Uyarıya Duyarlı Mikrojel İçeren Amfoter Hidrojellerin Sentez ve Karakterizasyonu

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Synthesis and Characterizations of Amphoteric Hydrogels Containing Stimuli-responsive Microgels

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by

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ETHICAL STATEMENT

I hereby declare that this thesis study titled “Synthesis and characterizations of amphoteric hydrogels containing stimuli-responsive microgels” has been prepared in accordance with the writing rules of Eskişehir Osmangazi University Graduate School of Art and Science under academic consultancy of my supervisor Prof. Dr. Selma Yarlıgan Uysal and Prof. Dr. Vural Bütün. I hereby declare that the work presented in this thesis is original. I also declare that, I have respected scientific ethical principles and rules in all stages of my thesis study, all information and data presented in this thesis have been obtained within the scope of scientific and academic ethical principles and rules, all materials used in this thesis which are not original to this work have been fully cited and referenced, and all knowledge, documents and results have been presented in accordance with scientific ethical principles and rules. 07/07/2021

Robina Maihan

ÖZET

Hidrojeller ve mikrojeller, biyouyumluluk, suda çözünebilme ve toksik olmamaları gibi belirli özelliklere sahip olmaları nedeniyle polimerik ilaç salım sistemlerinde yaygın olarak kullanılmaktadır. Bu çalışmada, pH ve sıcaklığa duyarlı olan Au/P(NIPAM-*co*-DEA) mikrojelin ilavesi ile pH duyarlı amfoterik hidrojeller hazırlanmıştır. Hidrojellerin ve mikrojelin, sıcaklık ve pH duyarlılığı ile şişme ve büzülme davranışları incelenmiş, kimyasal karakterizasyonu yapılmış ve bu sistemde 5-fluorourasil (5-FU) ilacının salım özellikleri araştırılmıştır.

İlk olarak, bu çalışmada, DMA ve AA monomerlerinin miktarını değiştirerek, istenen şişme/büzülme davranışına sahip bir hidrojel üretmenin mümkün olduğunu gösterilmiştir. İkinci olarak, PDEA ve P(NIPAM-*co*-DEA) mikrojellerinin sentezi ve bu mikrojellerin DLS ve TEM yöntemleri ile karakterizasyonlarına yer verilmiştir. Üçüncü olarak, pH ve sıcaklık duyarlı olan P(NIPAM-*co*-DEA) mikrojelinde bulunan Au nanopartiküllerinin sentezi gerçekleştirilmiş ve partiküllerin varlığı TEM analizi ile kanıtlanmıştır.

Son olarak, anti-kanser ilacı olan 5-FU hidrojel sistemine yüklenmiş ve Au/P(NIPAM-*co*-DEA) mikrojel içeriğinin değiştirilmesiyle 5-FU ilacının bu sistemden salımı kontrol edilmiştir. Elde edilen sonuçlar, pH ve sıcaklığa duyarlı olan Au/P(NIPAM-*co*-DEA) mikrojel içeren P(DMA/AA) hidrojelinin ilaç sektöründe kanser tedavisinde ilaç dağıtım sistemleri olarak kullanılabileceğini ortaya koymaktadır.

Anahtar kelimeler: pH duyarlı hidrojel, mikrojel, hidrofobik ilaç dağıtımı, 5-FU, ilaç salımı.

SUMMARY

Hydrogels and microgels, which have certain characteristics, including biocompatibility, water-solubility, and non-toxicity, are currently commonly utilized as polymeric medication release systems. In this study, we present the preparation of amphoteric hydrogels with the addition of Au/P(NIPAM-*co*-DEA) pH and temperature sensitive. The hydrogels and microgel temperature and pH behavior, chemical characterization, swelling and deswelling behavior, and 5-fluorouracil (5-FU) release properties were investigated.

