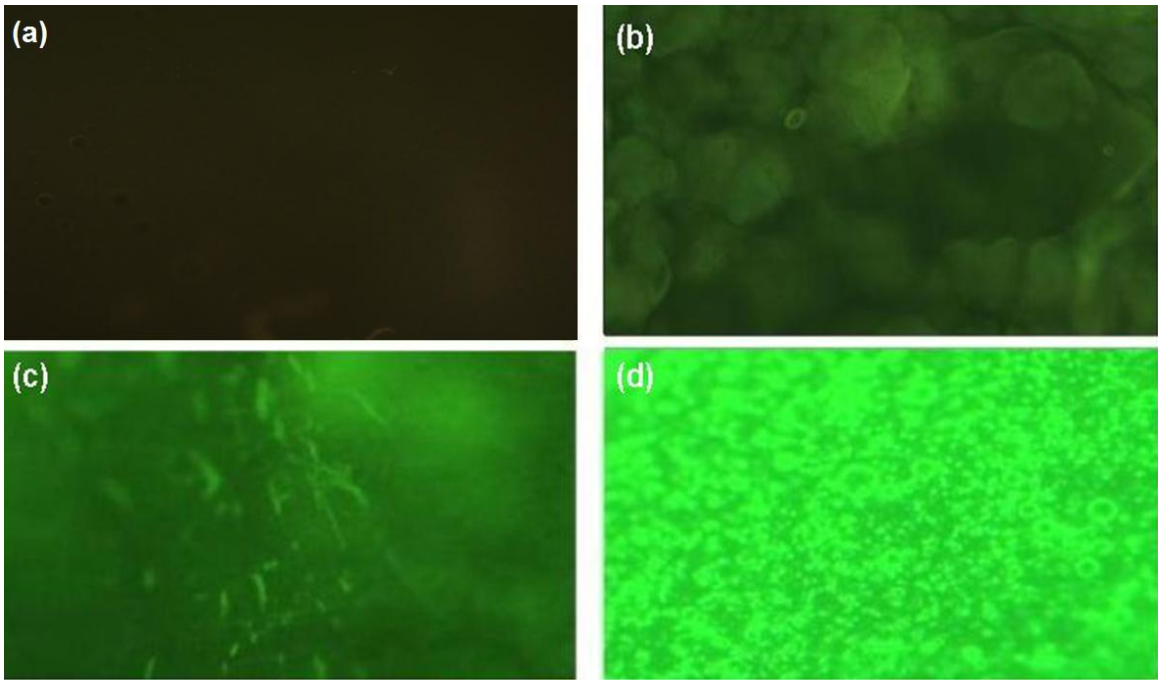


Figure 4.12. Fluorescence microscope images of hydrogels (a) P-10-2 (BODIPY-Br), (b)P-10-1, (c) P-10-2, and (d) P-10-3.

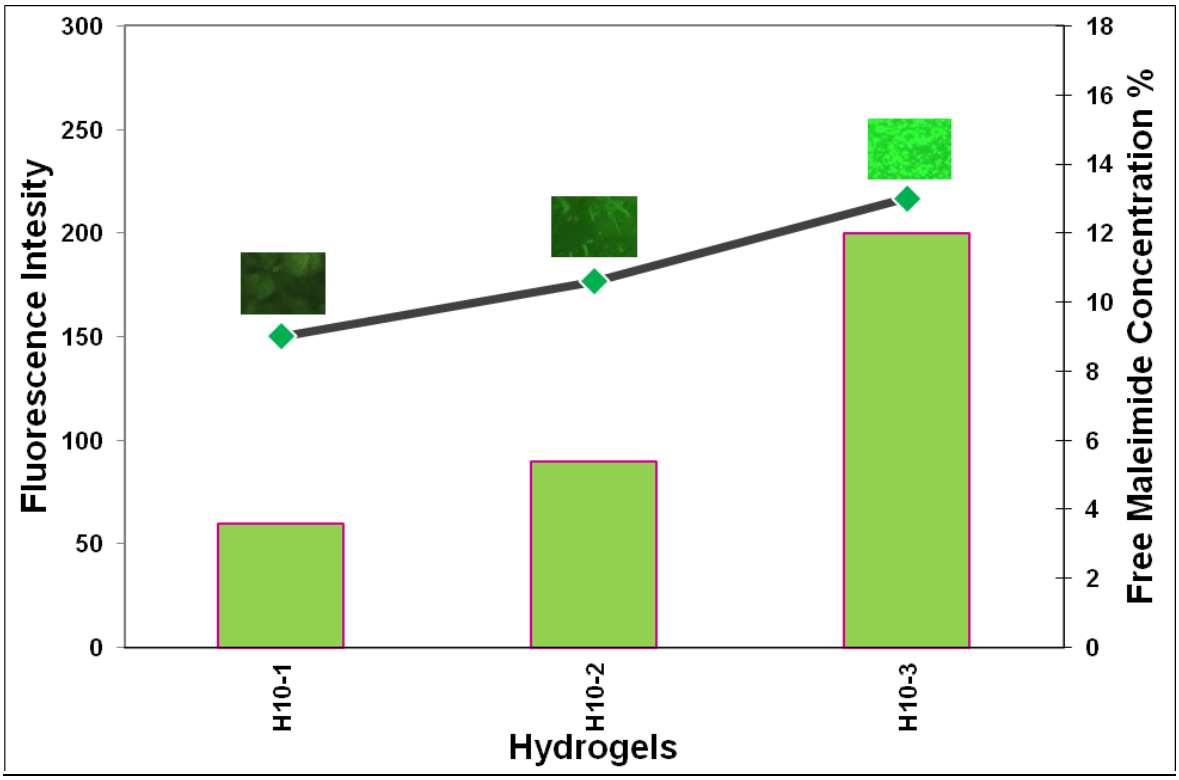


Figure 4.18. Fluorescence intensities of hydrogels.

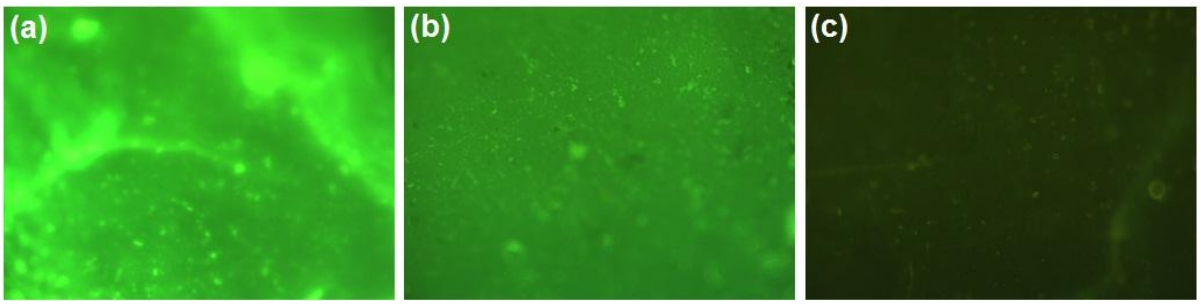


Figure 4.13. Fluorescence microscope images of hydrogels (a) P-6 , (b)P-10-2, and (c) P-20.

