ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

LIGHT INDUCED SYNTHESIS AND CHARACTERIZATION OF “CLICKABLE” POLYACRYLAMIDE HYDROGELS

M.Sc. THESIS

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“ÇIT ÇITLANABİLİR” POLİAKRİLAMİD HİDROJELLERİN IŞIK İLE UYARILMIŞ SENTEZİ VE KARAKTERİZASYONU

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A mi amada familia,

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ABBREVIATIONS

AAm	**:** Acrylamide
A-N_3	**:** (Azidomethyl)anthracene
BAPO	**:** Phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide
CuAAC	**:** Copper(I)-Catalyzed Azide-Alkyne Cycloaddition
DCM	**:** Dichloromethane
DMF	**:** Dimethylformamide
DMPA	**:** 2,2-Dimethoxy-2-phenylacetophenone
DMSO	**:** Dimethyl sulfoxide
EtOAc	**:** Ethyl acetate
FT-IR	**:** Fourier-transform infrared spectroscopy
ISC	**:** Intersystem crossing
ITU	**:** Istanbul Technical University
M	**:** Monomer
NMR	**:** Nuclear Magnetic Resonance
PA	**:** Propargyl acrylate
PAAm	**:** Poly(acrylamide)
PET	**:** Photoelectron transfer
PMDETA	**:** N,N,N’,N’’,N’’-Pentamethyldiethylenetriamine
PI	**:** Photoinitiator
Py-SH	**:** Pyrene-1-methanethiol
R.T.	**:** Room temperature
UV	**:** Ultraviolet
Vis	**:** Visible

SYMBOLS

$\bar{V}_r$	: Average molar volume of the repeat units
D_0	: Diameter of sample at after-preparation state
N_{exp}	: Experimentally determined network chain length
V_{eq}	: Equilibrium volume swelling ratio
ν_e	: Effective crosslink density
ν_2^0	: Volume fraction of the crosslinked polymer after-preparation state
ρ_P	: Density of the gel
D	: Diameter of sample at equilibrium swollen state
d_1	: Density of water
G	: Elastic moduli
h	: Planck's constant
m_0	: Weight of the gel sample as-prepared state
m_d	: Weight of dried the gel
S	: Initial cross-sectional area
α	: Deformation ratio
υ	: Radiation frequency
χ	: Polymer-solvent interaction parameter

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LIGHT-INDUCED SYNTHESIS AND CHARACTERIZATION OF "CLICKABLE" POLYACRYLAMIDE HYDROGELS

SUMMARY

Hermann Staudinger's groundbreaking study on macromolecules was recognized with the Nobel Prize in Chemistry on December 10, 1953. "High Polymers Bring High Honors" became a worldwide headline. Staudinger had identified the molecular blueprints of natural and synthetic "polymeric materials" with high molecular weights. His groundbreaking concept, which involved covalently bonding a large number of small monomer molecules to form macromolecules, ushered in a new age of molecular design of high molecular weight structural and functional polymeric materials. Polymeric materials are unique in their ability to combine an attractive cost to performance ratio, low energy demand during preparation and processing, flexible feedstock supply, simple processing with short cycle times typical of modern industrial mass production, and extraordinary versatility in terms of property profiles, application ranges, and waste recycling.

Step-growth (condensation) polymerization and chain-growth (addition) polymerization are the two types of polymerization processes that can be classified kinetically. The propagation methods of polymer chains can also be used to classify these procedures. The propagation of the chains in addition polymerization are formed by radical, cationic, and anionic reactions, whereas the propagation of the chains in step-growth polymerization are formed by polyaddition and polycondensation processes.

Polymers can be prepared by several techniques. Among theses techniques, photopolymerization is a rapidly growing technology since it offers several advantages over thermal polymerization. Higher rates of polymerization, temporal and spatial control, environmental benefits from the elimination of volatile organic compounds are some of the advantages of photopolymerization over thermal polymerization. In addition, probability of side reactions such as chain transfer to occur gets lower enabling the synthesis of more ordered macromolecules.

Although constituting a minimal amount in the formulation of a photopolymer, photoinitiators play a vital role by absorbing the light in ultraviolet-visible spectral range, typically between 250 and 450 nm, and converting this light energy into chemical energy in the form of reactive intermediates including free radicals, cations and anions, which then initiate photopolymerization. The two types of free radical initiators are *Type I* (α-cleavage) and *Type II* (H abstraction). Substituted carbonyl and aromatic compounds such as benzoin and its derivatives, benzyl ketals and acetophenones are the most common *Type I* (unimolecular) initiators. On the other hand, *Type II* photoinitiators, also known as bimolecular photoinitiators, are photoiniating systems that include a photoinitiator including benzophenone, thioxanthone, or quinone, as well as a co-initiator such as an alcohol or amine.

Since the first definition made by Staudinger in 1920s, in 100 years, polymer science has witnessed remarkable progress. “Smart polymeric materials” are an example of state-of-the-art polymers. Hydrogels belong to the smart polymeric materials subclass and defined as crosslinked polymers which can absorb large quantities of water due to their hydrophilic structure while not being dissolved as a consequence of their crosslinked structure. Today, they are widely used to produce groundbreaking smart gadgets, sensors, and actuators; their capabilities arise from their capacity to react to external stimuli with an observable reaction. According to their source, hydrogels can be classified as natural and synthetic. Some of the most common examples of natural and synthetic hydrogels are hyaluronic acid and polyacrylamide, respectively. One of the most important features of hydrogels is to possess suitable functionalities for post-synthetic modifications that can support their extended applications.

Chemical bonds, permanent or temporary physical entanglements, and secondary interactions, such as hydrogen bonds, can all be used to cross-link hydrogels. In physiological conditions, covalent cross-links are often stable. Mechanical properties can be tuned by altering the ratios of reactants in synthetic hydrogels. Toxic chemicals utilized in the manufacturing of these hydrogels, on the other hand, may diminish their biocompatibility.

Polyacrylamide, a polymer of the acrylamide monomer, is a colorless hydrogel that is durable, nonresorbable, nontoxic, and nonimmunogenic, as well as hydrophilic, viscoelastic, cohesive, and biocompatible. Due to these attractive properties, polyacrylamide hydrogels are among the most widely studied hydrogels. By applying appropriate modification methods, functional groups which can tune swelling and enhance mechanical properties can be incorporated into polyacrylamide hydrogels.

Coined by K. Barry Sharpless in 2001, “click chemistry” is a family of biocompatible reactions utilized in bioconjugation that allows specific biomolecules to be joined to specified substrates. Click chemistry is not a single specific reaction, but rather a method of producing compounds that mimic natural instances and molecules by connecting small modular parts. Click reactions connect a biomolecule with a reporter molecule in a variety of applications. The concept of a "click" reaction has been employed in chemoproteomic, pharmacological, and different biomimetic applications, and it is not confined to biological settings.

Following Sharpless' innovative classification of a number of idealized processes as click reactions, the materials science and synthetic chemistry research groups have followed a variety of paths to identify and execute these click reactions. Christopher N. Bowman has reviewed the radical-mediated thiol-ene reaction as an example. This reaction has all of the ideal characteristics of a click reaction: it is extremely efficient, simple to execute, produces no side products, and gives a high yield rapidly. Furthermore, the thiol–ene reaction is frequently photoinitiated, especially for photopolymerizations that result in highly uniform polymer networks, boosting unique spatial and temporal control capabilities of the click reaction.

Copper-catalyzed-azide-alkyne-cycloaddition reaction is another model example of a click reaction. In this reaction, an azide is reacted with a terminal alkyne to produce 1,2,3-triazole. This reaction is regiospecific since it only forms 1,4-disubstituted product, can be carried out at a wide range of temperatures and pH values, in a variety of solvents including water. Furthermore, it is 10^7 times faster than the uncatalyzed reaction, making it an ideal candidate for the modification of hydrogels.

In the present work, a facile synthesis of clickable polyacrylamide hydrogel by photopolymerization using acrylamide and propargyl acrylate in the presence of different photoinitiators and in the absence of any crosslinking agent under ultraviolet and visible light is reported. Clickable polyacrylamide hydrogels were synthesized in the presence of Irgacure 2959 (water soluble photoinitiator), BAPO (visible light photoinitiator) and DMPA. Afterwards, gel synthesized by water soluble initiator was irradiated for 3 h, 6 h, 9 h and 24 h. The effect of irradiation time on gel fraction, swelling degree and compressive elasticity was investigated. Thiol-ene, thiol-yne and copper catalyzed azide-alkyne cycloaddition click functionality of the produced hydrogels were confirmed by using respective fluorescent click components. Furthermore, the effect of hydrophobicity on swelling degree was examined by clicking 1,6- hexanedithiol onto hydrogels.

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ÖZET

Hermann Staudinger'in makromoleküller üzerine çığır açan çalışması 10 Aralık 1953'te Nobel Kimya Ödülü ile tanındı. "Yüksek Polimerler Yüksek Onur Getiriyor" dünya çapında bir manşet oldu. Staudinger, yüksek moleküler ağırlıklı doğal ve sentetik “polimerik malzemelerin” moleküler planlarını tanımlamıştı. Makromoleküller oluşturmak için çok sayıda küçük monomer molekülünün kovalent olarak bağlanmasını içeren çığır açan konsepti, yüksek moleküler ağırlıklı yapısal ve fonksiyonel polimerik malzemelerin moleküler tasarımında yeni bir çağı başlattı. Polimerik malzemeler, cazip bir fiyat/performans oranı, hazırlama ve işleme sırasında düşük enerji gereksinimi, esnek hammadde tedariki, modern endüstriyel seri üretimin tipik özelliği olan kısa çevrim süreleriyle basit işleme ve özellik profilleri açısından olağanüstü çok yönlülüğü bir araya getirme yeteneklerinde benzersizdir.

Adımlı büyüme (kondenzasyon) polimerizasyonu ve katılma (zincir büyümesi) polimerizasyonu, kinetik olarak sınıflandırılabilen iki tip polimerizasyon işlemidir. Bu prosedürleri sınıflandırmak için polimer zincirlerinin yayılma yöntemleri de kullanılabilir. Katılma polimerizasyona ek olarak zincirlerin yayılması radikal, katyonik ve anyonik reaksiyonlarla oluşturulurken, adımlı büyüme polimerizasyonunda zincirlerin yayılması poliadisyon ve polikondenzasyon işlemleri ile oluşturulur.

Polimerler birçok farklı teknikle hazırlanabilir. Bu teknikler arasında fotopolimerizasyon, termal polimerizasyona göre çeşitli avantajlar sunduğu için hızla büyüyen bir teknolojidir. Daha yüksek polimerizasyon oranları, geçici ve uzaysal kontrol, uçucu organik bileşiklerin ortadan kaldırılmasından elde edilen çevresel faydalar, termal polimerizasyona göre fotopolimerizasyonun avantajlarından bazılarıdır. Ayrıca zincir transferi gibi yan reaksiyonların oluşma olasılığı azalır ve daha düzenli makromoleküllerin sentezi sağlanır.

Bir fotopolimerin formülasyonunda minimum bir miktar oluşturmasına rağmen, fotobaşlatıcılar, tipik olarak 250 ile 450 nm arasındaki ultraviyole-görünür spektral aralıktaki ışığı soğurarak ve bu ışık enerjisini fotopolimerizasyonu başlatan serbest radikaller, katyonlar ve anyonlar. dahil olmak üzere reaktif ara ürünler biçiminde kimyasal enerjiye dönüştürerek hayati bir rol oynarlar. İki tip serbest radikal başlatıcı, *Tip I* (α-yarılması) ve *Tip II* (H koparılması)'dir. Benzoin ve türevleri, benzil ketaller ve asetofenonlar gibi ikame edilmiş karbonil ve aromatik bileşikler, en yaygın *Tip I* (unimoleküler) başlatıcılardır. Öte yandan, bimoleküler fotobaşlatıcılar olarak da bilinen *Tip II* fotobaşlatıcılar, benzofenon, tiyoksanton veya kinon içeren bir fotobaşlatıcıyı ve ayrıca bir alkol veya amin gibi bir yardımcı başlatıcıyı içeren fotobaşlatıcı sistemlerdir.

Staudinger'in 1920'lerde yaptığı ilk tanımlamadan bu yana, 100 yıl içinde polimer bilimi dikkate değer bir ilerleme kaydetti. "Akıllı polimerik malzemeler", son teknoloji polimerlerin bir örneğidir. Hidrojeller, akıllı polimerik malzemeler alt sınıfına aittir ve hidrofilik yapıları nedeniyle büyük miktarlarda suyu emebilen ve çapraz bağlı yapılarının bir sonucu olarak çözünmeyen çapraz bağlı polimerler olarak tanımlanır. Bugün, çığır açan akıllı araçlar, sensörler ve aktüatörler üretmek için yaygın olarak kullanılmaktadırlar; yetenekleri, gözlemlenebilir bir tepki ile dış uyaranlara tepki verme kapasitelerinden kaynaklanır. Kaynaklarına göre hidrojeller doğal ve sentetik olarak sınıflandırılabilir. Doğal ve sentetik hidrojellerin en yaygın örneklerinden bazıları sırasıyla hyaluronik asit ve poliakrilamiddir. Hidrojellerin en önemli özelliklerinden biri, genişletilmiş uygulamalarını destekleyebilen post-sentetik modifikasyonlar için uygun işlevselliklere sahip olmasıdır.

Hidrojelleri çapraz bağlamak için kimyasal bağlar, kalıcı veya geçici fiziksel karışıklıklar ve hidrojen bağları gibi ikincil etkileşimler kullanılabilir. Fizyolojik koşullarda, kovalent çapraz bağlar genellikle stabildir. Sentetik hidrojellerdeki reaktanların oranları değiştirilerek mekanik özellikler ayarlanabilir. Bu hidrojellerin üretiminde kullanılan toksik kimyasallar ise biyouyumluluklarını azaltabilir.

Akrilamid monomerinin bir polimeri olan poliakrilamid, dayanıklı, emilmeyen, toksik olmayan ve immünojenik olmayan, ayrıca hidrofilik, viskoelastik, yapışkan ve biyouyumlu olan renksiz bir hidrojeldir. Bu çekici özelliklerinden dolayı poliakrilamid hidrojeller en çok çalışılan hidrojeller arasındadır. Uygun modifikasyon yöntemleri uygulanarak, poliakrilamid hidrojellere şişmeyi ayarlayabilen ve mekanik özellikleri geliştirebilen fonksiyonel gruplar dahil edilebilir.

2001 yılında K. Barry Sharpless tarafından ortaya atılan "çıt çıt kimyası", biyokonjugasyonda kullanılan ve spesifik biyomoleküllerin belirli substratlara bağlanmasına izin veren biyouyumlu reaksiyonlar ailesidir. Çıt çıt kimyası tek bir spesifik reaksiyon değil, küçük modüler parçaları birleştirerek doğal örnekleri ve molekülleri taklit eden bileşikler üretme yöntemidir. Çıt çıt reaksiyonları, çeşitli uygulamalarda bir biyomolekül ile bir raportör molekülü birbirine bağlar. "çıt çıt" reaksiyonu kavramı kemoproteomik, farmakolojik ve farklı biyomimetik uygulamalarda kullanılmıştır ve biyolojik ayarlarla sınırlı değildir.