Firstly this research indicate that by varying amount of the DMA and AA monomers, it is possible to produce a hydrogel with a desired swelling/deswelling behavior. Secondly, the synthesis of PDEA and P(NIPAM-*co*-DEA) microgel and characterizations of these microgel by DLS and TEM methods. Thirdly, the synthesis of Au nanoparticles containing in P(NIPAM-*co*-DEA) pH and temperature responsiveness microgel and the result of these were proved by TEM analysis.

Last but not least, loading of anti-cancer drug to the hydrogel system and 5-FU release was controlled by the changing of Au/P(NIPAM-*co*-DEA) content. The results revealed that pH and temperature sensitive P(DMA/AA) hydrogels containing Au/P(NIPAM-*co*-DEA) microgel is applied as drug delivery systems in the pharmaceutical sector for cancer treatment.

Keywords: pH sensitive hydrogel, microgel, hydrophobic drug delivery, 5-FU, drug release

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LIST OF OBSERVATIONS AND SYMBOLS

Observations	**Descriptions**
AA	Acrylic acid
APS	Ammonium persulfate
CMC	Critical micelle concentration
DDS	Drug Delivery System
DEA	2-(diethyl amino)ethyl methacrylate
DMA	2-(dimethyl amino)ethyl methacrylate
DLS	Dynamic light scattering
M_n	The number average molecular weight
MBA	N, N'-methylene bis(acrylamide)
NIPAM	N-Isopropylacrylamide
pK_a	Acidity constant
PEGMA	Poly(ethylene glycol methacrylate)
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy

1. INTRODUCTION AND PURPOSE

In recent years, many researchers concerned about highly-absorbent polymer for producing modern utilization, likely conducting metals, biomaterials, sensors, discharge elements, and wave-sensing substances (Enas M. Ahmed et al., 2012). Additionally, modern progress in the development of neutral and ionic hydrogels for drug delivery purposes has focused on various features of their synthesis, characterization, and performance. Polymers with high molecular weights in sterically stable particles by building links can cause blocks but also cause positive effects. For instances, in purification processes, the addition of a polymer chain with a large molecular weight to the system causes impurities to groups and so it can make purification process harder. The gel can be considered as a three-dimensional material in the water or other solvents, while it is physically in the dried condition it is observed similar to solid material. When the appropriate solvent is added, the gel starts to swollen until the swelling reaches the equilibrium value (Harland and Prud‘homme, 1992).

Another impressive outlook of gels is that they perform like a single molecule. A single molecule is related to introduce to a large molecule in a macroscopic structure binding of monomer unit with each other and that makes up gel structure. These kinds of macroscopic structures act as a single polymer molecule (Dusek, 1993).

The hypothesis of this work is to produce a hybrid hydrogel system by combining an acidic monomer acrylic acid (AA), a basic monomer 2-(dimethylamino)ethyl methacrylate (DMA), and both temperature and pH responsive poly[(N-isopropyl acrylamide)-*co*-(2-(diethylamino)ethyl methacrylate)] P(NIPAM-*co*-DEA) microgels which contain both nanometals and drug molecules. The resulting combination could provide a new and effective drug delivery system with dual stimuli response.

The specific objectives of this study are to:

- **Prepare P(DMA/AA) hydrogel systems sensitive to pH.**
- **Synthesize PDEA and P(NIPAM-*co*-DEA) microgels having pH and temperature responsive nature.**
- **Locate gold nanoparticles (AuNPs) in P(NIPAM-*co*-DEA) microgels.**
- **Load 5-FU drug in AuNPs/P(NIPAM-*co*-DEA) microgel system.**
- **Add 5-FU-containing Au/P(NIPAM-*co*-DEA) microgels into the hydrogel system.**

2. LITERATURE REVIEW

Literature exists on hydrogel present that hydrogel is composed of homo or copolymers which is insoluble in water. The properties of hydrophilic polymer networks show that they can swell of their dry weight in water from 10-20% thousands of times. In addition, these polymeric hydrogels appear to perform swelling-shrinkage behavior by showing sensitivity to environmental parameters such as pH, temperature, magnetic field, and electric field. A summary of some of the studies in the literature regarding the monomers used in the thesis is presented below.