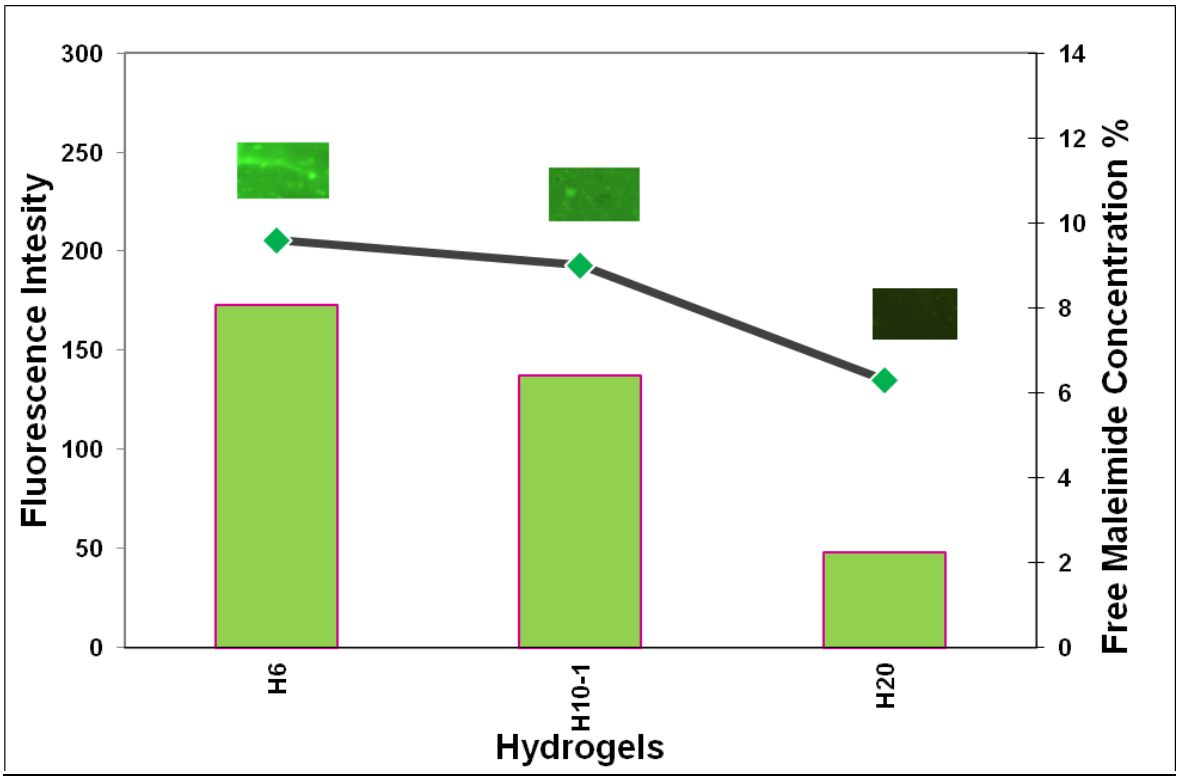


Figure 4.20. Fluorescence intensities of hydrogels.

4.6. Nature of the Crosslinking

In order to show that polymer crosslinks via Diels-Alder reaction between furan and maleimide group excess anthracene was added and same gelation procedure was applied. Since anthracene reacts with maleimide via Diels-Alder reaction and it is irreversible at 110°C, the unmasked maleimide is consumed and crosslinking is prevented. No gelation occurred and NMR of the resulting polymer shows addition of anthracene on to the unmasked maleimide side chains (Figure 4.21).

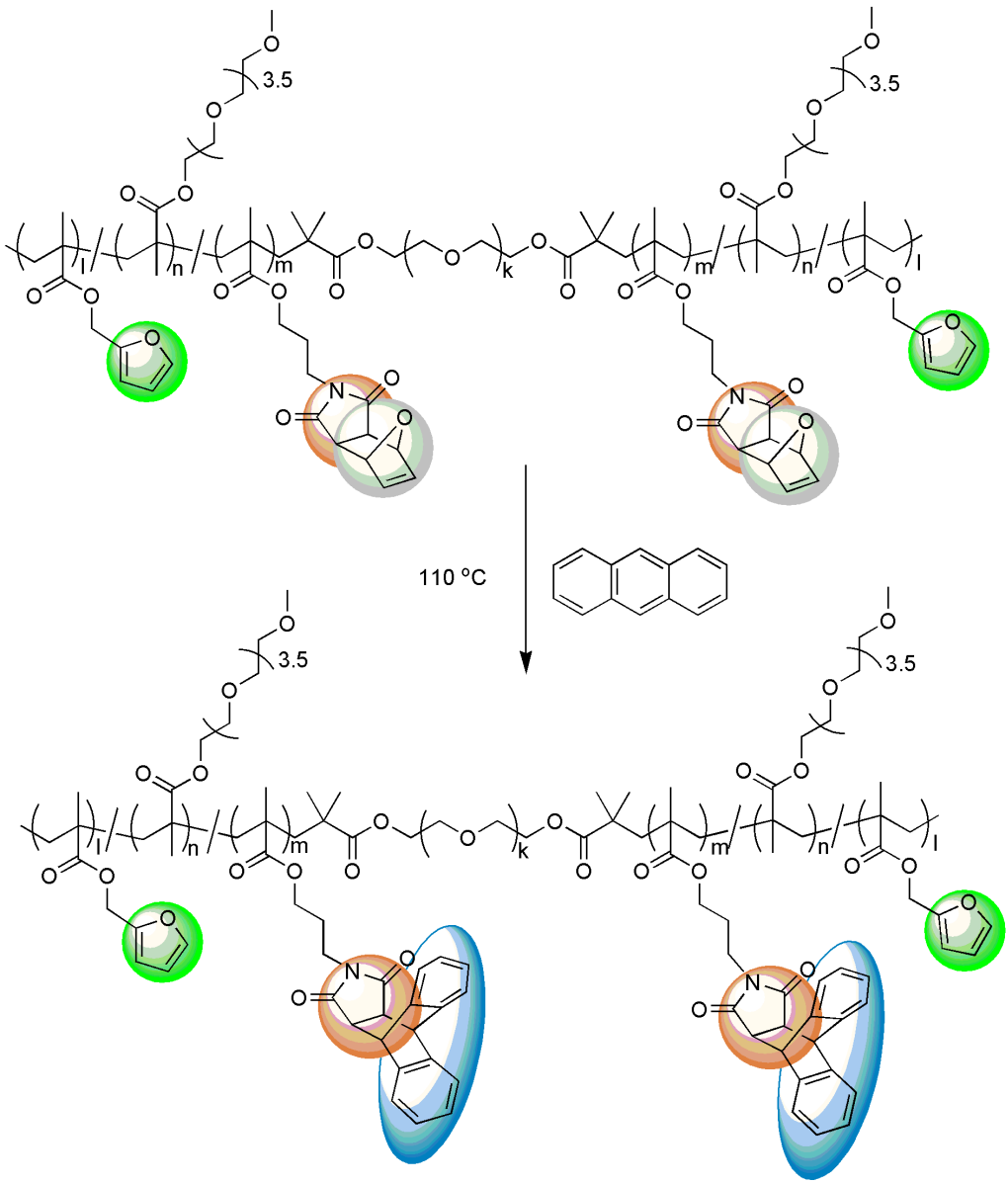


Figure 4.21. Inhibition of gelation in the presence of anthracene.

5. CONCLUSIONS

In the first part of the study, ABA type triblock polymers with different middle block length and side block length were synthesized. Characterizations of the polymers were done by GPC, IR, TGA and NMR. These polymers containing a furan unit and a masked maleimide unit were activated by heating via retro Diels-Alder temperature. Activated polymers were crosslinked via the Diels-Alder reaction. Water uptake capacity of hydrogels were examined and it is seen that with increasing PEG length the water uptake increases and with increasing hydrophobic side chain it decreases. The rubber like morphology of these hydrogels was characterized using SEM.