Sharpless'ın bir dizi idealize işlemi "çıt çıt" reaksiyonları olarak yenilikçi sınıflandırmasını takiben, malzeme bilimi ve sentetik kimya araştırma grupları bu çıt çıt reaksiyonlarını belirlemek ve yürütmek için çeşitli yollar izlemiştir. Christopher N. Bowman, bir örnek olarak radikal aracılı tiyol-en reaksiyonunu inceledi. Bu reaksiyon, bir çıt çıt reaksiyonunun tüm ideal özelliklerine sahiptir: son derece verimlidir, yürütülmesi kolaydır, hiçbir yan ürün üretmez ve hızlı bir şekilde yüksek verim verir. Ayrıca, tiyol-en reaksiyonu, özellikle yüksek oranda homojen polimer ağları ile sonuçlanan fotopolimerizasyonlar için sıklıkla ışık ile başlatılır, bu da çıt çıt reaksiyonunun benzersiz uzaysal ve zamansal kontrol yeteneklerini arttırır.

Bakır-katalizli-azid-alkin-siklokatılma reaksiyonu, çıt çıt kimyası reaksiyonlarının başka bir model örneğidir. Bu reaksiyonda, bir azid, 1,2,3-triazol üretmek için bir terminal alkin ile reaksiyona sokulur. Bu reaksiyon, sadece 1,4-disübstitüe ürün oluşturduğu için bölgeye özgüdür, su dahil çeşitli çözücülerde çok çeşitli sıcaklık ve pH değerlerinde gerçekleştirilebilir. Ayrıca, katalize edilmemiş reaksiyondan 10^7 kat daha hızlıdır, bu özellikleri de onu hidrojellerin modifikasyonu için ideal bir aday haline getirir.

Bu çalışmada, ultraviyole ve görünür ışık altında, farklı fotobaşlatıcıların varlığında ve herhangi bir çapraz bağlayıcı yokluğunda akrilamid ve propargil akrilat kullanılarak fotopolimerizasyon yoluyla çıt çıtlanabilir poliakrilamid hidrojelin kolay bir sentezi rapor edilmiştir. Çıt çıtlanabilir poliakrilamid hidrojelleri, Irgacure 2959 (suda çözünür fotobaşlatıcı), BAPO (görünür bölge fotobaşlatıcı) ve DMPA varlığında sentezlenmiştir. Suda çözünür başlatıcı ile sentezlenen jel 3 saat, 6 saat, 9 saat ve 24 saat boyunca aydınlatılmıştır. Aydınlatma süresinin jel fraksiyonu, şişme derecesi ve sıkıştırma elastikiyeti üzerindeki etkisi araştırılmıştır. Üretilen hidrojellerin tiyol-en, tiyol-in ve bakır katalizli azid-alkin siklokatılma çıt çıt işlevselliği, ilgili floresan çıt çıt bileşenleri kullanılarak doğrulanmıştır. Ayrıca, hidrojellerin üzerine 1,6-hekzanditiyol çıt çıtlanarak hidrofobikliğin şişme derecesi üzerindeki etkisi incelenmiştir.

1. INTRODUCTION

Hydrogels are physically and/or chemically crosslinked hydrophilic polymer networks that can swell in water and retain a great amount of water [1]. Hydrogels have intrinsic structures analogous to biological materials with tunable physical and chemical characteristics [2]. Highly hydrophilic nature of hydrogels makes them unique materials ideal for special applications.

Nowadays, *in situ* forming hydrogels have attracted great interest due their broad use in biomedical and pharmaceutical applications such as drug delivery systems [3–6], scaffolds [7–10], biosensors [11], biomolecular supporters [12–15] and coatings for medical devices [16]. *In situ* forming hydrogels may be injected using less invasive methods and display a sol-gel phase transition under external physical or chemical crosslinking [17]. Physically crosslinked *in situ* hydrogels have received a special attention as they can be prepared without crosslinking agents [18–20]. However, these agents are toxic chemicals which should be eliminated from the hydrogels prior to use which significantly limits the application of the formed hydrogels for biomedical purposes.

Conventional techniques for the preparation of hydrogels include photopolymerization [21–24], Michael-type addition chemistry [25,26], enzymatic cross-linking [27], Schiff-base [28,29] and oxime [30,31] reactions. Among these methods, photopolymerization techniques became favorable in order to address the world's rapidly increasing demand for the environmentally friendly conditions [32,33]. Photopolymerization reactions can be performed at room temperature and require small amount or no solvent [34]. Moreover, light induced reactions exhibit higher efficiency than conventional methods [35,36]. Synthesis of hydrogels under mild conditions without the use of any toxic metals or reagents is highly advantageous.

Polymer scientists have recently started to modify hydrogels with small or large molecules in order to control the hydrophilic/hydrophobic nature [37], to infuse drug carrying molecules [38], to incorporate ligands [39] and to add sensing properties to hydrogels [40]. The ability to post-modify hydrogels in a mild, metal-free environment is highly desirable, especially for the incorporation of sensitive biomolecules.

A straight-forward post-modification strategy is to take advantage of click chemistry. Click chemistry is often referred as carbon-hetero bond formation reactions that result in high yield and selectivity products [41]. Photoinduced thiol-ene click chemistry is a relatively fast and easy process that produces no side products and does not require the use of complex reagents or severe reaction conditions [42,43]. Due to the overall simplicity and robustness, photoinduced thiol-ene reaction has been classified as a member of click chemistry toolbox and is very often utilized in post-modification of hydrogels [44–46].

Another example of the most widely utilized click chemistry reaction for the preparation of polymer networks is the copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC) [47–52]. The high reaction yield [53], applicability at solid/liquid interface [54], tolerance to functional groups, and insensitivity to solvents [55] make CuAAC ideal for the synthesis of a wide range of materials [56–59]. CuAAC can also be considered as another useful technique for the post-modification of hydrogels [60].

Polyacrylamide (PAAm) hydrogels have many applications in drug delivery [61], water purification [62], food packaging [63] and biosensor fluids [64]. However, until recently, efficient strategies for the synthesis of reactive or functionalizable PAAm hydrogels were limited due to the lack of availability of functional free radical polymerizable monomers. A reliable approach to functionalize PAAm hydrogels is to incorporate reactive groups for the subsequent click reactions [65,66].

Herein, a facile synthesis strategy for PAAm hydrogels possessing olefinic and acetylenic click reactive sites by photoinduced free radical polymerization is reported. For this purpose, light-induced copolymerization of acrylamide (AAm) and propargyl acrylate (PA) was applied at different reaction times in the absence of a crosslinker. In this approach, PA plays a crucial role since it undergoes selective polymerization to form network structure with the click reactive sites. Several physico-chemical and mechanical properties of the hydrogels such as the gel fraction, the equilibrium volume swelling ratio and the compressive elasticity were evaluated as a function of the irradiation time. Clickability of the hydrogels was confirmed by conducting thiol-ene and thiol-yne, and CuAAC reactions by using fluorescent click components, namely thiol-pyrene and azide-anthracene, respectively. The tunability of the hydrophilicity and mechanical properties were also demonstrated by clicking 1,6-hexanedithiol onto the hydrogels.

2. THEORETICAL PART

2.1 Photochemistry

Photochemistry provides a potent toolkit for a wide range of chemical transformations, and it is truly unique in its capacity to meet the energetic requirements of such transformations that would otherwise be unachievable with thermal analogues. In most cases, the absorption of a mole of UV photons produces 130 times more energy than ambient conditions. Many chemical processes would be able to take place due to the massive quantity of energy input offered by light. In addition, the fact that this energy for propelling chemical reactions forward can be obtained easily from light, ideally unlimited solar light, bodes well for long-term development and a greener future. As a result, chemists have been inspired to utilize the benefits of ubiquitous light and create photochemical techniques by the green nature of photochemistry [67–69].

A photochemical reaction requires a number of factors to take place: (a) there must be overlap between the spectral emission of light source and absorption spectra of the targeted chromophore, (b) the absorbed photon must have enough energy to enable the desired reaction, and (c) the energy must eventually lead to appropriate orbital transitions in the target molecule to cause the desired reaction. As a result, photoinitiated process formulations must include a photosensitive molecule with absorption in the wavelength range in which the process is to be carried out. A photon must be absorbed either directly by a molecule that also contains the desired reactive functional group or indirectly by a molecule that forms reactive intermediates such as formation of radical/ionic intermediates in polymerization/crosslinking processes [70].

A Jablonski diagram, named after Aleksander Jabłoński, is a diagram that shows the electronic states of a molecule and the transitions between them in molecular spectroscopy [71]. In this diagram, there are several ways for a molecule to return to its ground state (the lowest energy state of an atom or other particle) after it has received energy in the form of electromagnetic radiation. As shown in Figure 2.1, photon absorption excites an electron from its ground state (S_0) to its excited singlet state (S_1). In the absence of photochemical events, the excited singlet state of the chromophore, S_1, deactivates to S_0 either by fluorescence emission or through internal heat dissipation. Intersystem crossing (ISC) to a triplet state is another possibility (T_1). T_1 deactivates to S_0 either by phosphorescence emission or ISC. Photochemical processes, which include the numerous targeted reactions, compete with these photophysical events.

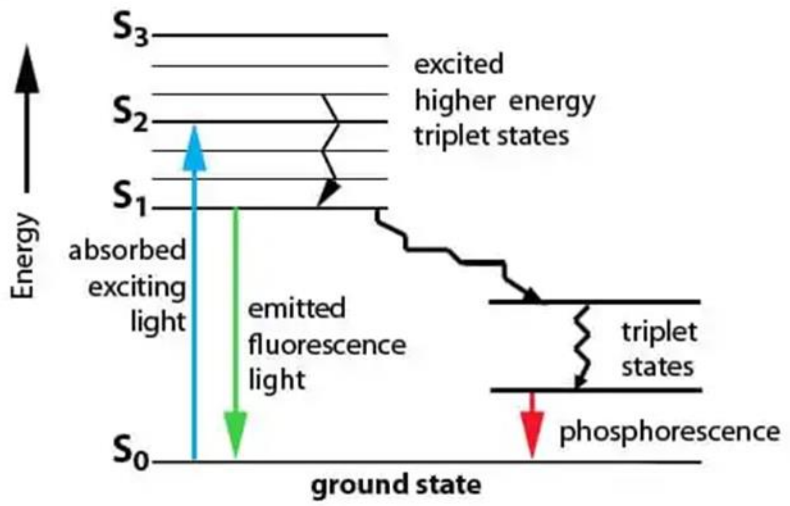


Figure 2.1 : A Jabłoński diagram.

2.2 Photopolymerization

Photoinitiated polymerization has drawn attention in recent years, since it brings together a diverse set of economic and environmental benefits. Photopolymerization has been the basis of many traditional applications in coatings, adhesives, inks, printing plates, optical waveguides, and microelectronics for more than 30 years [72]. Unlike thermally based applications, which often need high temperatures, photopolymerization can be performed at ambient temperature or even lower. This is a significant benefit for both traditional monofunctional monomer polymerization and contemporary curing applications. When monofunctional monomers are photopolymerized, fewer side reactions such as chain transfer occur, however in thermal polymerization, the likelihood of chain transfer is high, resulting in large number of branched macromolecules. Hence, syndiotactic polymers which are difficult to be obtained by thermal polymerization can be synthesized using photopolymerization. Furthermore, photopolymerization can be used for monomers having low ceiling temperature. These monomers can only be polymerized at low temperatures since at hot temperatures, depolymerization predominates. Low temperatures are also required for biological applications such as enzyme immobilization via polymerization. Thermal initiation is often not practicable for curing coatings or surfaces, especially large areas or fine structures are to be cured, or if the curing formulation is used in an application where heating is disallowed, such as dental fillings [73].

As illustrated in Figure 2.2, in photoinitiated polymerization, growing chain ends and initiating species can be radicals [74], cations [75], anions and weak bases [76]. Nevertheless, the photoinitiated condensation polymerization, which involves the step-growth addition of low molecular-weight components to produce a macromolecule, is still emerging.

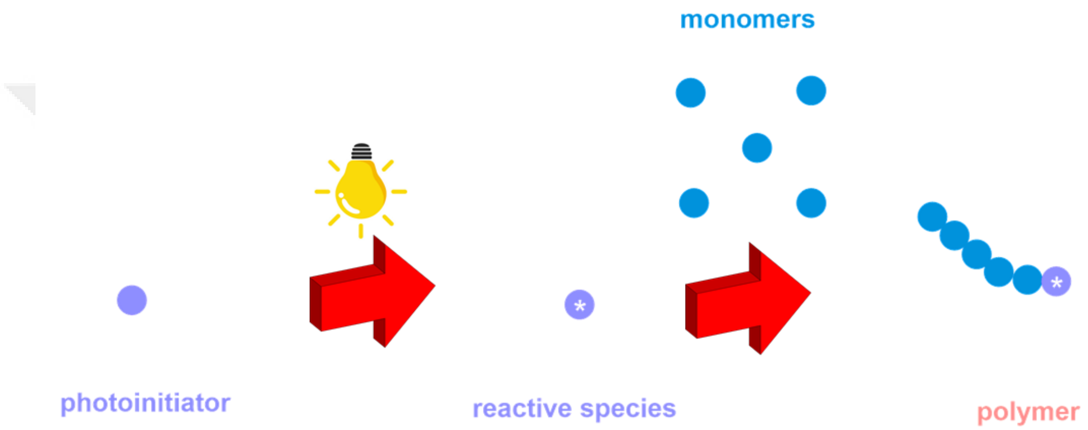


Figure 2.2 : General representation of photopolymerization.

2.2.1 Free radical polymerization

Free radical polymerization is one of the most important methods for polymerizing unsaturated compounds. It has been used to polymerize certain monomers such as ethylene, vinyl chloride, acrylates, and their derivatives in the industry. Since molecular weight distribution is difficult to regulate and low-molecular-weight polymers are frequently generated, this approach is the top choice for synthesizing random or statistical polymers and copolymers with an uncontrolled size chain.

Free radical photopolymerization can be divided into four stages: (i) photoinitiation, (ii) propagation, (iii) chain transfer and (iv) termination (Figure 2.3).