Hydrogels have been around since 1960, when Wichterle and Lim suggested using hydrophilic networks of poly(2-hydroxyethyl methacrylate) (PHEMA) in contact lenses (Wichterle and LiM., 1960).

A new composed of poly ampholyte hydrogel using AA and DMA was synthesized by Maolin Zhai and co-workers. Furthermore, P(DMA-*co*-AA) was produced by UV-induced copolymerization techniques and the swelling kinetics of hydrogel were investigated. The equilibrium swelling ratios of DMA and AA hydrogel changes on the variety of pH since DMA and AA monomer contain multiple proportions groups considering the protonation of the tertiary amine in DMA or the deprotonation of the carboxyl group in AA the swelling ratios of hydrogel in different pH media were studied.

The provision of that gel includes acidic pendant groups in temperature-stimuli gels as the pH of the system is raised the swelling of gels also increases. Correspondingly, the dramatic increase of pH caused the expansion of gel while having a weakly basic group in temperature-

sensitive gel system. Given such characteristics, the NIPAM and AA gels which have both temperature and pH responses were synthesis by M.K. Yoo and co-workers (2000) using free radical solution polymerization methods. Also, the effect of polyelectrolyte solute in the swelling of P(NIPAM-*co*-AA) hydrogel was studied which is used in drug delivery applications.

In a study employing AA and 2-hydroxyethylacrylate (HEA) monomers, Sanna and his colleagues used benzoyl peroxide and tetraethylenglycol diacrylate respectively as an initiator and cross-linker. Conversions were observed to be around 90% to 96%. In addition, the sensitivity of synthesized hydrogels to pH was studied by settling the balance values of different pH's (Sanna, et al., 2012).

In the past few years, researchers concerned more about removing and recovering organic impurity from wastewaters by using polymeric adsorbents. Karthika and colleagues synthesized gellan gum-infused-g-PDMA (GG-*g*-PDMA) hydrogel by free-radical polymerization in a water environment for elimination of methyl orange (MO) from aqueous solution with supreme adsorption using the microwave irradiation method. The production of GG-*g*-PDMA is done by using microwave radiation. The pH and temperature sensitivity of crosslinked graft copolymer has been investigated. Furthermore, the kinetics of the adsorption and efficiency for anionic dyes MO have been studied.

One of the essential and undeniable problems is the oil/water separation for the reason of oil spill events in marine transportation, sewage produces during refinery and sewage of oil industry. To solve this problem several methods have been promoted to compound the intelligent sensitive separation substances. Tingting Li and co-workers published smart dual environment responsiveness controllable separation materials by free radical polymerization technique. DMA and methacrylic acid (MAA) based hydrogel is prepared using MBA as cross linker and potassium persulfate ($K_2S_2O_8$) as initiator. The P(DMA-*co*-MAA) hydrogel is both temperature and pH stimulus and used for dual-controllable oil/water separation materials.

Nowadays, the usage of synthetic polymers as biomaterials has gained interest, however, the biocompatibility of natural biological materials is pretty good, and their mechanical strength is quite poor.

Over the past few years, tertiary amine monomers such as DMA and 2-(diethylamino)ethyl methacrylate (DEA) have been concerned due to their pH-sensitive also their block copolymers usually present attractive associative responses. The block copolymer of DMA and DEA micelles has been first reported by Bütün and co-workers in 1997. The related diblock copolymers were characterized by DLS, SANS, and fluorescence spectroscopy (Bütün, et al., 2001). The detailed studies were carried out systematically by Bütün et al by using DMA, DEA 2-(diioopropylamino)ethyl methacrylate (DPA) and 2-(N-morpholino)ethyl methacrylate (MEMA) monomers and their diblock copolymer micelles which were synthesized by group transfer polymerization (GTP) techniques. All of the PDMA-*b*-PDEA, PDMA-*b*-PDPA, PDMA-*b*-PMEMA block copolymers had surface-active character and formed core-shell micelles in water (Bütün et al. 1997, 1998, 2001).