In the second part of the study, functionalizations of the hydrogels were accomplished by thiolene reaction between free maleimide groups and BODIPYC10SH. Using fluorescence microscopy, the relative degrees of functionalizations were obtained. The nature of functionalization was examined by control experiments with non-thiol containing dye BODIPY-Br and it was found that fluorescent dyes are attached to hydrogel by covalent bond. .

APPENDIX A: SPECTROSCOPY DATA

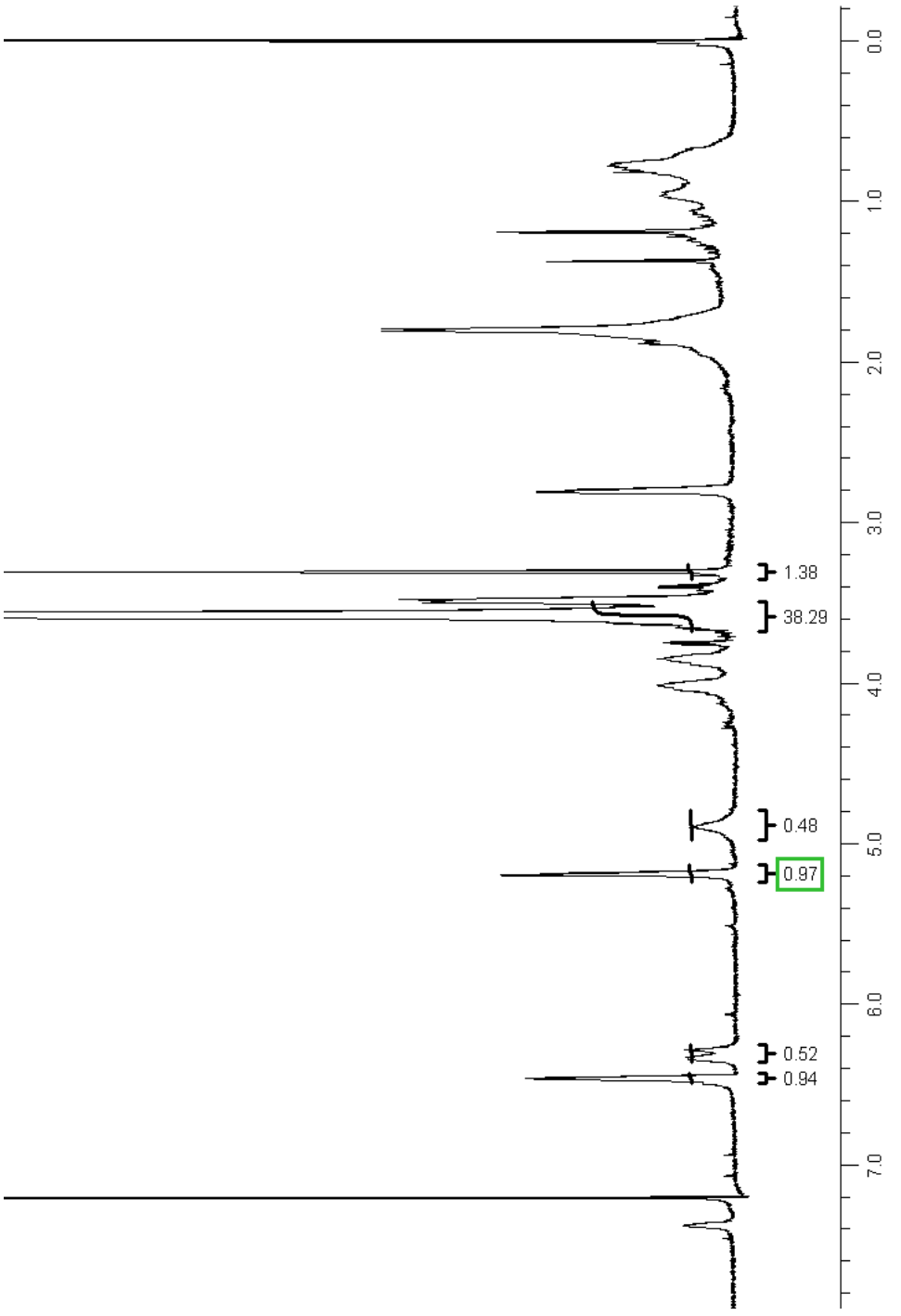


Figure A.1. NMR of P-6.

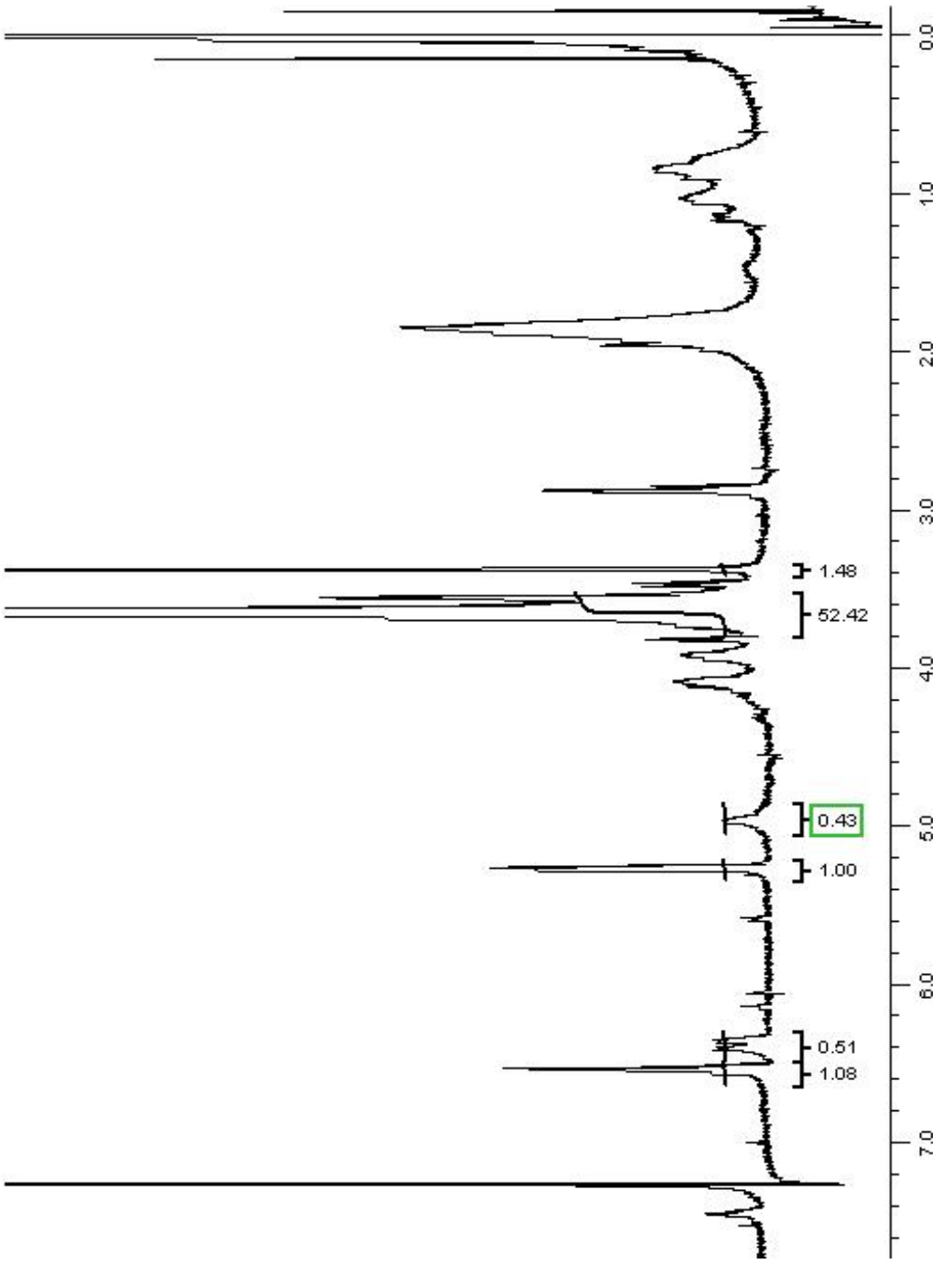


Figure A.2. NMR of P-10-1.