(i) Photoinitiation:

$$PI \xrightarrow{h\nu} PI^{*}$$

$$PI^{*} \longrightarrow R_1^{\cdot} + R_2^{\cdot}$$

$$R_1^{\cdot} + M \longrightarrow R_1{-}M^{\cdot}$$

(ii) Propagation:

$$R_1{-}M^{\cdot} + M \longrightarrow R_1{-}M{-}M^{\cdot}$$

$$R_1{-}M{-}M^{\cdot} + (n\text{-}2)\,M \longrightarrow R_1{-}M_n^{\cdot}$$

(iii) Chain transfer:

$$R_1{-}M_n^{\cdot} + YZ \longrightarrow R_1{-}M{-}Y + Z^{\cdot} \qquad \textit{to chain transfer agent}$$

$$R_1{-}M_n^{\cdot} + M{-}H \longrightarrow R_1{-}M_n{-}H + M^{\cdot} \qquad \textit{to monomer}$$

$$R_1{-}M_n^{\cdot} + H{-}\text{solvent} \longrightarrow R_1{-}M_n{-}H + \text{solvent}^{\cdot} \qquad \textit{to solvent}$$

$$R_1{-}M_n^{\cdot} + P{-}H \longrightarrow R_1{-}M_n{-}H + P^{\cdot} \qquad \textit{to polymer}$$

(iv) Termination:

$$R_1{-}M_n^{\cdot} + R_1{-}M_m^{\cdot} \longrightarrow R_1{-}M_{n+m}{-}R_1 \qquad \textit{by combination}$$

$$R_1{-}M_n^{\cdot} + R_1{-}M_m^{\cdot} \longrightarrow R_1{-}M_n + R_1{-}M_m \qquad \textit{by disproportionation}$$

Figure 2.3 : The general mechanism of free radical photopolymerization.

(i) Photoinitiation is the first step of photoinitiated free radical polymerization. In this step, light is absorbed by a photosensitive compound or electronic excitation energy is transferred from photosensitizer to the photosensitive compound.

(ii) Propagation is the step consists of addition of radical initiator to the monomer. When a chain is initiated, it will continue to propagate until all monomers are consumed, a chain transfer reaction has taken place or the chain is terminated.

(iii) Chain transfer, unlike the other mechanisms of termination, chain transfer leads to the extinction of a radical while simultaneously creating another one.
(iv) Termination is a chemical process that stops forming reactive intermediates. This can be via either combination or disproportionation. In combination, two chain ends are simply connected to produce a single long chain. A hydrogen atom from one chain end is abstracted to the other during disproportionation, resulting in a polymer with a terminal unsaturated group and a polymer with a terminal saturated group.

2.2.1.1 Photoinitiators

While having the smallest volume in a photopolymerization formulation, a photoinitiator plays a critical function in polymerization. Photoinitiator is a molecule that initiates polymerization when exposed to light. It affects crucial parameters including cost, curing speed and yellowing. Other criteria to consider are commercial availability, solubility, especially in a wide variety of monomers, storage stability, low migration, low odor, and nonyellowing characteristics in cured films. For free radical polymerization, photoinitiators are classified as *Type I* (cleavage type) and *Type II* (H-abstraction type) photoinitiating systems [77,78].

***Type I* photoinitiators**

Type I photoinitiators can generate initiating radicals directly upon bond cleavage due to the absorption of light. This initiation system involves only one molecule and therefore called as unimolecular photoinitiation system. *Type I* photoinitiators are generally aromatic carbonyl compounds with proper substitution. Figure 2.4 shows photolytic α-cleavage of benzoin. Benzil ketals, acetophenones, benzyl oximes, benzoin ethers, acylphosphine oxides all spontaneously undergo "α-cleavage" when exposed to light, leading to the generation of free radicals (Table 2.1). Due to their lower absorbencies, the other cleavable initiators such as azo, peroxy and halogenated compounds are not extensively employed [73].

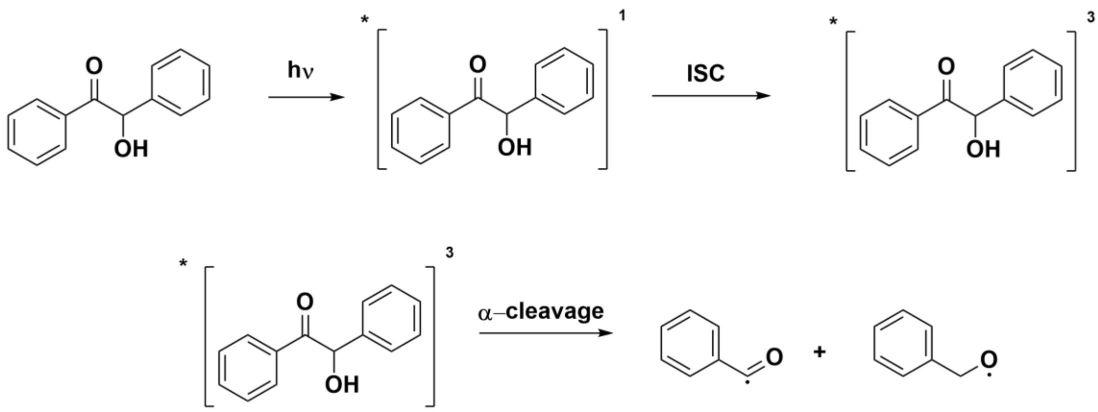
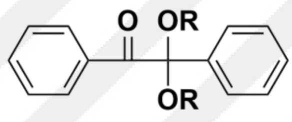
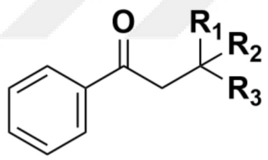
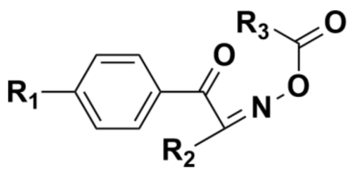
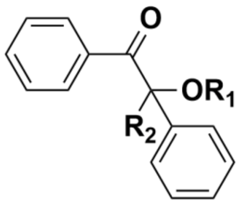
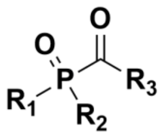


Figure 2.4 : Photolytic α-cleavage of benzoin.

Table 2.1 : Chemical structures and maximum absorption wavelengths of typical *Type I* radical photoinitiators.

Phoinitiator	Chemical Structure	λ_{max}
Benzil ketals	 R= CH_3, C_3H_7, CH_2	365
Acetophenones	 R_1= OCH_3, OC_2H_5 R_2= OCH_3, H R_3= C_6H_5, OH	340
Benzyl oximes	 R_1= H, SC_6H_5 R_2= CH_3, C_6H_{13} R_3= C_6H_5, OC_2H_5	335

Benzoin ethers	 R_1= H, alkyl R_2= H, substituted alkyl	323
Acylphosphine oxides	 $R_{1,2}$= alkyl, aryl, alkyl halides, alkoxy R_3= alkyl, aryl	380

***Type II* photoinitiators**

In collaboration with a variety of co-initiators, *Type II* photoinitiators generate radicals. In Type II photoinitiation, initiating radicals are formed via light absorption by a photoinitiator (e.g., ketone type photoinitiators), followed by interaction with co-initiator molecules, resulting in radical formation, as depicted in Figure 2.5.

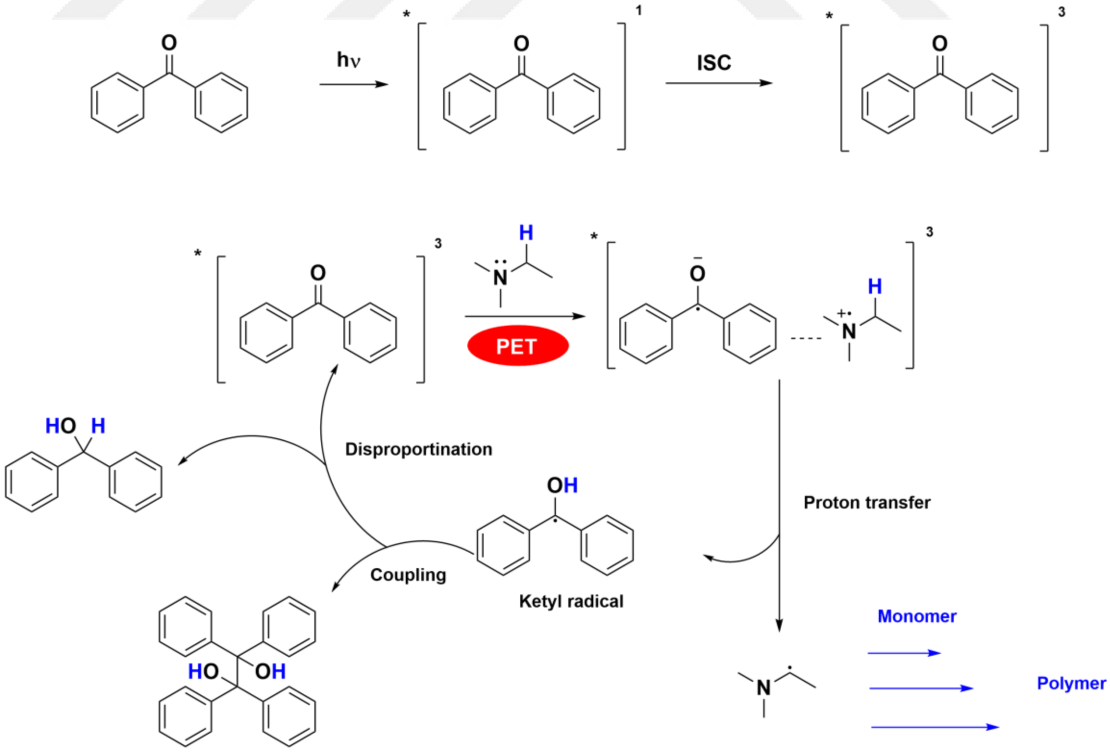
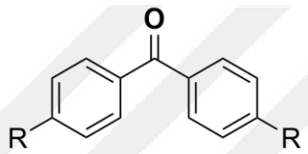
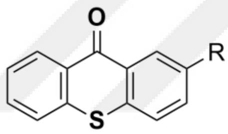
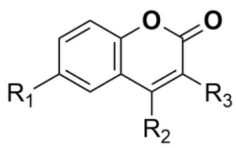
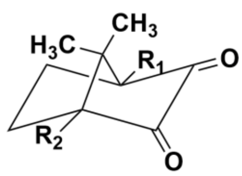
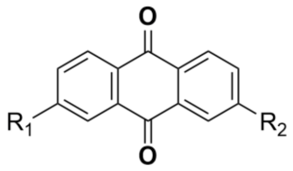


Figure 2.5 : Representation of *Type II* photoinitiation of benzophenone with a tertiary amine co-initiator.

Depending on the nature of the co-initiator, the interaction of the excited photoinitiator with the co-initiator can take several forms, including hydrogen abstraction, electron transfer, or a combination of both electron transfer and hydrogen abstraction processes [79]. Amines, for example, are frequently utilized as co-initiators in *Type II* photoinitiation due to their good reductant characteristics. The most extensively used *Type II* photoinitiators include benzophenone, thioxanthone, ketocoumarin, camphorquinone, anthraquinone (Table 2.2), and their derivatives while amines, alcohols, ethers and thiols are used as co-initiators (Figure 2.6).

Table 2.2 : Typical *Type II* (bimolecular) photoinitiators

Phoinitiator	Chemical Structure	λ_{max}
Benzophenones	 R= H, OH, $N(C_2H_5)_2$, C_6H_5	335
Thioxanthones	 R= H, Cl, $CH(CH_3)_2$	390
Coumarins	 R_1= OH, OCH_3, $N(CH_3)_2$, $N(C_2H_5)_2$, halides R_2= CH_3 R_3= H, benzothiazole	370
Camphorquionones	 R_1= H, CH_3 R_2= H, CH_3	470

Anthraquinones	 R_1= H, CH_3 R_2= CH_3, CH_2OH, OCH_3, CO_2H	350

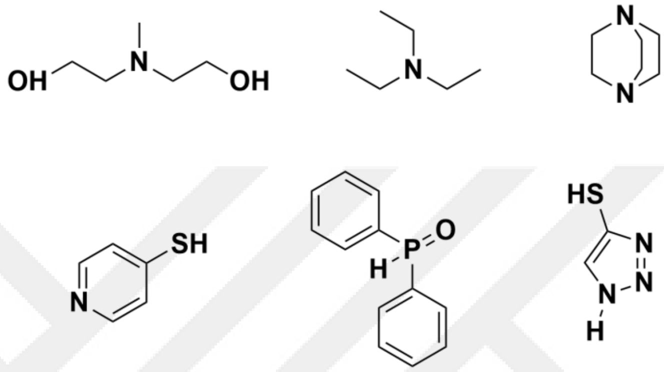


Figure 2.6 : Examples of co-initiators.

2.2.2 Free radical crosslinking polymerization

Free radical polymerization is one of the most widely used of the different methods to synthesize crosslinked polymer networks. The wide range of molecules that can be polymerized and cross-linked by free radical polymerization, from thermosetting acrylate-based adhesives to hydrophilic monomers for contact lenses or protein electrophoresis gels, is undoubtedly a driving force behind its success. Moreover, the rate of polymerization may be well controlled by selecting the suitable amount and type of initiator, as well as the process temperature [80].

The crosslinking species produced by the free radical copolymerization of a monomer with a bis-unsaturated monomer is of particular interest since it has a wide range of applications. As illustrated in Figure 2.7 the system is binary when the process begins, and it can be regarded like a conventional free radical copolymerization. When some bis-unsaturated monomer units are introduced to radical sites, however, pendent double bonds develop, which have their own reactivity. The system therefore contains three polymerizable constituents: monomer, bis-unsaturated monomer, and pendant unsaturation, resulting in a ternary system [81].

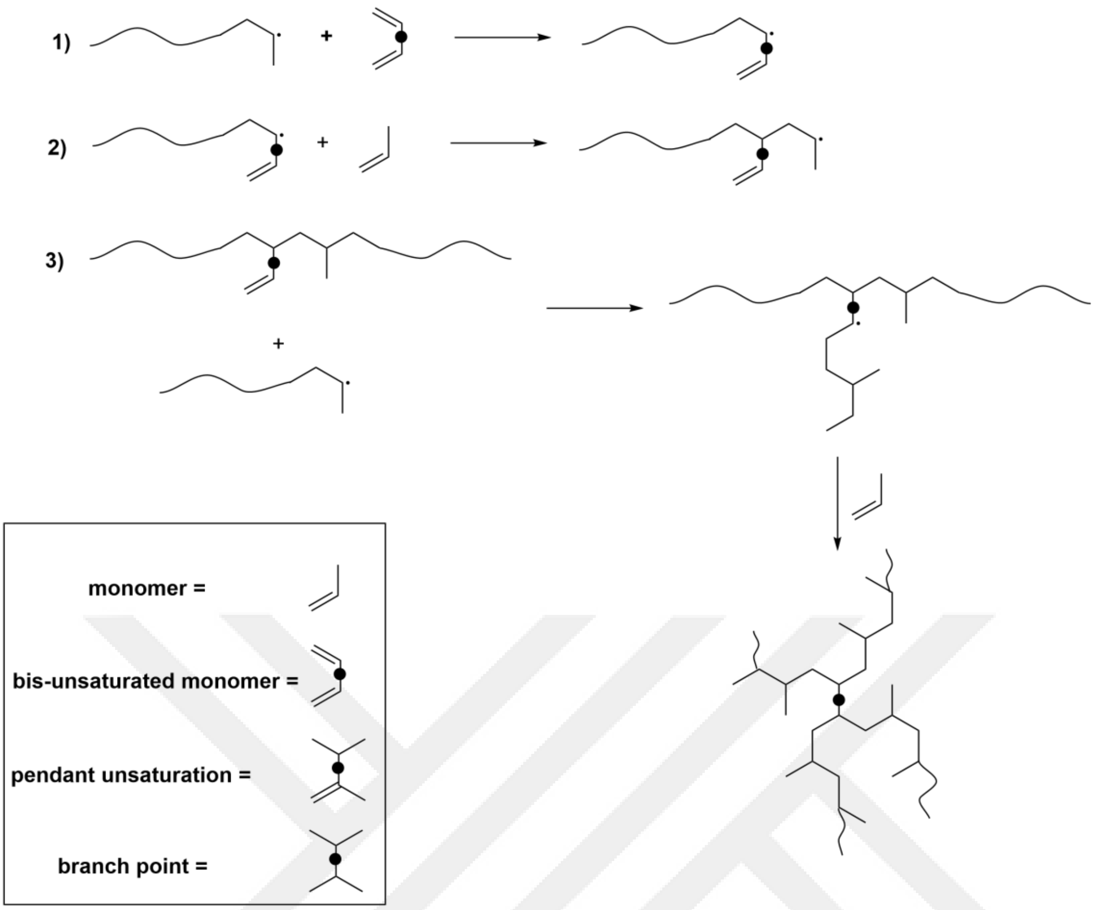


Figure 2.7 : Mechanism of crosslinking free radical polymerization.