The ethyl cellulose grafted poly(2-(dimethylamino)ethyl methacrylate) (EC-*g*-PDEA) copolymers that have hydrophobic and pH-responsive groups were produced by Deqian Wang and co-workers. The (EC-*g*-PDEA) copolymers were synthesized through the atom transfer radical polymerization (ATRP) method and the reaction was living and controllable. The characterization of (EC-*g*-PDEA) has been done by DLS, meanwhile and drug release was characterized by rifampicin (RIF).

Danial and co-workers have synthesized amphiphilic diblock copolymers of P(DEA-*co*-DMA)-*b*-PDMA with different amounts of DEA in the core block by the RAFT polymerization technique. Moreover, the pH studies of P(DEA-*co*-DMA)-*b*-PDMA have been investigated by light scattering measurements. The main purpose to produce the amphiphilic diblock

copolymers was to clarify the pH region and degree of aggregation since it is a pH-responsive copolymer.

There are many studies in the literature on microgels sensitive to environmental effects by emulsion polymerization. Amalvy and co-workers synthesized novel DEA microgel in the existence of bifunctional cross-linker by emulsion polymerization techniques while the pH of the system was set 8-9. This pH-responsive was characterized by DLS, proton HNMR studies (Amalvy et al. 2004). PDEA-based nanogel was also synthesized by Pikabea and coworkers for the purpose of the loading of anticancer drug doxorubicin which presents a pH-dependent in drug delivery systems.

B. Karabacak reported the preparation of the PDEA microgel emulsifier-free method in the existence of DMA (Karabacak, 2016). DMA is a cationic ammonium salt that functions as a comonomer and surfactants at the same time. Based on the amount of DEA, DMA, and water, type of copolymer the pH-sensitive performance of DEA microgel was examined in dilute aqueous solution.

Mixed micelles of methoxy capped polyethylene glycol-*block*-PDEA-*block*-poly(methyl methacrylate) MPEG-*b*-PDEA-*b*-PMMA with poly[poly(ethylene glycol)methyl ether methacrylate]-*block*-PDEA (PEGMA-*b*-PDEA) have been developed for the DOX delivery at the tumorous pH, which afforded excellent potential in the controlled deliverance of antitumor treatment (Feng 2017).

2-(Diethyl amino)ethyl methacrylate-*block*-poly(hydroxyl ethyleneglycol methacrylate) PDEA-*b*-PHEGMA amphiphilic block copolymers have been synthesized by (Christodoulakis et al. 2009) which is responsive to pH. For synthesizing the block copolymer-metal complex at least one block polymer is required which usually produces micelle core and can correlate with

metal composites. Meanwhile, in the dissolvent media second block polymer produce the coronate which presents great stability. The diblock copolymer has been synthesized by group transfer polymerization (GTP) under nitrogen gas applying standard schlenk methods, then the characterization of diblock copolymer has been done by DLS, TGA ^{1}H NMR spectroscopy.

In another study conducted by Bütün and his colleagues (2001), a series of homopolymers (M_w/M_n <1,5) of four different tertiary amine methacrylate monomers were reported by using GTP. In this study, 2-(dimethylamino) ethyl methacrylate (DMA), 2-(diethylamino)ethyl methacrylate (DEA), 2-(diiopropylamino)ethyl methacrylate (DPA) and 2-(N-morpholino)ethyl methacrylate (MEMA) were used as monomers. By changing the ratio of monomer and initiator the molecular weights of the polymers were varied. Both PDMA and PMEMA homopolymers have been found to dissolve in water at 20 ºC in both acidic and neutral media. Furthermore, at pH 8, these PDMA and PMEMA homopolymers were found to exhibit reversible solubility behavior at high temperatures depending on their molecular weight. PDEA and PDPA homopolymers were soluble at neutral and basic pH at 20 ºC, while in an acidic environment, they dissolve as cationic polyelectrolytes, as tertiary amine parts are protonated. Furthermore, the DMA monomers are copolymerized separately with the other three tertiary amine methacrylate comonomers. This diblock copolymer can be dissolved as unimers in aqueous media at 20 ºC but forms micelles and reverse micelles depending on the changing parameters such as pH, temperature, or electrolyte concentration (Bütün et al., 2001).