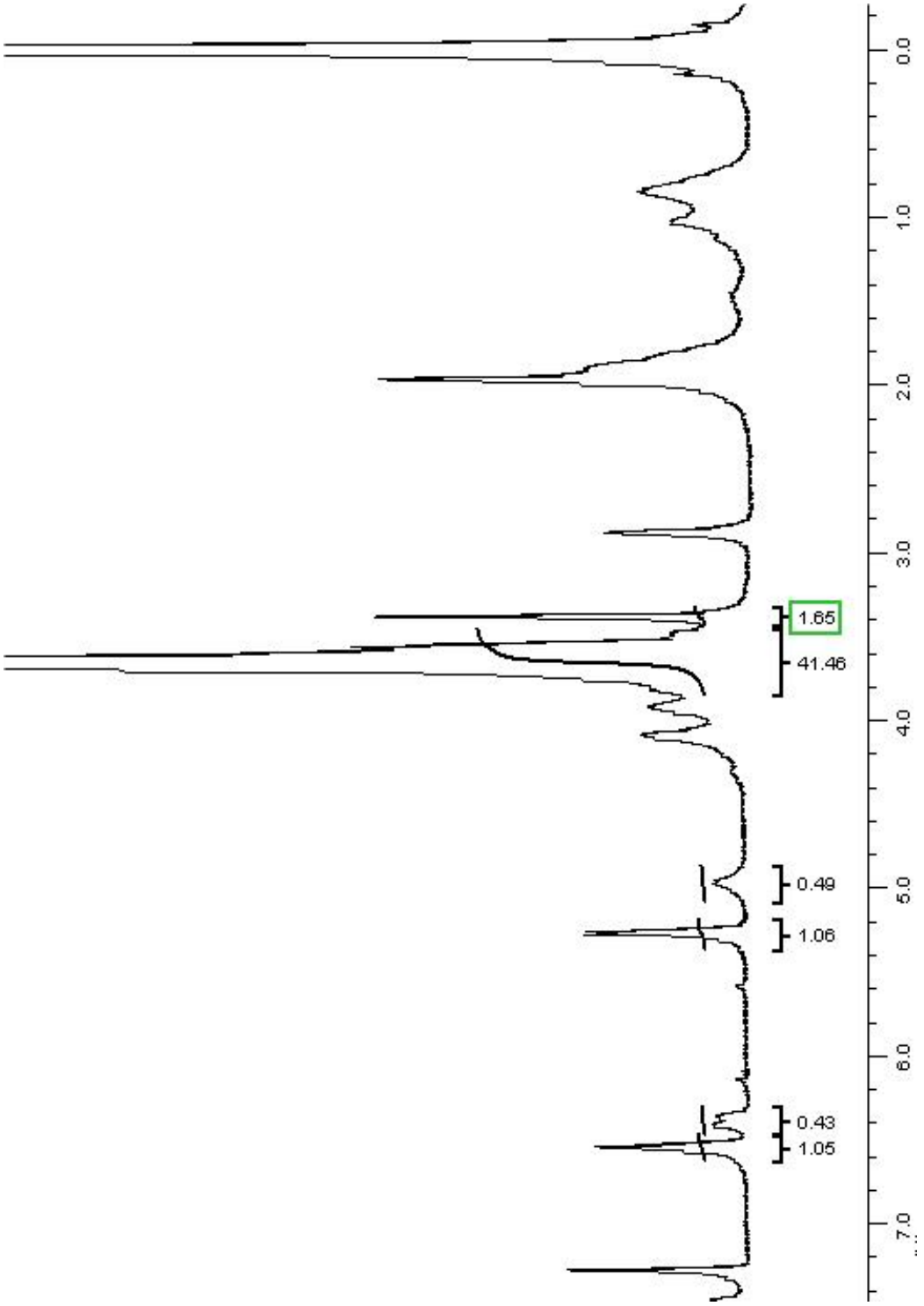


Figure A.3. NMR of P10-2.