2.3 Hydrogels

Hydrogels are hydrophilic, insoluble in water polymeric macromolecules that are described as semi-open network systems composed of entangled chains or short strands of variable lengths linked together by crosslinks [82]. They can entrap a significant amount of the solvent inside the pores or interstitial spaces and acquire a completely swollen state when exposed to a thermodynamically compatible solvent such as water or any biological fluid [83]. Swelling is followed by dimensional change [84], which results in a significant change in rheological properties and, eventually, phase transition (Figure 2.8). Hydrogels, whether natural or synthetic, have inherent physicochemical properties such as hydrophilicity, swellability, gelation, mechanical strength, porosity, biocompatibility, and biodegradability, allowing them to be used in a variety of applications such as water purification, ion exchange chromatography, enhanced oil recovery, sensor development, removal of azo dye pollutants or toxic materials and the development of immotherapy [37,85,86].

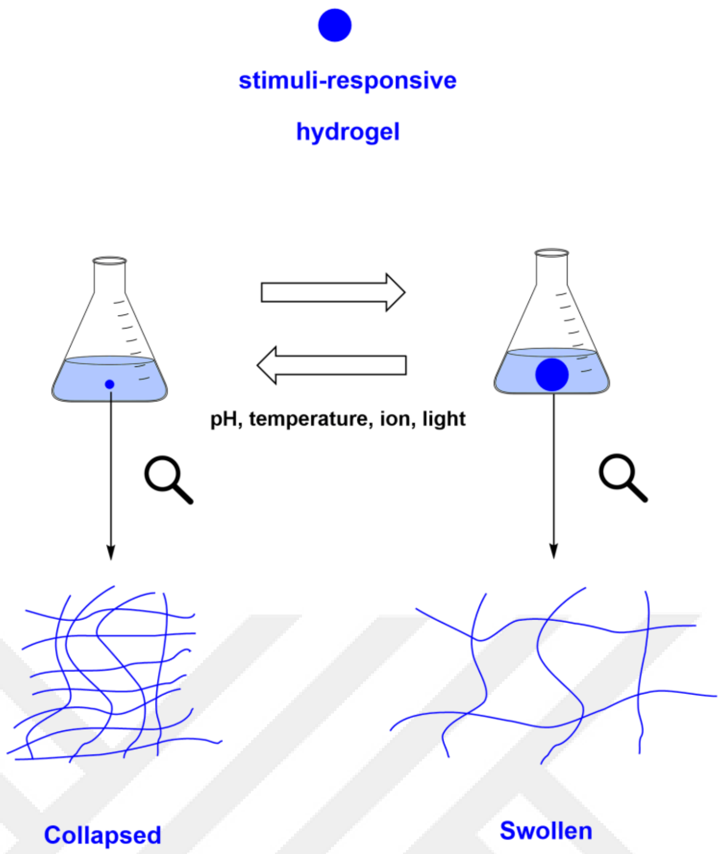


Figure 2.8 : Swelling and deswelling process of a stimuli-responsive hydrogel.

Hydrophilic functional groups, such as alcohols, carboxylic acids, and amides, are responsible for the stiffness and water absorption capacity of hydrogels, while the presence of crosslinks gives them the resistance to dissolution in aqueous media [87]. Agarose, alginate, chitosan, collagen, fibrin, gelatin, hyaluronic acid, poly(vinyl alcohol), poly(acrylic acid), poly(acrylamide), poly(N-isopropylacrylamide), poly(ethylene glycol), poly-2-hydroxyethyl methacrylate (PHEMA) are some of the most common examples of natural and synthetic hydrogels, respectively (Table 2.3).

Table 2.3 : Examples of natural and synthetic hydrogels.

Natural	Synthetic
Agarose	Poly(vinyl alcohol)
Alginate	Poly(acrylic acid)
Chitosan	Poly(acrylamide)
Fibrin	Poly(N-isopropylacrylamide)
Gelatin	Poly(ethylene glycol)
Hyaluronic acid	Poly-2-hydroxyethyl methacrylate

2.3.1 Poly(acrylamide) (PAAm) hydrogels

Polymerization of acrylamide monomer leads to the formation of polyacrylamide (PAAm), which is a colorless hydrogel widely used in many applications due to its nontoxic, nonimmunogenic biocompatible properties with high viscoelasticity and hydrophilicity [88]. PAAm hydrogel is comprised of 2.5 % PAAm and 97.5 % nonpyrogenic water and was originally used in plastic surgery in Ukraine in the 1980s [89]. It has since been found to have promising results in cell-based research, ophthalmic surgeries, drug delivery, wound dressing, food packaging goods, and water purification [90,91]. As shown in Figure 2.9, the repeating unit for PAAm is $[–CH_2–CH(CONH_2)–]_n$.

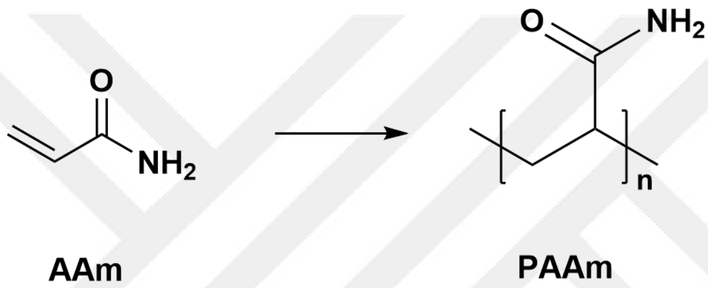


Figure 2.9 : Polymerization of acrylamide.

Hydrogen bonding holds water together between cross-linked polymer chains. The synthesis of PAAm hydrogels have been reported to be nonallergenic, nontoxic, non-absorbable and nonbiodegrable [92].

2.3.2 Modification of hydrogels

Although the materials used to synthesize hydrogels are hydrophilic and flexible, most hydrogels fracture easily, even under low stress levels, due to their poor mechanical strength and toughness. As a result, improving mechanical strength is crucial from a practical point of view. Recently, there has been significant effort put into modifying the mechanical properties of these hydrogels to make them ideal for various applications [93]. Conventional techniques, such as physical mixing or chemical grating, can achieve this goal to a certain degree [94]. Another effective method is the incorporation of inorganic materials into hydrogel networks as reinforcing agents to form hydrogel composites [95]. In particular, for modifications that involve sensitive biomolecules, click chemistry is a powerful toolbox since it enables post-modification of hydrogels under mild and metal-free conditions.

2.4 Click chemistry

In 2001, K. Barry Sharpless et al. coined the term "click chemistry" which refers to the creation of a group of potent, highly reliable, and selective heteroatom link reactions enabling the rapid synthesis of important novel molecules and combinatorial libraries [47]. Click chemistry has emerged as a potential chemistry strategy in the search for new and efficient methods for the synthesis of a wide range of biologically active scaffolds. For the fabrication of molecules, click chemistry is an effective approach.
The ideal chemical reaction, according to Sir John Cornforth, a Nobel Laureate in Chemistry, is "one which a one-armed operator can conduct by pouring the reactants into a bath-tub and collecting the pure product from the drain hole" [96]. Synthetic procedures in click chemistry are aimed to boost the incorporation of all resources utilized in the process into the final product. They have a high atom economy due to the inclusion of addition and rearrangement reactions. The products are made with the goal of maximizing efficacy while minimizing cytotoxicity [97]. In Figure 2.10, the greenness of click chemistry is illustrated.

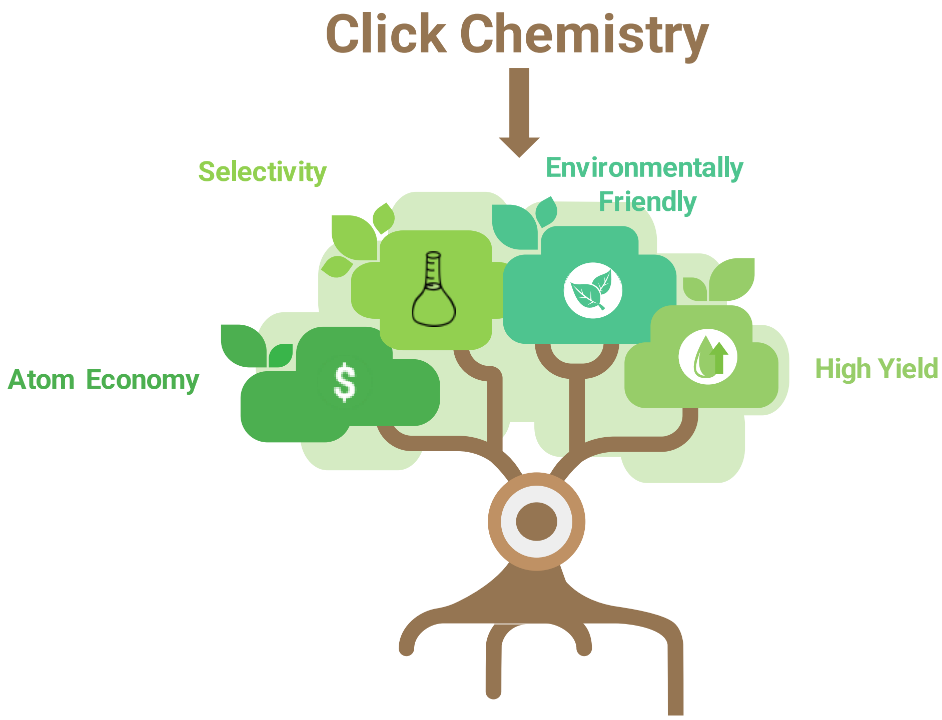


Figure 2.10 : Features of click chemistry.

Although there is no standard way to categorize click reactions, the main requirement for "click chemistry" is well met by reactions that occur in nature, and its mimic in the laboratory is the closest and most attractive to most synthetic organic chemists. The unofficial classification of click reactions is as follows (Figure 2.11):

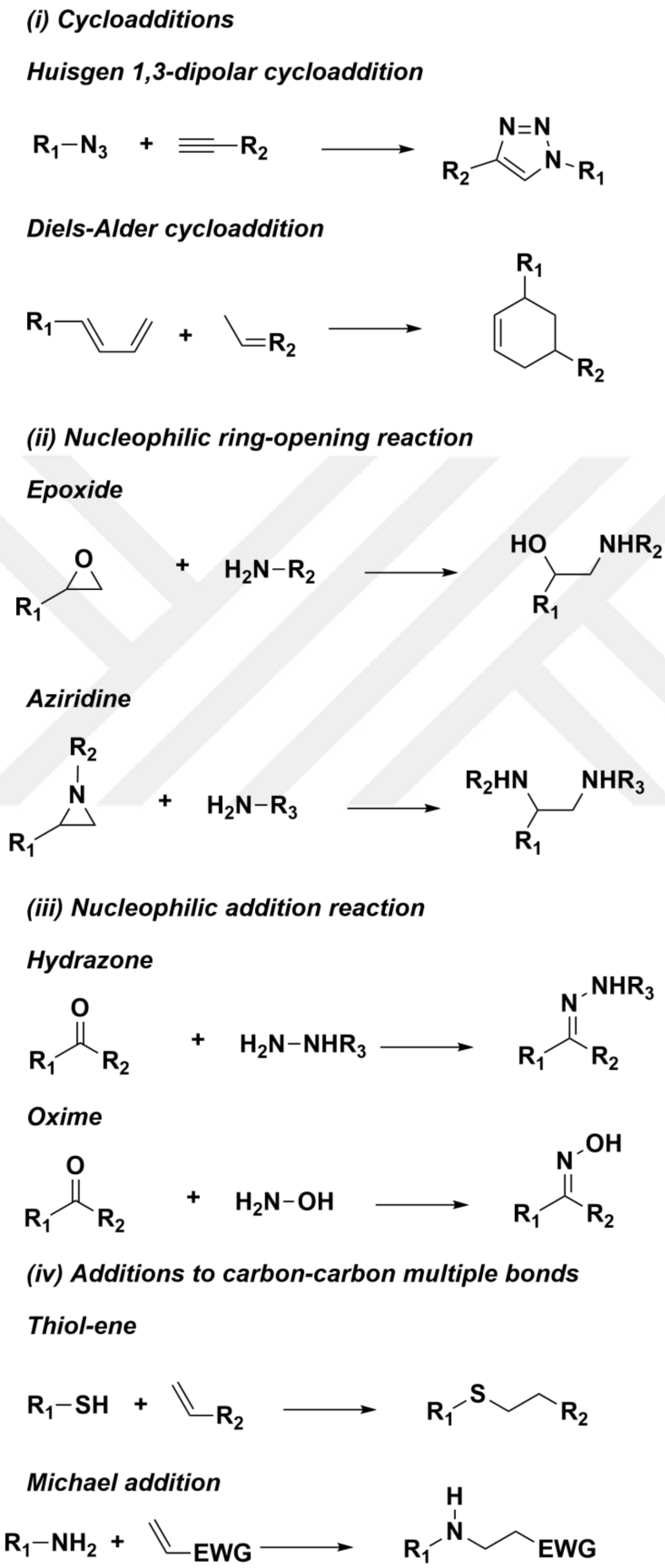


Figure 2.11 : Classification of click reactions.

(i) Cycloadditions refer to 1,3-dipolar cycloadditions and hetero-Diels-Alder cycloadditions. These include the classical Huisgen 1,3-dipolar cycloaddition and metal catalyzed cycloaddition reactions.

(ii) Nucleophilic ring-opening reactions include opening of strained heterocyclic electrophiles such epoxides, aziridines, cyclic sulfates, aziridinium ions and episulfonium ions.

(iii) Nucleophilic addition reaction involves the carbonyl functional group reactions, such as formation of hydrazones, oxime ethers, thiourease, urease, aromatic heterocycles and amides.