Studies in the literature show that a new type of microgel has been synthesized from acrylamide (AAm), 1-vinyl-2-pyrrolidone (NVP), and DEA by free radical precipitation polymerization applied in controlled drug release. As the model of drug 5- fluorouracil (5-FU) has been used which has high and faster drug release at pH 7.4 in contrast to both pH 5.5 and pH 2.1. As a result, the authors (Özbaş, et al., 2018) mentioned controlled drug release of 5-FU from P(AAm-*co*-NVP-*co*-DEA) processed nearly 10 h also the main parameter which can be affected on microgel drug delivery systems is the changes based on the pH of the system.

Zhang and co-workers (2005) synthesized, P(NIPAM-*co*-AA-*co*-HEA) microgels, and they used it as a pattern in the synthesis of magnetic and semiconductor nanoparticles. The AA units of the copolymer microgel were ionized in the basic region and then semiconductor nanoparticles were obtained by adding cations to the medium.

In hydrogel synthesis the NIPAM monomer is the most used monomer. In numerous publications in the literature has been investigated based on P(NIPAM) hydrogel. The production of P(NIPAM) hydrogel and its impact on the swelling-shrinkage conduct were examined by Biswas and his colleagues. In this study, PNIPAM hydrogels were synthesized at different rates in ethanol/H_2O environment. The temperature rise impact of gels was first discovered at low critical solution temperature (LCST) and the swelling properties of PNIPAM gels were found to decrease with an increase in temperature. Secondly, it has been observed that the pores of the gel structure are tiny with ethanol reduction and that the swelling property reduces when the quantity of ethanol in the gels tested at 20 ^{o}C in proportion to the contraction. The decrease in swelling is explained by the separation of free water molecules between P(NIPAM) chains from the gel structure (Biswas, et al., 2011).

Chaikara and colleagues synthesized 2-(dimethyl acrylamide) (DMAAm), AAm and itaconic acid (IA) - based ionic hydrogels at four different charge densities. In buffer solutions with varied pH values, the hydrogel swelling profiles produced by the free radical polymerization method using MBA cross-linker and (APS) initiator (Çankaya ve Akçakaya, 2006).

5-FU is the medicine used since 1957 for many forms of cancer, including colorectal and breast cancer. The medication is a commonly used medicine for anti-metabolized diseases. Several studies are now being conducted to enhance the sensitivity of 5-FU in cancer cells and its remedial efficacy through the use of new combination treatments, encapsulated medicines, and so on. In some cancers, combining 5-FU with other treatment agents improves medication response rates.

Drug release systems were studied and hydrogels were shown to be environmentally sensitive in a research done by Gutowska and his colleagues in literature investigations of acrylic hydrogels. In this study, P(NIPAM/AA), and poly(N-isopropylacrylamide/butylmethacrylate/2-(diethylamino)ethylmethacrylate, (NIPAM/BMA/DEA) hydrogels were synthesized. Temperature, pH, and temperature/pH variables were also used to examine the swelling-shrinkage behavior of gels. The P(NIPAM/AA) gel had a temperature-sensitive release profile between 35 and 40 ^{o}C, but the P(NIPAM/BMA/DEA) gel's release profile at 37 ^{o}C was heavily influenced by the pH changes. At pH 2, the P(NIPAM/BMA/DEA) gel showed delayed release, whereas at pH 7.4 , it showed controlled release. The researchers discovered that medication release from hydrogels is influenced by the gel's composition as well as environmental stimuli (Gutowska, et al., 1997).