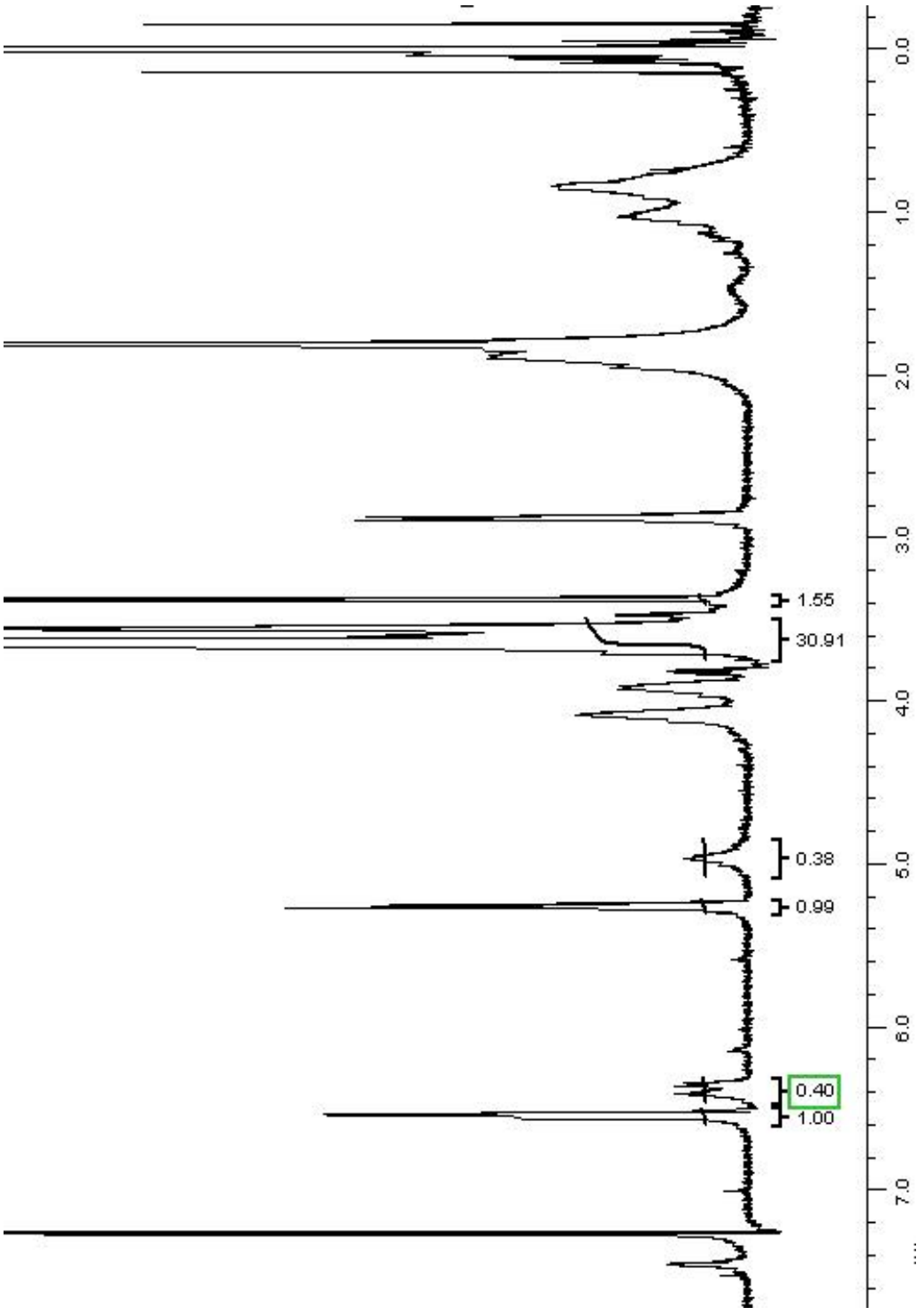


Figure A.4. NMR of P-10-3.

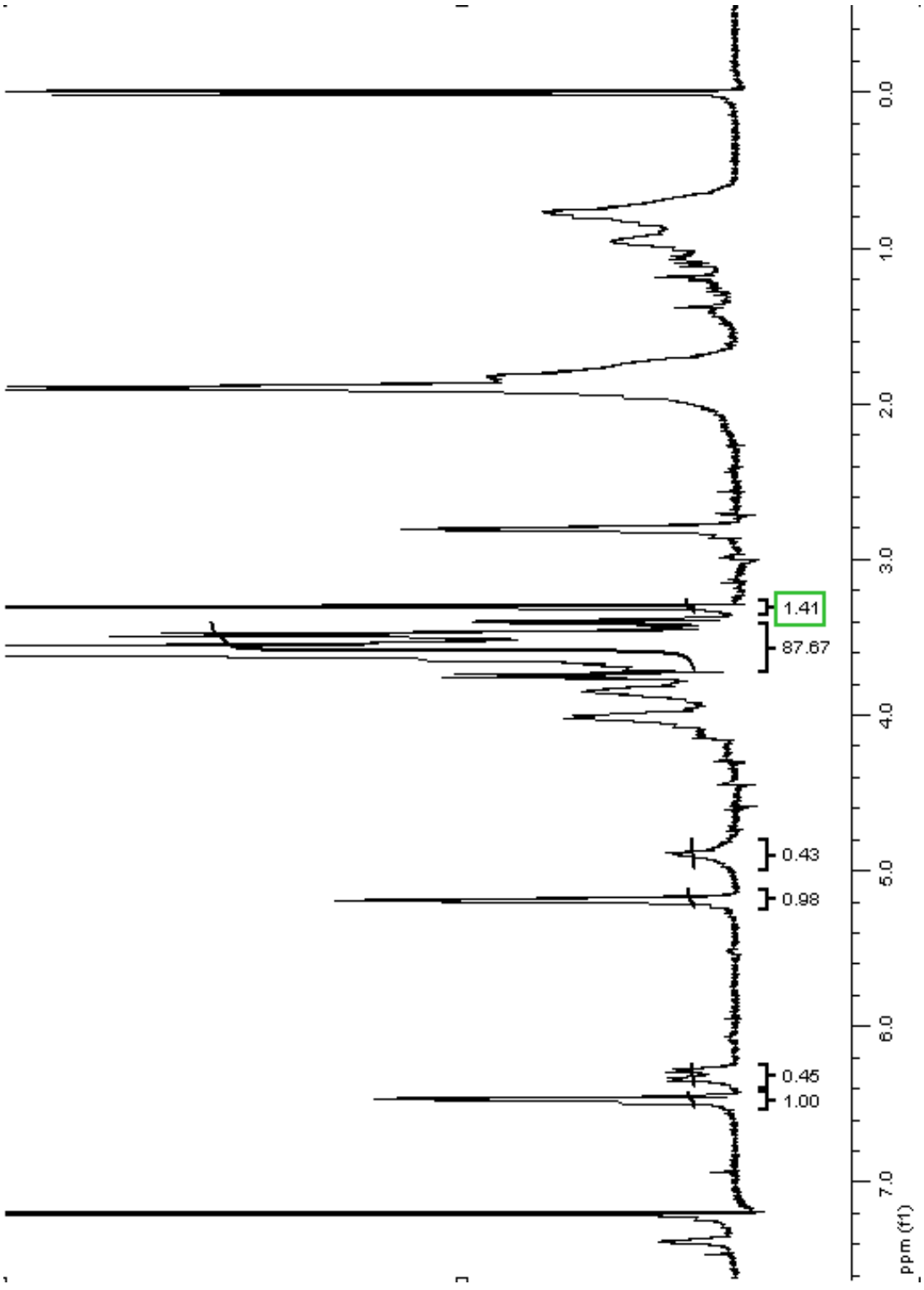


Figure A.5. NMR of P-20.

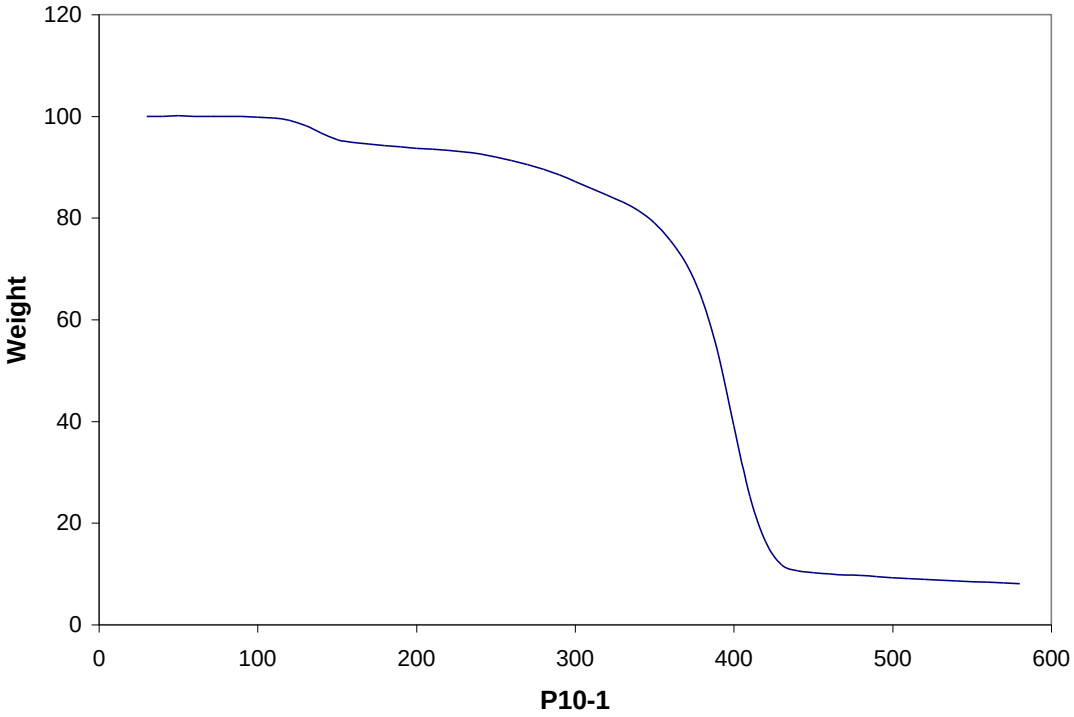


Figure A.6. TGA of P-10-1.