(iv) Additions to carbon-carbon multiple bonds are classified as the reactions which include epoxidation, nitrosyl halide addition, dihydroxylation and some Michael addition reactions.

2.4.1 Thiol-X click reactions

The thiol-ene/yne reaction is the hydrothiolation of a carbon-carbon double/triple bond with anti-Markovnikov orientation (Figure 2.12), which has been known for almost a century. In the literature, this reaction has been commonly used in polymer research to prepare nearly perfect networks and films, as shown by Hoyle *et al.* and Bowman and coworkers [98].

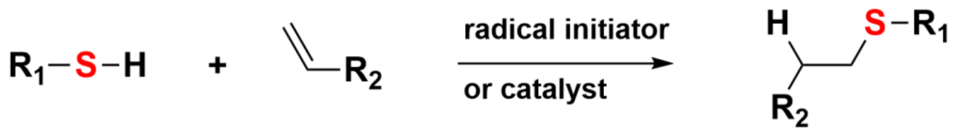


Figure 2.12 : The hydrothiolation of a carbon-carbon double bond with anti-Markovnikov addition.

Nonetheless, primarily as a result of its "click" properties, the thiol-ene reaction has recently attracted researchers in other areas of synthesis. Thiol-ene reaction has various characteristics that make it a highly appealing, simple, and adaptable method [99]. First and foremost, such hydrothiolation reactions can occur via a radical pathway [100], catalytic processes driven by nucleophiles, acids and bases [101], in the lack of an additional catalyst in highly polar solvents such as water or DMF [102], or by supramolecular catalysis employing β-cyclodextrin [103], for instance. Furthermore, a wide range of enes and ynes may serve as suitable substrates, such as activated and non-activated species and olefinic bonds which are multiply-substituted. Moreover, almost any thiol, even highly functional molecules, can be used, however reactivity varies greatly depending on the strength of the S–H bond and the cleavage method (homolytic vs. heterolytic cleavage). Lastly, this reaction is exceptionally rapid at room temperature and atmospheric pressure, insensitive to oxygen, moisture and air as long as oxygen concentration is lower than that of thiol and regioselective.

2.4.2 Copper-catalyzed azide–alkyne cycloaddition (CuAAC) click reaction

First introduced independently by Sharpless [104] in the United States and Meldal [105] in Denmark, copper catalyzed azide-alkyne cycloaddition (CuAAC) is a great illustration of how transition metal catalysis may help in synthetic organic chemistry. The conventional reaction, which does not employ copper catalyst, is a slow and high temperature process that is unselective. The reaction is accelerated dramatically (10^7 times) in the presence of catalytic quantities of copper(I) species [106]. Furthermore, the regioselectivity was improved in a spectacular way, with the 1,4- disubstituted isomer being the only product produced unlike the uncatalyzed version which produces an equimolar mixture of 1,4- and 1,5-disubstituted 1,2,3-triazole regioisomers as shown in Figure 2.13.

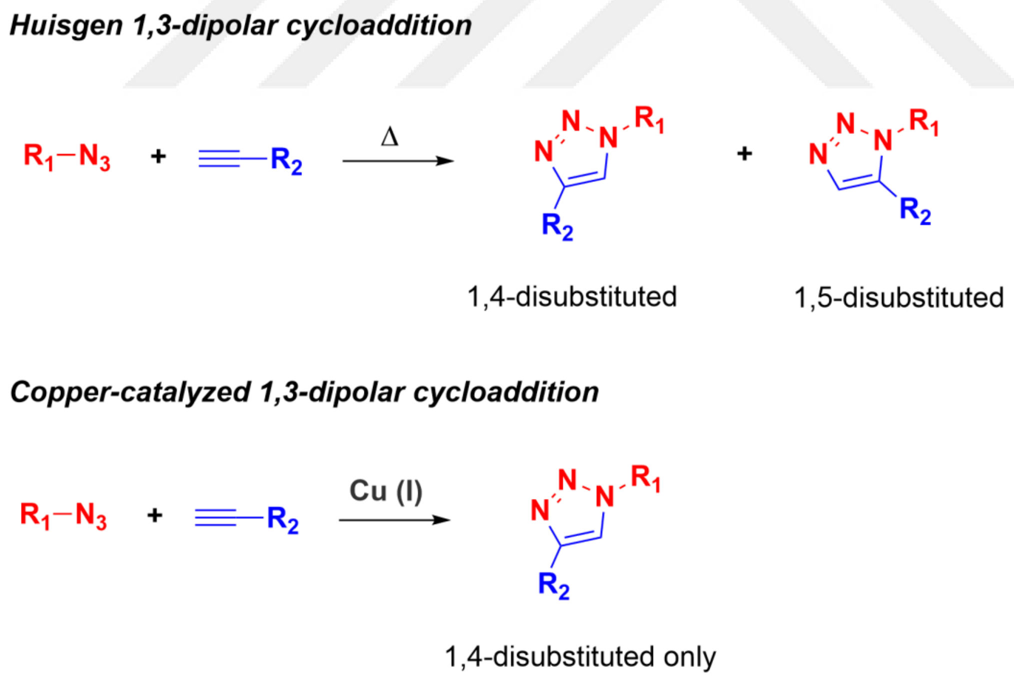


Figure 2.13 : Thermal and Cu(I)-catalyzed-azide-alkyne cycloaddition.

Synthetic compounds having the 1,2,3-triazole core have fascinating biological features, regardless of the fact that this heterocycle does not occur naturally [107]. Furthermore, the 1,2,3-triazole is chemically inert against oxidation, reduction and hydrolysis under acidic or basic conditions resembling the characteristics of the amide bond which enables it to participate in hydrogen bonding and dipole-dipole interactions [57]. As a consequence, the CuAAC has found useful applications in a variety of fields, including drug discovery [108], dendrimer [109], biochemistry [110], polymer chemistry [111] and materials science [112].

3. EXPERIMENTAL PART

3.1 Materials and Solvents

3.1.1 Materials

Propargyl acrylate (98%, Sigma-Aldrich) was passed through a short neutral activated aluminium oxide (0.063−0.200 mm) column before use in order to remove the inhibitor and later stored in the refrigerator. Acrylamide (AAm, Sigma-Aldrich), 1-[4-(2hydroxyethoxy) phenyl]-2-hydroxy-2-methyl-1-propane-1-one (Irgacure 2959, Ciba), sodium azide (NaN_3, Panreac), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, Sigma-Aldrich), copper (II) chloride ($CuCl_2$, Merck), 1-pyrenemethanol (98%, Sigma-Aldrich), Phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide (BAPO, 97%, Sigma-Aldrich), 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99%, Sigma-Aldrich), were used as purchased.

3.1.2 Solvents

Dimethyl sulfoxide (DMSO, Merck), dimethyl formamide (DMF, Merck), dichloro methane (DCM, Merck) were used as received.

3.2 Characterization

3.2.1 ^{1}H Nuclear magnetic resonance spectroscopy (^{1}H-NMR)

^{1}H NMR spectra were recorded on an Agilent NMR System VNMRS 500 spectrometer at room temperature in $CDCl_3$ with tetramethylsilane as internal standard.

3.2.2 Fourier-transform infrared spectroscopy (FT-IR)

Fourier-transform infrared (FT-IR) analyses were performed on a Perkin-Elmer FTIR Spectrum One B spectrometer.

3.2.3 Fluorescence spectroscopy

The fluorescence spectra were recorded on a Varian Cary Eclipse fluorophotometer with excitation wavelengths at 317 and 350 for pyrene and anthracene modified hydrogels, respectively.

3.3 Synthesis

3.3.1 Synthesis of (azidomethyl)anthracene (A-N_3)

A synthesis procedure from the literature was followed (Figure 3.1) [113, 114]. 9-Hydroxymethylanthracene (3.70 mmol, 1 eq.) was dissolved in DCM (25 mL) and cooled down to 0˚C in an ice bath. $SOCl_2$ (0.75 eq.) was then slowly added to the reaction mixture and stirred for 1 h while warming up to room temperature. Under vacuum, the solvent was removed and the remaining material was redissolved in DMF (5 mL). After the compound was dissolved, NaN_3 (0.75 eq.) was added and the reaction mixture was stirred at 50 °C. The reaction mixture was allowed to cool for 1 h before being diluted with water and extracted with EtOAc. The combined organic phases were filtered and collected under vacuum after being washed with brine and dried with anhydrous $MgSO_4$. The resulting product was brown-yellow crystalline solid, yield= 93% ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.44 (dd, 2H), 8.14 (dd, 2H), 7.64 (td, 2H), 7.56 (td, 2H), 5.51 (s, 2H); ^{13}C{^{1}H} NMR (DMSO-d_6, 125 MHz) δ 131.39, 130.72, 129.51, 129.06, 127.28, 126.96, 125.88, 124.51, 45.96.

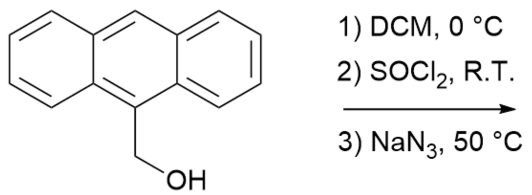


Figure 3.1 : Synthesis of (azidomethyl)anthracene (A-N_3).

3.3.2 Synthesis of pyrene-1-methanethiol (Py-SH)

The synthesis of thiol functionalized pyrene (Py-SH) was adapted from literature (Figure 3.2) [115]. In a 50 mL round-bottom flask pyrene methanol (1.000 g, 4.31 mmol) was added to a stirring solution of thiourea (0.328 g, 4.31 mmol) and 48% HBr (1400 μL) in 10 mL acetonitrile and 3 mL DCM. The mixture was homogenized within approximately 30 min and was refluxed at 90 °C for 9 h. Small quantities of the heated reaction mixture were placed into a cold (ice bath) 15% aqueous NaOH solution (50 mL) and stirred overnight at room temperature. While keeping the temperature below 10 °C, the sodium thiolate solution was cooled (ice bath) and acidified with 50 % aqueous H_2SO_4 to pH 2-3. The crude product was extracted using chloroform (3-15 mL), and the combined extracts were washed with aqueous $NaHCO_3$ solution (10 mL) and brine before being dried over Na_2SO_4. The resulting product was yellowish solid, yield= 55 % ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.43–8.41 (m, 2H), 8.32–7.23 (m, 4H), 8.15 (s, 2H), 8.06 (m, 1H), 4.50 (s, 2H), 2.07 (s,1H).

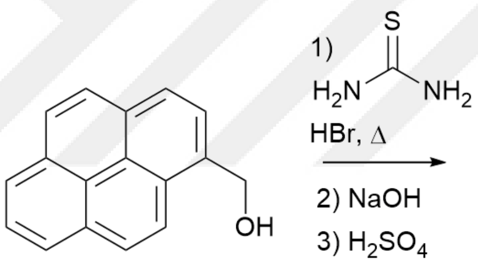


Figure 3.2 : Synthesis of pyrene-1-methanethiol (Py-SH).

3.3.2.1 Synthesis of clickable poly(acrylamide-co-propargyl acrylate) hydrogel

An aqueous solution of AAm (142 mg, 2.00 mmol), PA (11 μL, 0.1 mmol) and Irgacure 2959 (5.0 mg, 0.02 mmol) was prepared in 1 mL of water. The solution was purged with nitrogen gas for 5 min and then transferred into a 1 mL polypropylene syringe. Irradiation of the syringe at room temperature resulted in formation of clickable hydrogel. After polymerization, syringe was gently broken and the hydrogel was transferred into a beaker of deionized water for 2 days to remove unreacted reagents in the reaction mixture.

3.3.2.2 Modification of the hydrogels with photoinduced thiol-ene click reactions

Pristine hydrogel, pyrene-1-methanethiol (12.42 mg, 0.05 mmol, 1 eq.) or 1,6-hexanedithiol (7.50 mg, 0.05 mmol, 1 eq.), DMPA (0.01 eq.) in 2 mL of DMF were combined in a Schlenk tube. The reaction mixture was bubbled with nitrogen gas for 5 min. The reaction mixture was then irradiated by a Rayonet merry-go-round photoreactor emitting light nominally at 350 nm with a light intensity of around 3.0 mW/cm^{-2} at room temperature for 2 h. After the click reaction, functionalized hydrogel was washed with excess water and DMF in order to remove unreacted reagents.

3.3.2.3 Modification of the hydrogels with photoinduced CuAAC

To a Schlenk tube equipped with magnetic stirrer, pristine hydrogel, $CuCl_2$ (6.75 mg, 0.05 mmol, 1 eq.), PMDETA (10.44 μL, 0.05 mmol, 1 eq.), and BAPO (20.92 mg, 0.05 mmol, 1 eq.) were added and dissolved in 5 mL of DMSO. The reaction mixture was degassed by three freeze – pump – thaw cycles for two times and irradiated by a Ker-Vis blue photoreactor with a circle of six lamps (Philips TL-D 18 W) which emit light at 400-500 nm for 24 h. Following the click reaction, the modified hydrogel was immersed in deionised water, ammonia solution in order to remove any unreacted reagents.

3.4. Swelling and mechanical properties of hydrogels

3.4.1 Equilibrium swelling testing of hydrogels

Once the reaction was over, the hydrogels were removed from the polypropylene syringes and were first divided into small pieces. Equilibrium swelling was tested using conventional volumetric procedure. Before immersing into excess amount of water, the diameter of the samples was measured using a calibrated digital compass (Mitutoyo Digimatic Caliper, Series 500, resolution: 0.01 mm). The hydrogels were allowed to swell in excess amount of water until they reached the equilibrium state, which was determined by monitoring the change in the diameter of the samples. At swollen-state, the diameter of the swollen samples was measured and the equilibrium volume swelling ratio V_{eq} and the volume fraction of the crosslinked network chains in the equilibrium swollen network ν_2 were calculated by Eq. (1) [116]:

$$V_{\mathrm{eq}} = (D/D_0)^3 \text{ and } \nu_2 = \nu_2^0 / V_{eq} \tag{1}$$

where D and D_0 are the diameters of sample at equilibrium swollen state and after-preparation state, respectively. Note that ν_2 and ν_2^0 are the structural parameters that characterize the hydrogel in the post-synthesis and swollen state.