Yuan and coworkers studied thermosensitive PLGA–PEG–PLGA hydrogels filled with 5-FU. This hydrogel system was utilized as an injectable physical barrier to limit tissue adhesion during tendon treatment as well as to reduce fibroblast growth. The 5-FU-loaded hydrogels demonstrated a sol–gel–precipitation phase change as temperature increased, with the hydrogels exhibiting the highest storage properties at physiological temperature. The hydrogel's continuous release of 5-FU lasted 7 days. The hydrogels deteriorated after 4 weeks after being injected into the subcutaneous layer of rats and demonstrated satisfactory in vivo biocompatibility. This study showed that therapy with 5-fuororacil-loaded PLGA–PEG–PLGA hydrogels might be an effective method for avoiding postoperative tendon adhesion (Yuan et al., 2105).

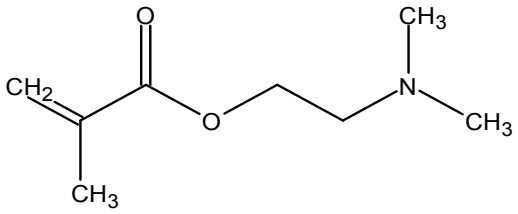
3. MATERIALS AND METHODS

3.1. Materials

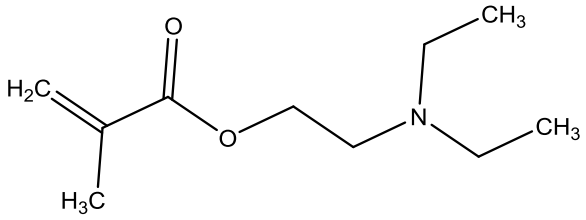
Monomers are stored in the presence of inhibitors due to their easy polymerization properties in the case of heat, light, or metal exposure. To remove the hydroquinone methyl ether inhibitor contained in the 2-(dimethyl amino)ethyl methacrylate (DMA) and 2-(diethylamino)ethyl methacrylate (DEA) monomers are used in the reaction, basic alumina (Merck) was passed through the colon and cleared of inhibitors and impurities. Monomers are stored in the freezer at -18 °C.

Table 3.1. Chemical materials properties of monomers

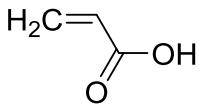
	DMA	AA	NIPAM	DEA
Molecular Formula	$C_7H_{13}O_2$	$C_3H_4O_2$	$C_6H_{11}NO$	$C_{10}H_{19}NO_2$
Molecular Weight	157.21 g·/mol	72.063 g/mol	113.16g/mol	185.26 g/mol
Density	0.93 g/ml at 20 °C	1.051 g/mL	1.1 g/cm^3	0.92 g/ml at 20 °C



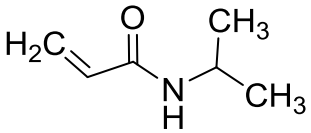
2-(dimethyl amino) ethyl methacrylate (DMA)



2-(diethyl amino) ethyl methacrylate (DEA)



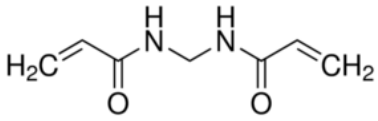
Acrylic acid (AA)



N-isopropyl acrylamide (NIPAM)

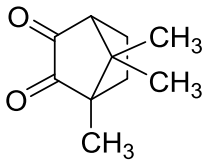
Solvent: Double distilled w ater was used as a solvent in some hydrogel synthesis. Furthermore, the mixture of ethyl alcohol (Merck) and double distilled water ($EtOH/H_2O$) has been used as the solvent.

Cross-linker: N,N'-methylene bis(acrylamide) was used as cross-linker in hydrogel synthesis.