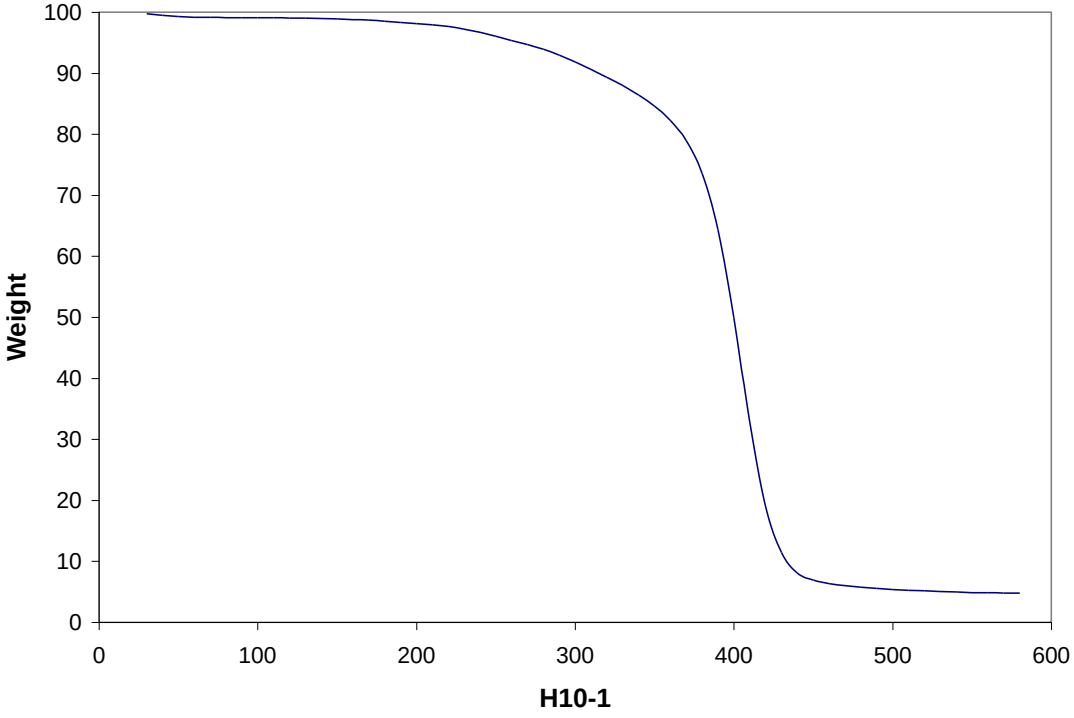


Figure A.7. TGA of H-10-1.

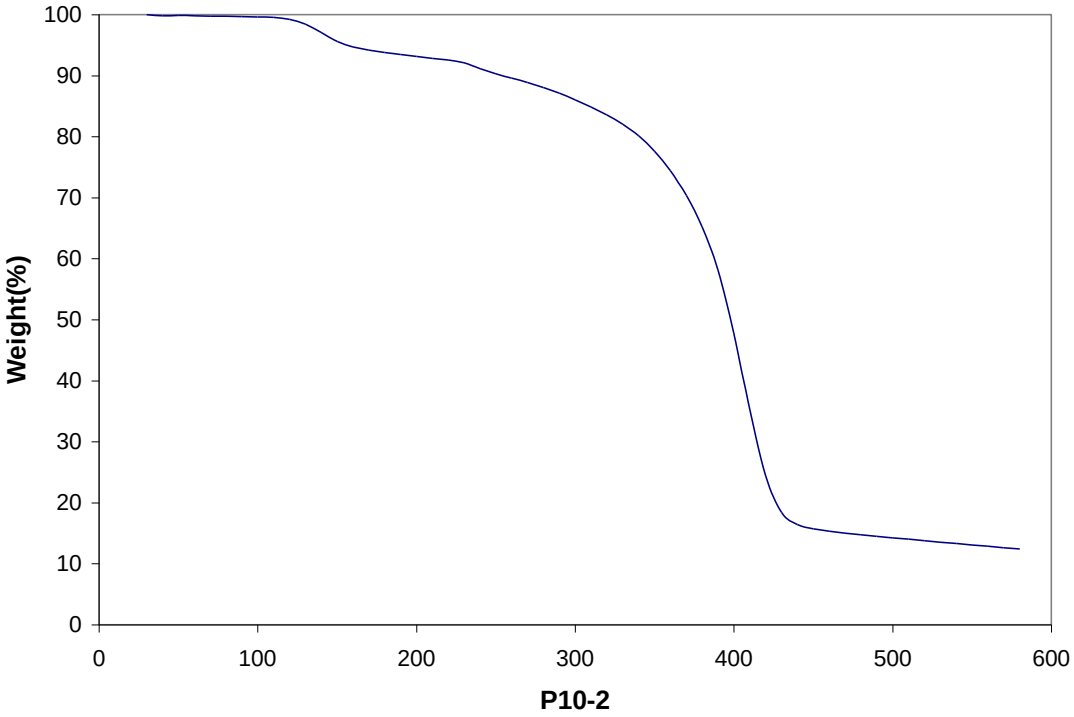


Figure A.8. TGA of P-10-2.

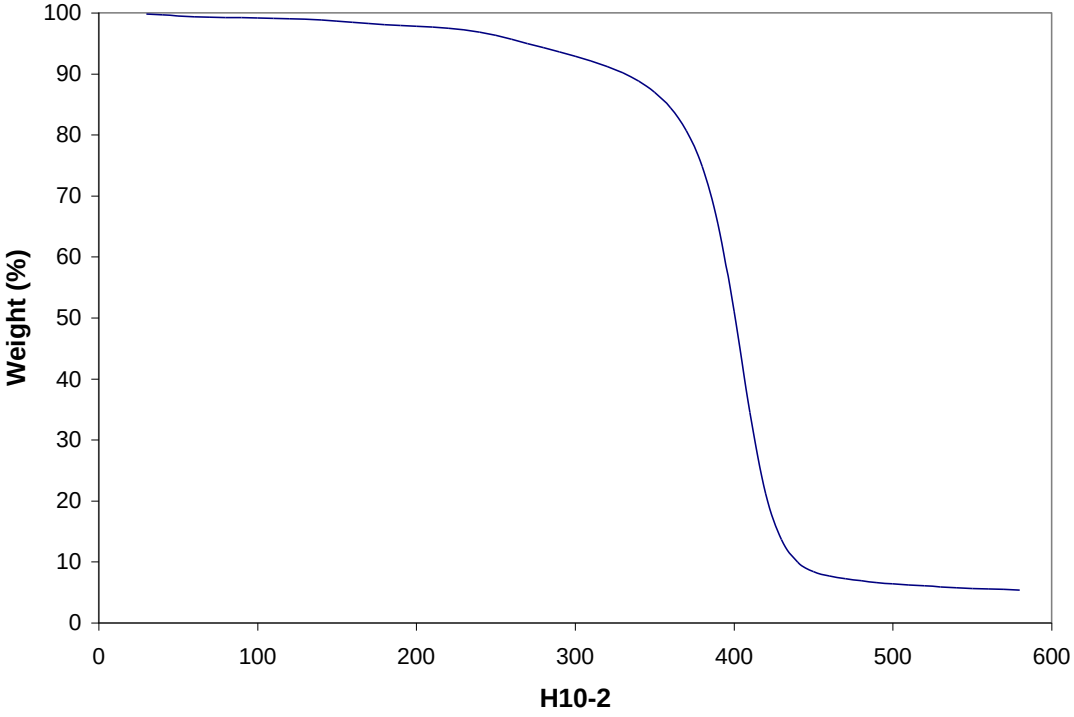


Figure A.9. TGA of H-10-2.