3.4.2 Compressive testing

Mechanical properties were determined by compressive testing of the hydrogels in uniaxial compression mode both after-preparation and after attaining the equilibrium swelling state. A cylindrical hydrogel sample of 4 mm in diameter and 4-5 mm in length, as measured prior to testing, was compressed by a vertically placed circular probe with a diameter of 40 mm. The change in the length and weight of the hydrogel sample was monitored during the uniaxial compression. After 20 s of chain relaxation at each loading, the change in length of the compressed sample was monitored as $\Delta L = L_0 - L$, where L_0 and L are the initial and deformed sample lengths along the vertical axis, respectively. For uniaxial compression tests, the crosshead speed was set at 1 mm/min and the test was performed corresponding to approximately 20% compression over the sample length. The nominal stress σ was determined as the ratio of compressive force per unit area of sample from Eq. (2):

$$\sigma = f/S = G(\alpha - \alpha^{-2}) \qquad (2)$$

where G is the compressive modulus, S is initial cross-sectional area; $S = \pi\left(D_0/2\right)^2$ determined using the diameter of sample and α is the deformation ratio determined as $\alpha = 1 - \Delta L / L_0$. The measurements were repeated with at least three samples and the results were averaged. From the stress–compression curves, the compressive modulus was determined as slope of the linear portion of curves (1–3%) by using the linear regression method. Based on the assumption that the polymer network is a collection of Gaussian chains, statistical theory of rubber elasticity [117, 118] defines the elastic modulus, G, at thermodynamic equilibrium swelling state from Eq. (3):

$$G = A\nu_e \mathrm{RT}\left(\nu_2^0\right)^{2/3}\nu_2^{1/3} \qquad (3)$$

where the front factor A equals 1 in an affine network when each chain satisfies Gaussian assumption and A equals 1- 2 / ϕ for a phantom network, in which ϕ is the functionality of the crosslinks. As the number of crosslinked chains remains unchanged during the swelling process, assuming $\nu_2 = \nu_2^0$ as-prepared state, Eq. (3) can be rearranged to determine the compressive modulus after-preparation state G_0 by Eq. (4):

$$G_0 = A\nu_e RT\nu_2^0 = A\frac{1}{NV_1}RT\nu_2^0 = A\frac{\rho}{M_C}RT\nu_2^0 \tag{4}$$

which gives the characteristic network parameters known as the effective crosslinking density, ν_e to estimate the mole number of elastically active chains in the polymer network, N is the network chain length, and M_C is the molecular weight between cross-links. The swelling and compressive elasticity results were combined to determine the magnitude of interactions between P(AAm-co-PA) and water by solving Flory–Huggins polymer–solvent interaction parameter, χ from the following Eq. (5) [4];

$$\ln(1-\nu_2)+\nu_2+\chi(\nu_2)^2 + 0.5\nu_e\left((\nu_2)^{1/3}(\nu_2^0)^{2/3} - \nu_2/2\right) = 0 \tag{5}$$

By using the experimentally determined values of ν_2^0, ν_2, and ν_e of P(AAm-co-PA) hydrogels, χ parameter was calculated to evaluate the extent of interaction between copolymer network and water.

4. RESULTS AND DISCUSSION

We have previously shown that photochemically formed radicals react with double and triple bonds at different rates [119]. Laser flash photolysis studies on acylphosphine oxides revealed that the rate of reaction of the radicals with double bonds is higher than that of the structurally resembling triple bonds by almost two orders of magnitude. In the present work, we used AAm and PA as monofunctional monomer and bifunctional monomer possessing both double and triple bonds, respectively. Upon irradiations of the solutions in the presence of various photoinitiators, in addition to the acrylate chain propagation, the reaction of the photochemically formed radical with a small portion of propargyl group of PA leads to the formation of branched and essentially networked structures. While the reaction with the triple bond results in the formation of double bonds, some portion of the triple bond remains unreacted because of the relatively lower reactivity. The overall process is presented in Figure 4.1.

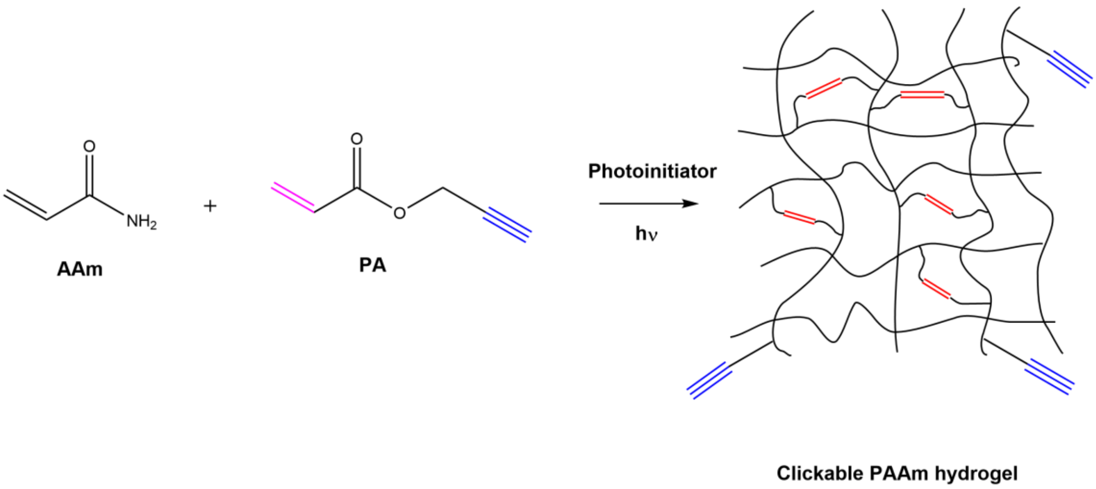


Figure 4.1 : Photoinduced synthesis of clickable PAAm hydrogel.

In the process, several photoinitiators, namely 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959), phenylbis(2,4,6-trimethylbenzoyl) phosphine oxide (BAPO) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) which undergo α-cleavage photoscission (Figure 4.2) were used in organic and aqueous media. The results are presented in Table 4.1.

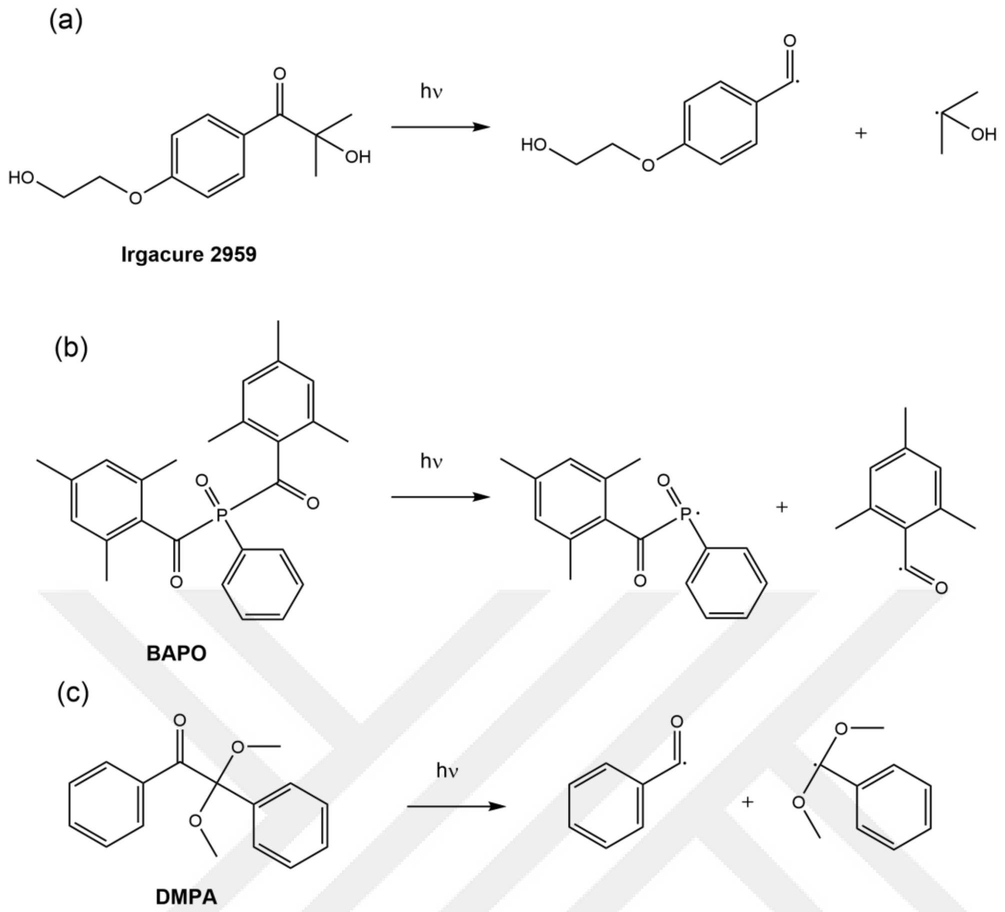


Figure 4.2 : Photoinduced radical generation of photoinitiators used in this study, (a) Irgacure 2959, (b) BAPO and (c) DMPA.

Table 4.1 : Photoinduced synthetic conditions for PAAm hydrogels using acrylamide (2.00 mmol) and propargyl acrylate (0.01 mmol) monomers in 1 mL of solvent at room temperature.

Photoinitiator	Irradiation wavelength (nm)	Solvent	Gelation time[a] (h)
Irgacure 2959	365	Water	3 h
BAPO	405	Acetone	1 h
DMPA	350	Acetone	1 h

[a]Time for obtaining tack free polymer film.

In order to evaluate the gel properties such as gel fraction, swelling degree and elasticity at different time intervals, PAAm hydrogels were prepared in aqueous media by using Irgacure 2959 as a water soluble photoinitiator.

Figure 4.3 shows the experimentally determined values of volume fraction of the crosslinked polymer after-preparation state, ν_2^0 as a function of the irradiation time. Following the gelation, the structural characteristics of PAAm hydrogels were determined. All the hydrogels were immersed in distilled water for one-week, and then dried at room temperature until constant weights are attained. Monomer conversion in terms of the volume fraction of the network at the stage of gel preparation ν_2^0 is experimentally determined by Eq. (6):

$$\nu_{2,\exp}^{0} = \left[1 + \frac{\left(m_0/m_{dry} - 1\right)\rho_p}{d_1}\right]^{-1} \tag{6}$$

where m_0 is the weight of the gel sample as-prepared state, m_d is the weight of dried the gel. ρ_P and d_1 are the density of the gel (1.35 g/mL) and water (1.0 g/mL), respectively. In Figure 4.3, the dashed line represents the theoretical ν_2^0 values calculated from the feed composition by $\nu_{2,theo}^0 = 10^{-3} C_0 \overline{V}_r$, where $\overline{V}_r$ is the average molar volume of the repeat units (54.1 mL / mol) and C_0 is the initial concentration of the monomers (w/v). The experimentally calculated values of ν_2^0 of hydrogels are in good agreement with the theoretical ones. The gel fraction w_{gel} as the conversion of total monomers to the insoluble network was obtained in the range of 81-82%.

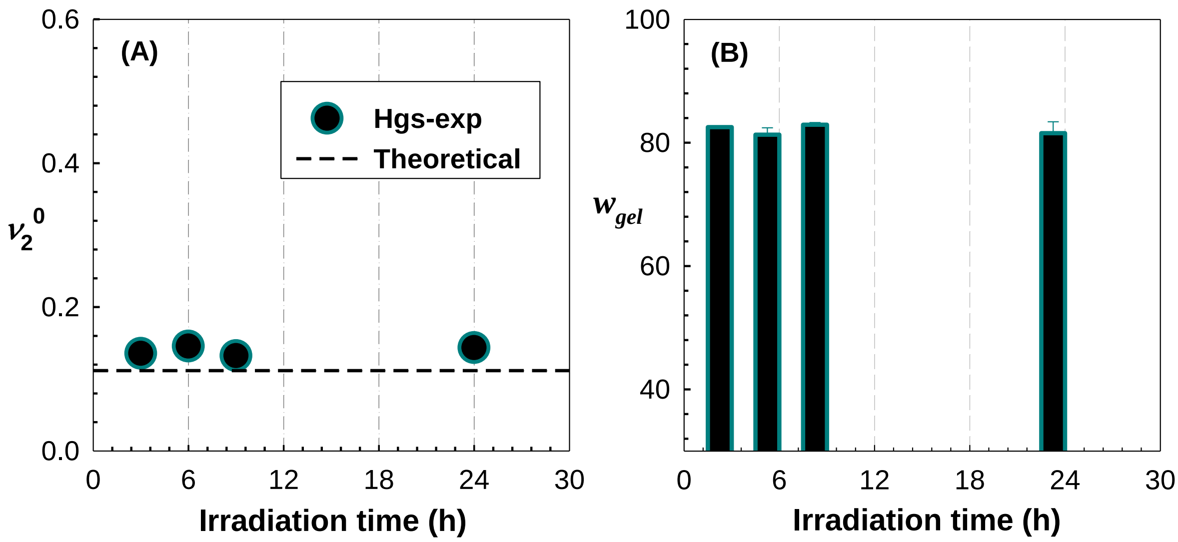


Figure 4.3 : (A) Experimentally determined values of volume fraction of crosslinked polymer after preparation ν_2^0 (symbols) as a function of irradiation time. The dashed line represents the theoretical ν_2^0 values calculated from the feed composition. (B) Gel fraction w_{gel} of the hydrogels.

To determine the equilibrium volume swelling ratio of the hydrogels using Eq. (1), all samples were allowed to attain their thermodynamic equilibrium state at room temperature which takes approximately two weeks. The effect of the irradiation time on the swelling degree of the hydrogels was shown in Figure 4.4. With increasing the irradiation time, the equilibrium volume swelling ratio of the hydrogels in water decreased continuously as the volume fraction of the gel in the equilibrium swollen network $\nu_2$increased. The lower degree of swelling observed with the increase in irradiation time indicates a higher crosslink density, which means that the copolymer chains in the network matrix become stiffer. Figure 4.4(B) shows χ parameter of PAAm-water system determined by solving the Eq. (5) as a function of ν_2. The decrease in the degree of swelling of the hydrogel as the irradiation time increases is reflected by the continuous increase in χ parameter. This increase is also indicative of weak interactions between copolymer chains and water molecules.

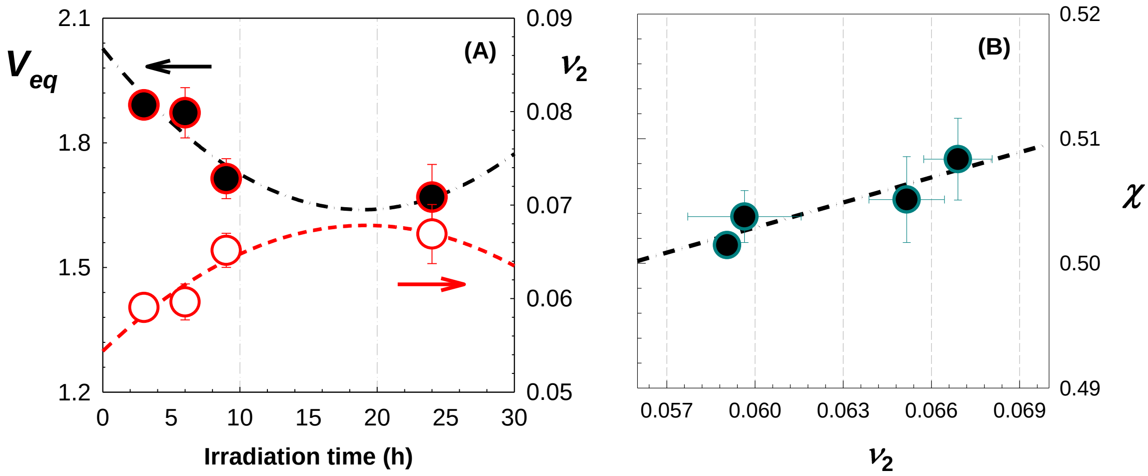


Figure 4.4 : (A) Equilibrium volume swelling ratio V_{eq} (solid symbols) in water and the volume fraction of the crosslinked polymer in the equilibrium swollen network ν_2 (empty symbols) as a function of irradiation time. (B) Polymer-solvent interaction parameter χ as a function of the volume fraction of copolymer ν_2.