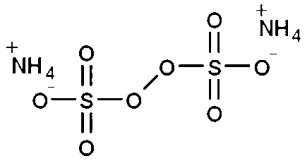


N, N'-methylene bis(acrylamide) (MBA)

Initiator: Ammonium persulfate (APS) (Merck) was used as initiator in hydrogel synthesis and for microgel synthesis DL- Camphor Quinone has been used as initiator.



DL-Camphor Quinone



Ammonium persulfate (APS)

3.2. Methods

Hydrogels are typically produced from hydrophilic monomers, however in rare situations, hydrophobic monomers are employed to improve the properties of hydrogels for wide variety of applications. The hydrogel elastic formation formed by the interconnection of hydrophilic polymer chains. For hydrogel preparation, chemical and physical cross-linkers are employed. To produce high-grade mechanical strength and durable hydrogels, chemical cross-linking of polymers can be accomplished using a variety of polymerization techniques, including free radical, condensation, UV radiation, and small molecule cross-linking methods. (Sennakesavan et al. 2020). Solution polymerization, suspension polymerization, free radical polymerization, and radiation polymerization are all methods of producing hydrogels.

3.2.1. Free radical polymerization

Free-radical polymerization is the conventional technique of all forms of polymerization. Several operators can control or alter the pace of cross-linking in this process, including temperature, cross-linker concentration, initiator and catalyst model. The production of nanocomposite hydrogels based on chain-growth polymerization is improved by radical polymerization. The polymer is created with this approach by the frequent addition of free radical groups, which act as an initiator in the process. (Akhtar,et al., 2016).

The four chemical stages described below are followed by free radical polymerization:

(a) Initiation: The formation of active monomeric centers (initiation) is the first stage in the process (in some sources, the decomposition reaction of the initiator is also evaluated within this step).

(b) Growth: The process through which monomers interact with active centers (by preserving the active center)

(c) Termination: The step that loses the function of the active center

(d) Chain transfer: The stage where the active center is transferred to a different molecule.

As initiators, we used ammonium persulfate (APS). Free radicals that are produced from the initiator attack the double bonds existing in the monomers of polymers like AA or DMA. Furthermore, these radicals attack the double bond of cross-linking agent (MBA), and consequently, double bonds of monomers and cross-linker will be executed. These monomers connect commonly by covalent force and build a longer polymer chain.

3.2.2. Synthesis of microgel

Two techniques have been provided in the literature for the synthesis of non-aquatic microgels. The initial technique was to synthesize microgels in the aqueous environment by emulsion polymerization. At the end of the process, the tiny particles have been transformed into an organic habitat. Chemical solvent washing. The preparation form of the final microgel might be freeze-drying, rotary evaporation. In theory, this technique might be utilized for nearly all form of microgel particles.

The second technique to create microgel particles in non-water solutions is to perform the direct reaction through polymerization or precipitation polymerization in non-aqueous solutions. Despite this, producing the particles directly in non-aqueous solutions has some challenges since it is impossible to predict whether the particles would swell or de-swell during synthesis in the non-aqueous solvent, and the outcome is not always well-defined spherical particles (Bonham, Faers, and Van Duijneveldt 2014).

Three major systems for preparing microgel can be considered:

1) Usage of the Monomer techniques: Anionic, cationic, or non-ionic monomers are some of the most frequent techniques that may be used in this system. Furthermore,

cross-linkers may be made from monomers with two-function groups, and radical polymerization can be utilized to polymerize systems based on their composition.

2) Using polymer techniques: Chemical crosslinking of aqueous polymer solutions or the formation of emulsions in oil medium could be used to create this system.
3) Using microgels to synthesis other microgels: includes mechanically grinding a microgel to produce microgels.