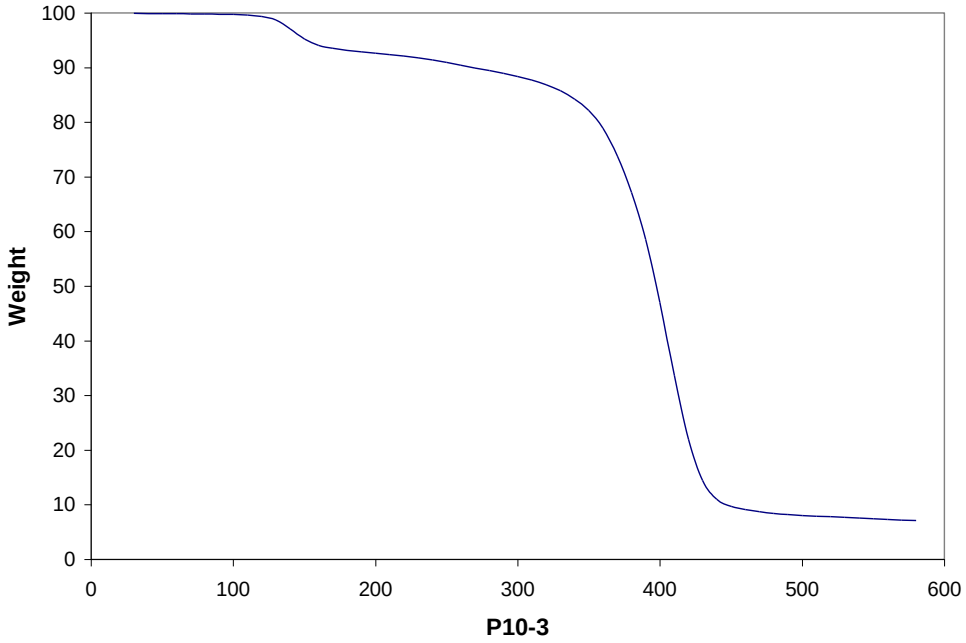


Figure A.10. TGA of P-10-3.

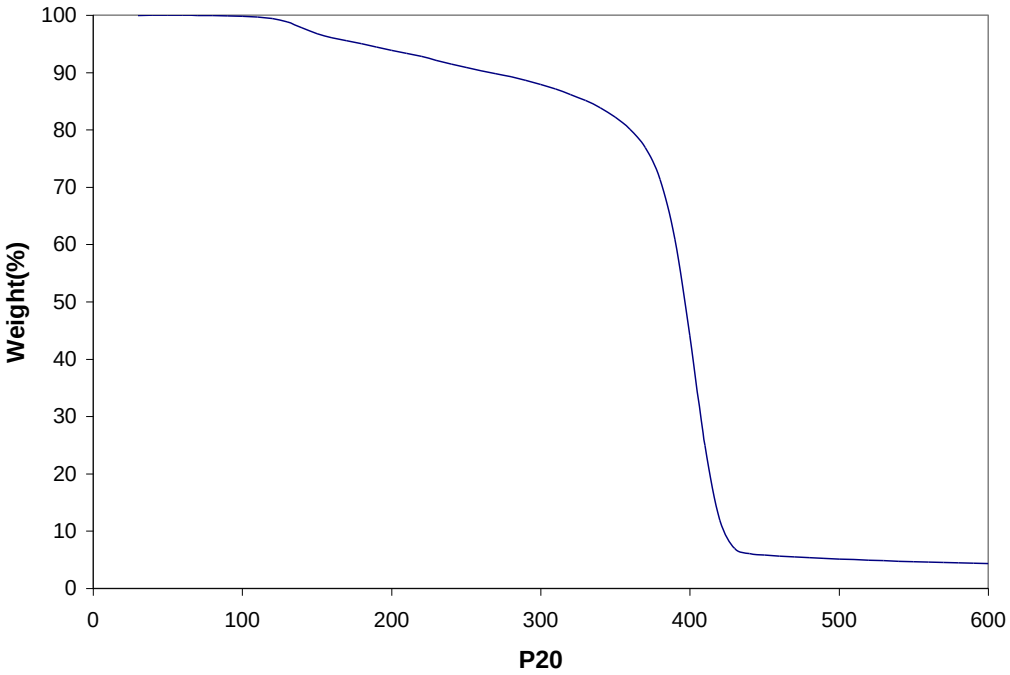


Figure A.11. TGA of P-20.

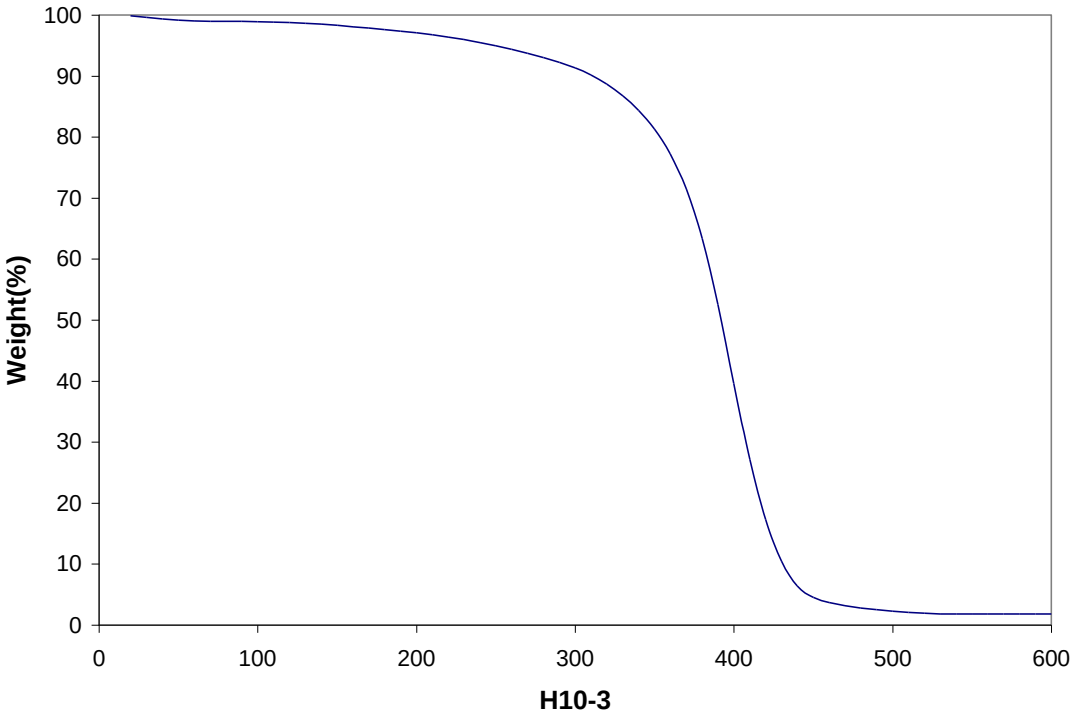


Figure A.12. TGA of H-10-3.