Figure 4.5 presents the stress - compression isotherms of PAAm hydrogels after their preparation and after equilibrium swelling in water. The effect of irradiation time is also presented. The slope of the stress-compression isotherms varies depending on the irradiation time while the composition is the same. The elastic moduli of the hydrogels, G_0 after preparation-state and after equilibrium swelling in water, G determined from the slope of stress-strain curves as a function of the irradiation time were shown in Figure 4.6A. The compressive modulus is one of the network parameters to evaluate the stiffness of the material, which is directly proportional to the crosslinking density. The influence of the irradiation time on the elasticity of the hydrogels indicate a continuous increase in the elastic modulus. The elastic modulus of the hydrogels after equilibrium swelling in water is lower than that of the hydrogels after preparation at all irradiation times as a consequence of the swelling process. The most striking observation from the stress-compression curves of the hydrogels is 1.2-fold increase in elastic modulus observed by increasing the irradiation time from 3 h to 24 h. As can be seen from Figure 4.6B, this increase in the irradiation time led to a significant increase in the effective crosslink density, $\nu_e$of the hydrogels. Consequently, the observed low swelling degree is due to the highly cross-linked network structure. In Figure 4.6 C and D, the experimentally determined network chain length, N_{exp} and the molecular weight between cross-links, M_C for phantom (ϕ = 4) and affine network models, respectively, determined by Eq. (4), were shown as a function of the irradiation time. An increase in the crosslinking density led to a decrease of N_{exp} and M_C of the hydrogels in all cases. A denser network resulting in higher compressive modulus is responsible for higher crosslinking density and also results in the presence of shorter copolymer chains between crosslinks and lower average molecular length and therefore weakens the mobility of the polymer chains.

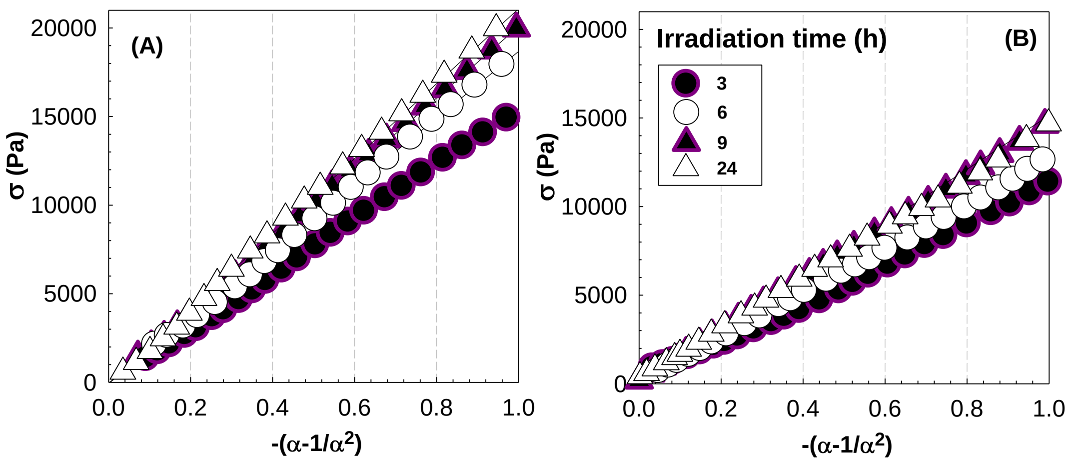


Figure 4.5 : Stress - strain isotherms of PAAm hydrogels after their preparation (A) and after equilibrium swelling in water (B). The irradiation time of the hydrogels is indicated in the figure.

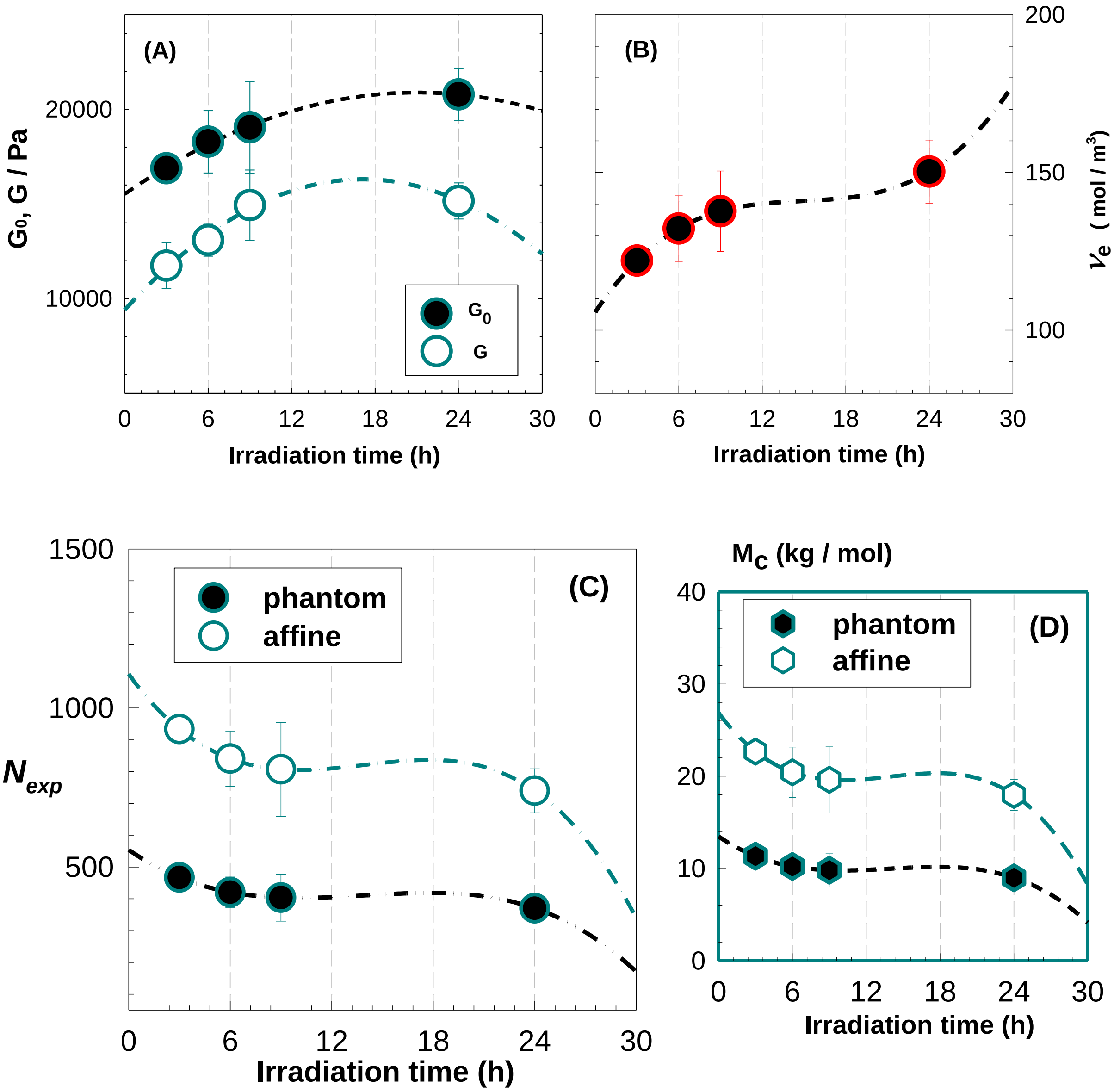


Figure 4.6 : (A) Compressive elastic moduli from the uniaxial compression tests after preparation (solid symbols) and after equilibrium swelling in water (empty symbols), (B) effective crosslink density ν_e, (C) experimentally determined network chain length N_{exp} and (D) molecular weight between cross-links M_C of PAAm hydrogels as a function of the irradiation time. The filled and open symbols indicate the calculation results for phantom (φ = 4) and affine network models, respectively.

Clickability of the hydrogels was also verified with different click chemistry reactions namely, thiol-ene, thiol-yne and CuAAC. Thiol functional pyrene (Py-SH) and azide functional anthracene (A-N_3) were deliberately chosen as the respective click components so as to facilitate efficient characterization of the modified gel through their attractive fluorescence features (Figure 4.7).

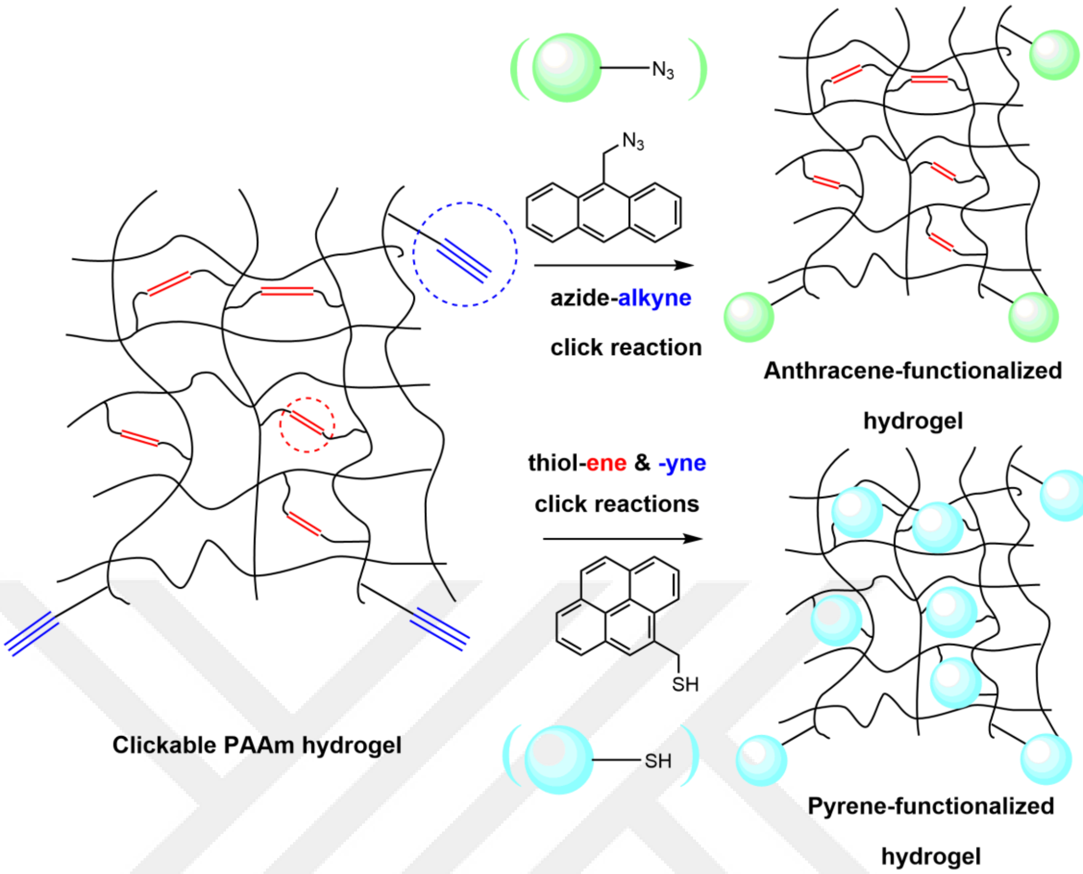


Figure 4.7 : CuAAC, and thiol-ene and -yne click reactions of the PAAm hydrogels by (azidomethyl)anthracene, and pyrene-1-methylthiol, respectively.

Thiol click component, Py-SH was obtained by the reaction of 1-pyrenemethanol with thiourea. The azido click component A-N_3 was synthesized from 9-hydroxymethylanthracene. Spectroscopic data are shown in Figure 4.8 and 4.9. FT-IR Spectroscopy was employed to monitor click reactions. The effective fabrication of the hydrogels by click reactions were confirmed by the disappearance of the peak at around 2100 cm^{-1} which corresponds to the triple bonds (Figure 4.10). Successful click reactions were further confirmed by fluorescence spectroscopic studies. As can be seen from Figures 4.11 and 4.12, pristine hydrogels show no emission at all, while anthracene and pyrene modified hydrogels exhibit broad emission bands.

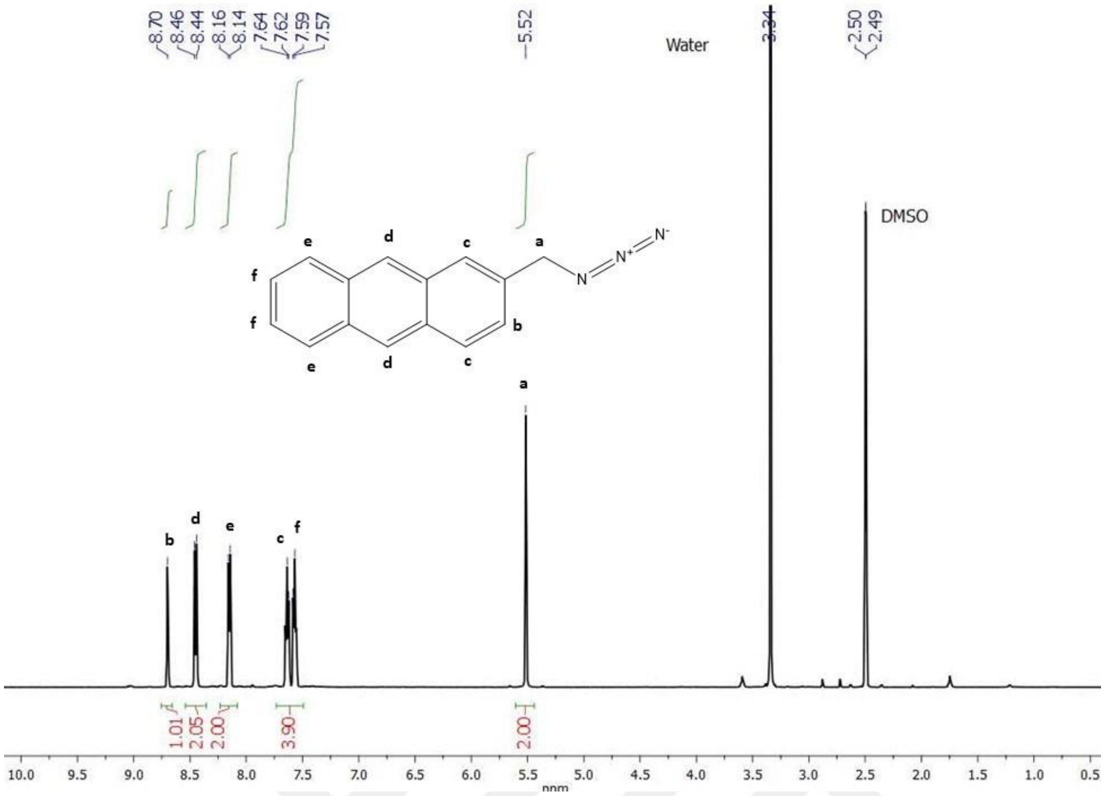


Figure 4.8 : ^{1}H NMR spectrum of A-N_3.

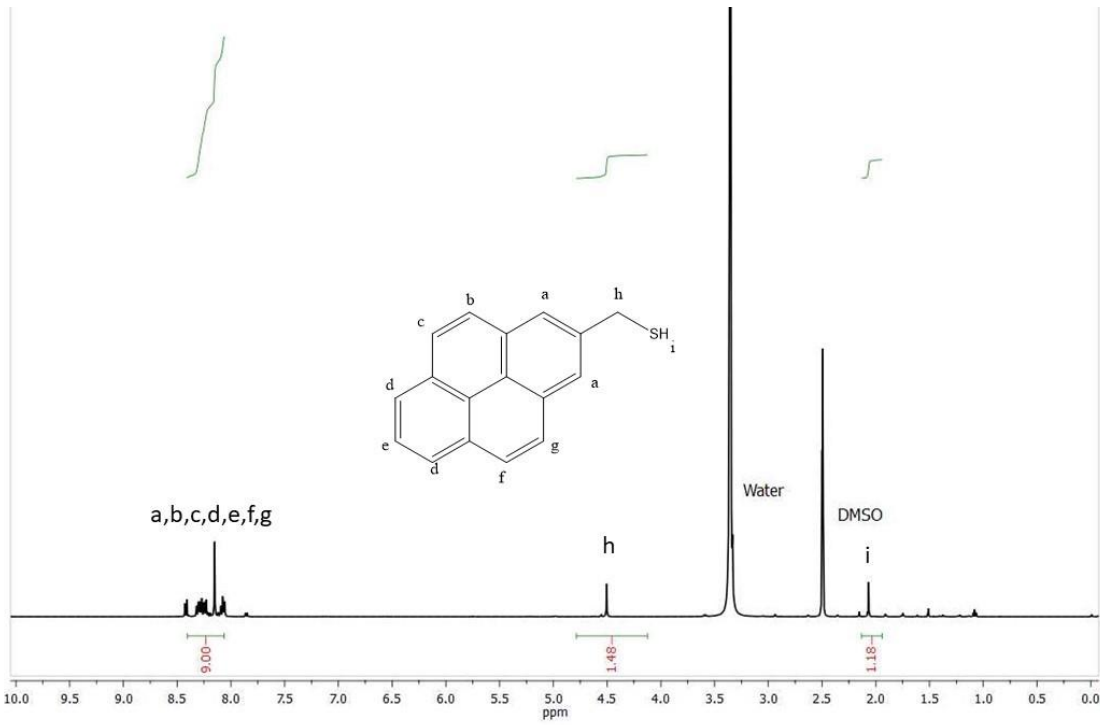


Figure 4.9 : ^{1}H NMR spectrum of Py-SH.

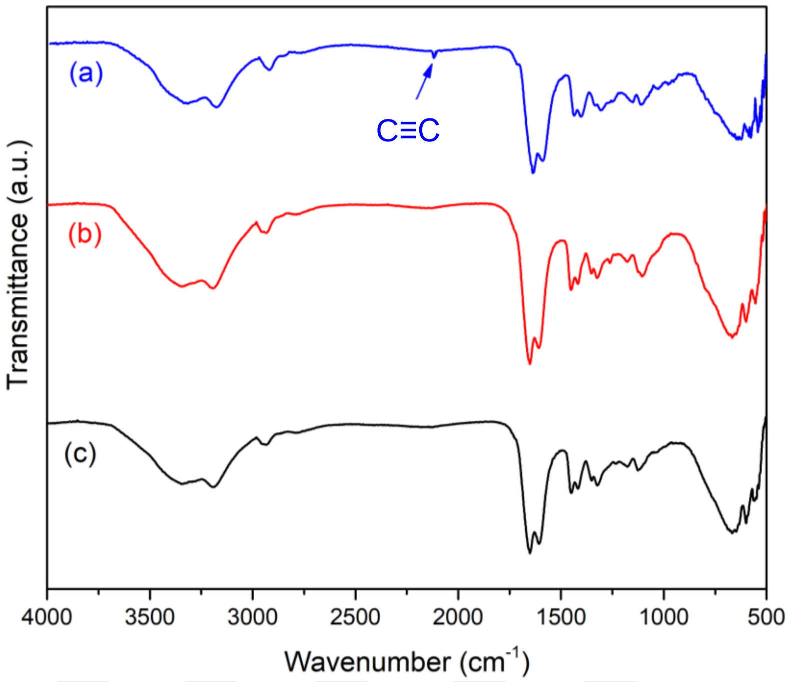


Figure 4.10 : FT-IR spectra of (a) hydrogel before click reactions, (b) after thiol-ene reaction with Py-SH (c) and after azide-alkyne click reaction with A-N_3.

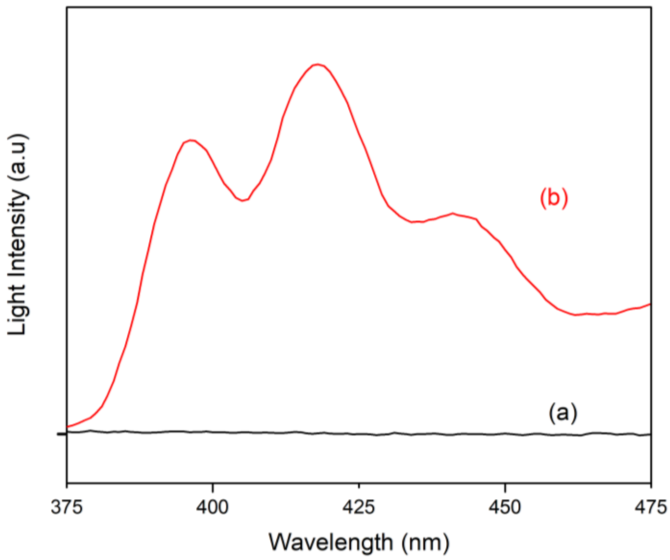


Figure 4.11 : Fluorescence emission spectra of hydrogels before (a) and after (b) click reactions with A-N_3.

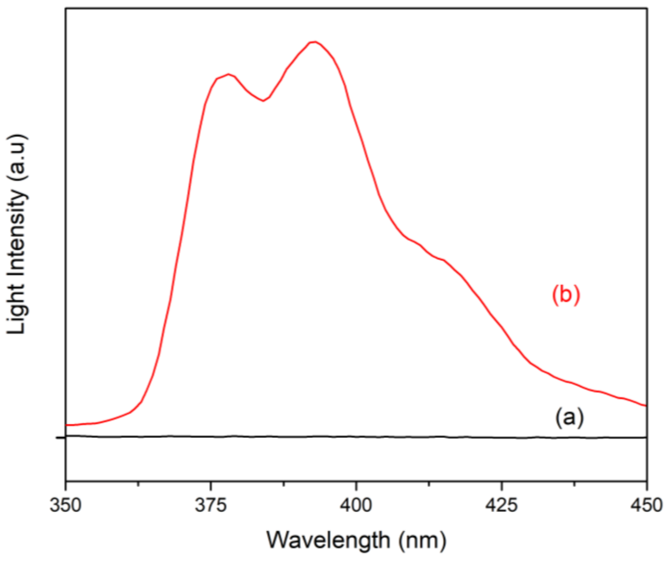


Figure 4.12 : Fluorescence emission spectra of hydrogels before (a) and after (b) click reactions with Py-SH.

Figure 4.13 represents the visual image of anthracene functionalized hydrogels under irradiation with a 366 nm UV lamp.

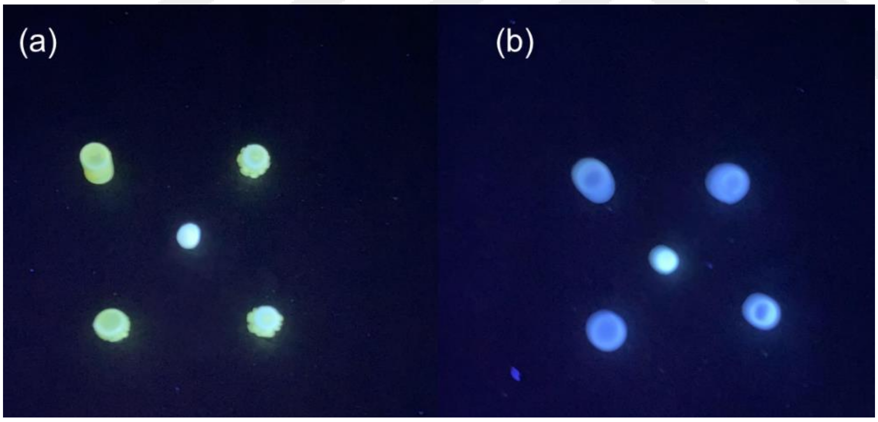
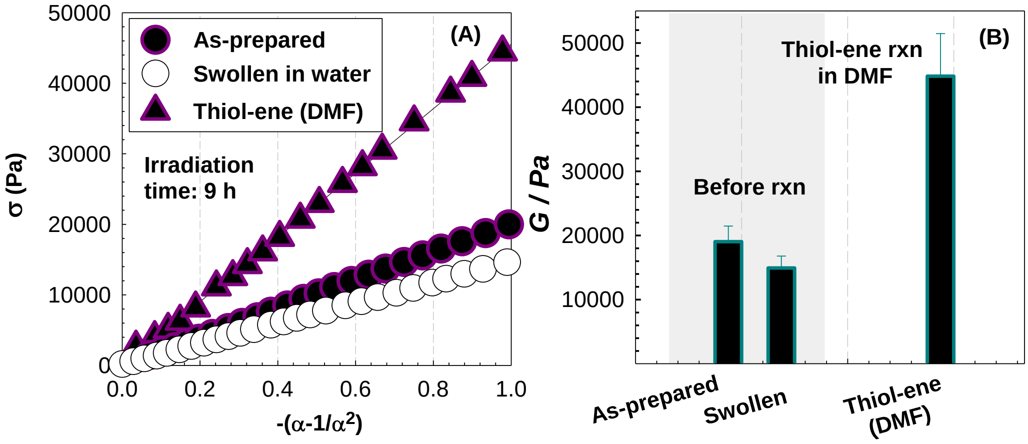


Figure 4.13 : Photo of the hydrogels after reaction with (a) A-N_3 (b) Py-SH.

In order to demonstrate the possibility to tune hydrogel properties by click post-modification process, thiol–ene reaction was conducted by using PAAm hydrogel prepared by 9 h irradiation and long alkyl chain thiol compound. For this purpose, pristine hydrogel at swelling equilibrium in water was placed in a solution of 1,6-hexanedithiol and photoinitiator in DMF, irradiated for 4 hours, and then subjected to mechanical testing. Figures 4.14A shows the stress - strain isotherms of the hydrogels before and after click reaction. Similar comparison of the compressive elastic moduli of hydrogels is presented in Figure 4.14B. Notably, the compression modulus of the initial gel is reduced from 19.0 kPa to 14.9 kPa after swelling in water. Interestingly, after the thiol-ene reaction, its elastic modulus increased to 44.8 kPa. The effective crosslink density ν_e, network chain length N_{exp} and molecular weight between cross-links M_C of the hydrogel before and after thiol-ene reaction were compared in Figure 4.14C. It is clearly seen that while the effective crosslink density ν_e increases, a significant decrease in the network chain length N_{exp} and the molecular weight between cross-links M_C is observed after the click reaction.



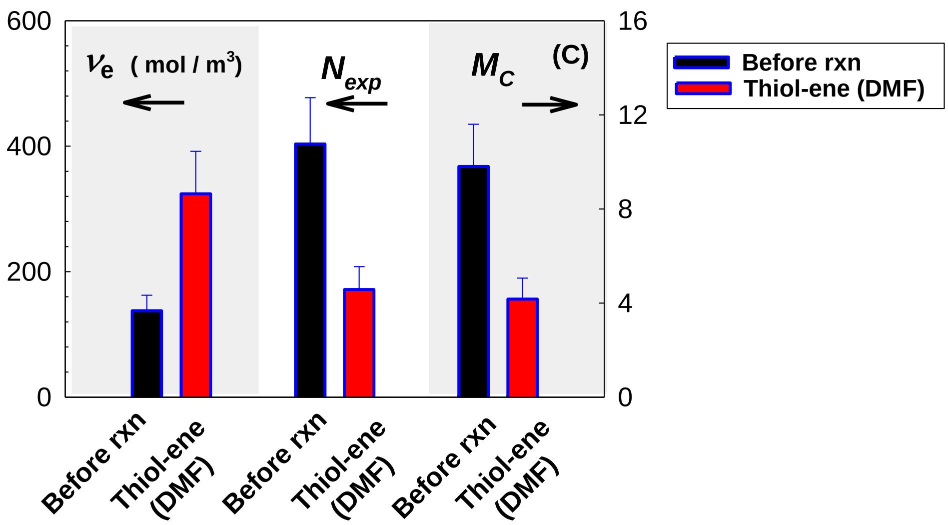


Figure 4.14 : (A) Stress - strain isotherms of PAAm hydrogels prepared with crosslinking time of 9 h after preparation (●), after equilibrium swelling in water (○) and after thiol-ene rxn in DMF for 4h (▲). (B) Comparison of compressive elastic moduli of PAAm hydrogels prepared with irradiation time of 9 h after preparation, after equilibrium swelling in water and after thiol-ene rxn in DMF for 4h. (C) Effective crosslink density ν_e, network chain length N_{exp} and molecular weight between cross-links M_C of PAAm sample before and after thiol-ene reaction.

5. CONCLUSION

In conclusion, a novel photopolymerization approach acting at UV and visible range in the absence of conventional crosslinker for the preparation clickable PAAm hydrogels is reported. The method pertains to the use of AAm and PA as monomers in the photopolymerization using cleavable photoinitiators. In the process, PA acts as both monomer and precursor for introducing click functionalities through the reactivity difference of the photochemically derived radicals towards double and triple bonds. The possibility of and thiol-ene, thiol-yne and CuAAC click reactions was confirmed by using suitable fluorescent click components. The effect of irradiation time and click modification on the gel fraction, swelling degree, equilibrium volume swelling ratio and elasticity were evaluated. The applied click reactions facilitate to tune swelling and mechanical properties of the gels. This approach to form hydrogels having abilities to be functionalized is broadly applicable particularly in bioapplications as biologically active molecules can be incorporated by either or both click reactions.

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OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS:

- **Bilgic M. B.**, Kaya K., and Yagci Y. 2022: A Versatile Cu(I) Metal-Organic Framework (MOF) as a Photoinitiator for Light-Induced Polymerizations and CuAAC Catalyst- 3rd European Young Chemists' Meeting January 19-21, 2022 Fribourg, Switzerland.
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