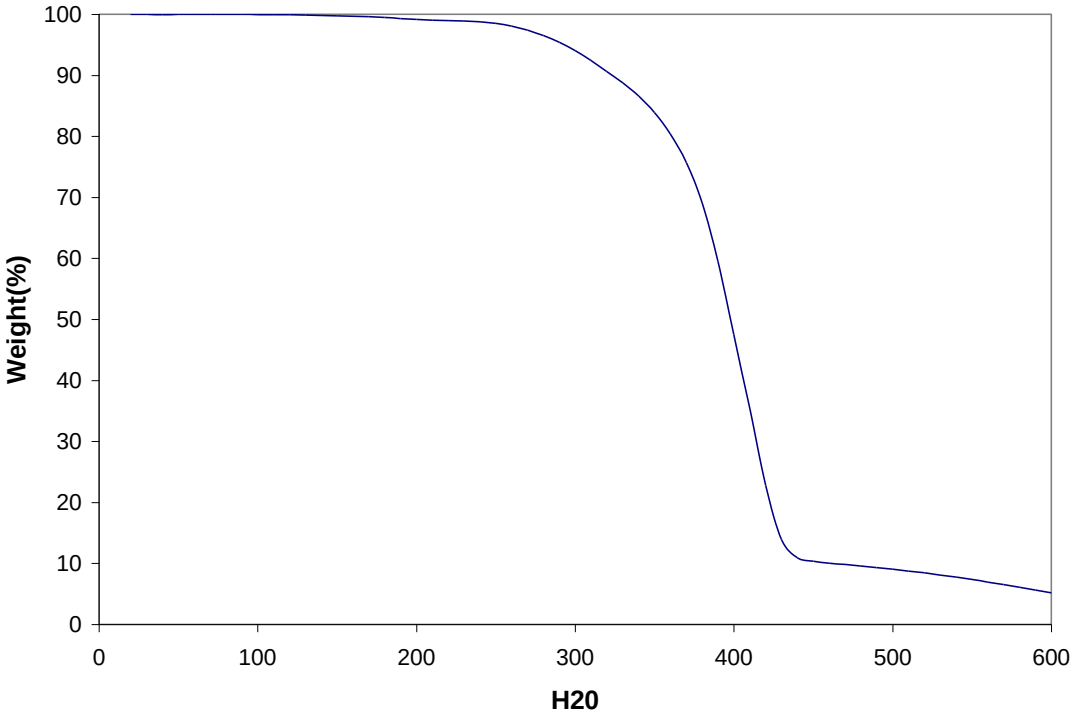


Figure A.13. TGA of H-20.

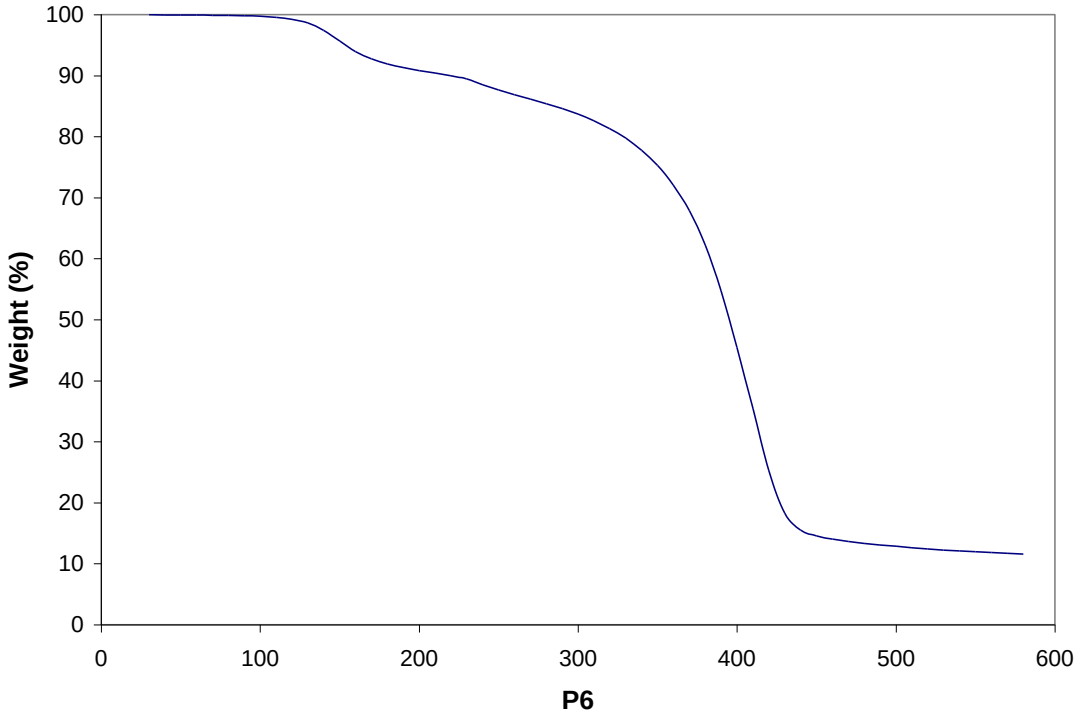


Figure A.14. TGA of P-6.

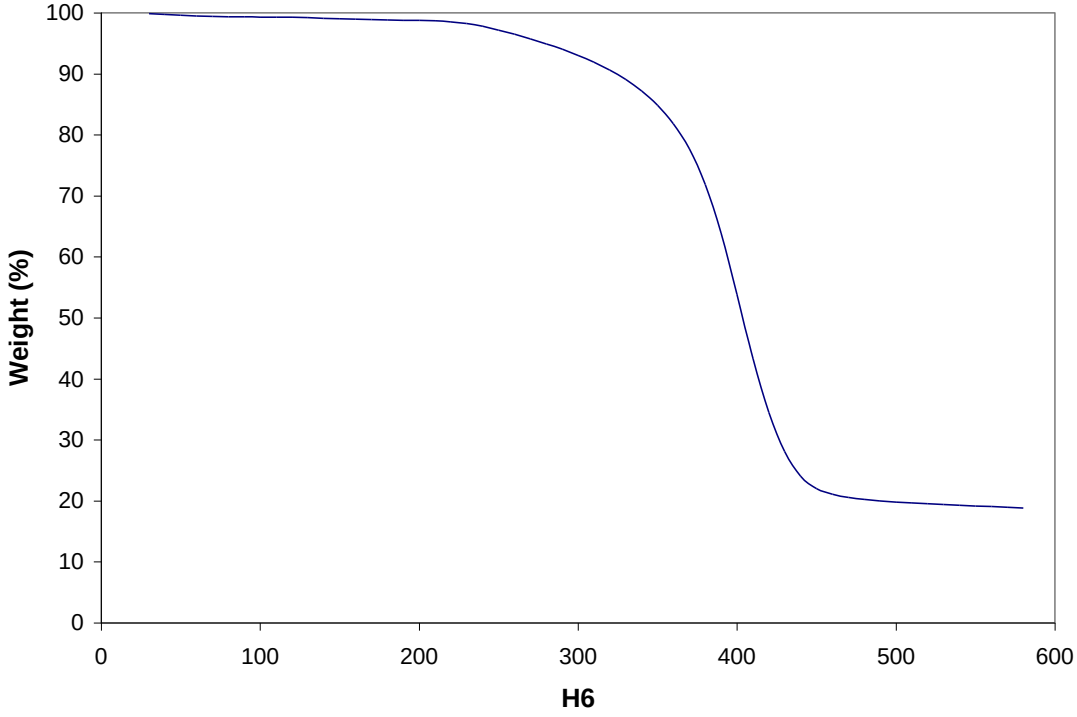


Figure A.15. TGA of H-6.

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