3.2.3. Emulsion polymerization

Various polymerization processes exist, such as dispersion, emulsion, suspension, atomic transfer, group transfer, and free radical polymerization. Emulsion polymerization is a new type of free radical polymerization that produces polymer chains at micro/nanoparticle sizes. The quantity and size of particles in an emulsion polymerization are both dependent on stabilizer variables, particularly the stability of interfacial layers. By using emulsion polymerization, we may regulate the polymerization rate, for example, the increase in molecular weight of the generated latexes. The degree of polymerization can be adjusted by lowering the initiator concentration or the reaction temperature. There are two types of polymerization systems in emulsion polymerization:

a) Conventional emulsion polymerization: The emulsification of a hydrophobic monomer in water happened in this system (oil-in-water), and then polymerization will initiate by a water-soluble initiator.
b) Inverse emulsion polymerization, in this system (water-in-oil), the hydrophobic monomer could be emulsified in too much low polarity environment (e.g. paraffin or xylene media) and then the polymerization process gets completed by a hydrophobic initiator.

One of the advantages of emulsion polymerization is comfortable to produce non-aqueous microgels. However, after the reaction, this technique needs removing of surfactant, preparation of core-shell morphologies, and occurring of non-uniform cross-linking. For an emulsion system, required one or more than one monomer, surfactant or stabilizers, cross-linkers, and initiators.

Monomer: In the process of emulsion polymerization, for instance acrylamide, acrylic acid, diethylaminoethyl methacrylates, styrene, acrylonitrile, 2-diethylaminoethyl methacrylate etc. are generally free-radically polymerized monomers. These common monomers might be categorized according to their solubility in the water phase:

1) High solubilization monomer like diethylaminoethyl methacrylate

2) Medium solubilization monomer such as methyl methacrylate

3) Insoluble monomers in the aqueous phase for instance styrene

Polymers produced by the emulsion process could be utilized in a variety of application namely, plastic pigments, rheological transformers, films, coating, thermoplastics materials, synthetic rubbers, adhesives, and binders.

In the dispersion medium, microgel particles, the collision movements (Brownian motions) between the particle and the molecule get occurred. Which affects the facilitates particle stabilization (Myers, et al., 1999). Besides that, too swollen suspensions microgels can pass more light in contrast to less swollen microgels. Extremely swollen suspension microglia has a close appearance to the counter, while a less swollen microgel suspension has a structure that looks like milk. In common, microgels are more stable due to the decreased effect of Van der Walls and steric restrictions of groups on the particle surface. Chemical properties of the structure can be used to classify the surfactants which are used in particle synthesis.

P(DMA/AA) hydrogels were synthesized separately with AA, DMA monomers using ammonium persulfate (APS) free radical initiator and N, N'-methylene bis(acrylamide) (MBA) cross-linker by free radical polymerization method. PDEA microgel were synthesized using DL- Camphorquinone photoinitiator $PDMA_{68}$-$PDEA_{29}$ diblock copolymer stabilizer, (B4, M_n= 25000 g/mol and P.D.I= 1,86) and MBA cross-linker by emulsion polymerization method. Emulsion polymerization was also used to synthesis the P(NIPAM-*co*-DEA) microgels. P(DMA/AA) hydrogels were synthesized in the presence of P(NIPAM-*co*-DEA) microgel, APS free radical initiator and MBA cross-linker by free radical polymerization method.

3.2.4. $P(DMA_{0.46}/AA_{0.54})$ Hydrogel Preparation

DMA and AA monomers with different ratio were used in preparation of P(DMA/AA) hydrogel by using different amounts of (MBA cross-linker). For the synthesis of $P(DMA_{0.46}/AA_{0.54})$ hydrogel system, first, 0.95 ml of AA and 2 ml of DMA monomers were mixed in the appropriate volume glass bottle, in the second step 1.5 ml of distilled H_2O and MBA (for RM81, RM87, and RM93 the amount of MBA respectively: 10, 5 and 1 mg) were dissolved and added to the monomers. Finally, 0.018 g of APS, 1% moles of DMA monomer, was dissolved in 0.5 ml of distilled water for each of the experiments and added to the prepared mixture.

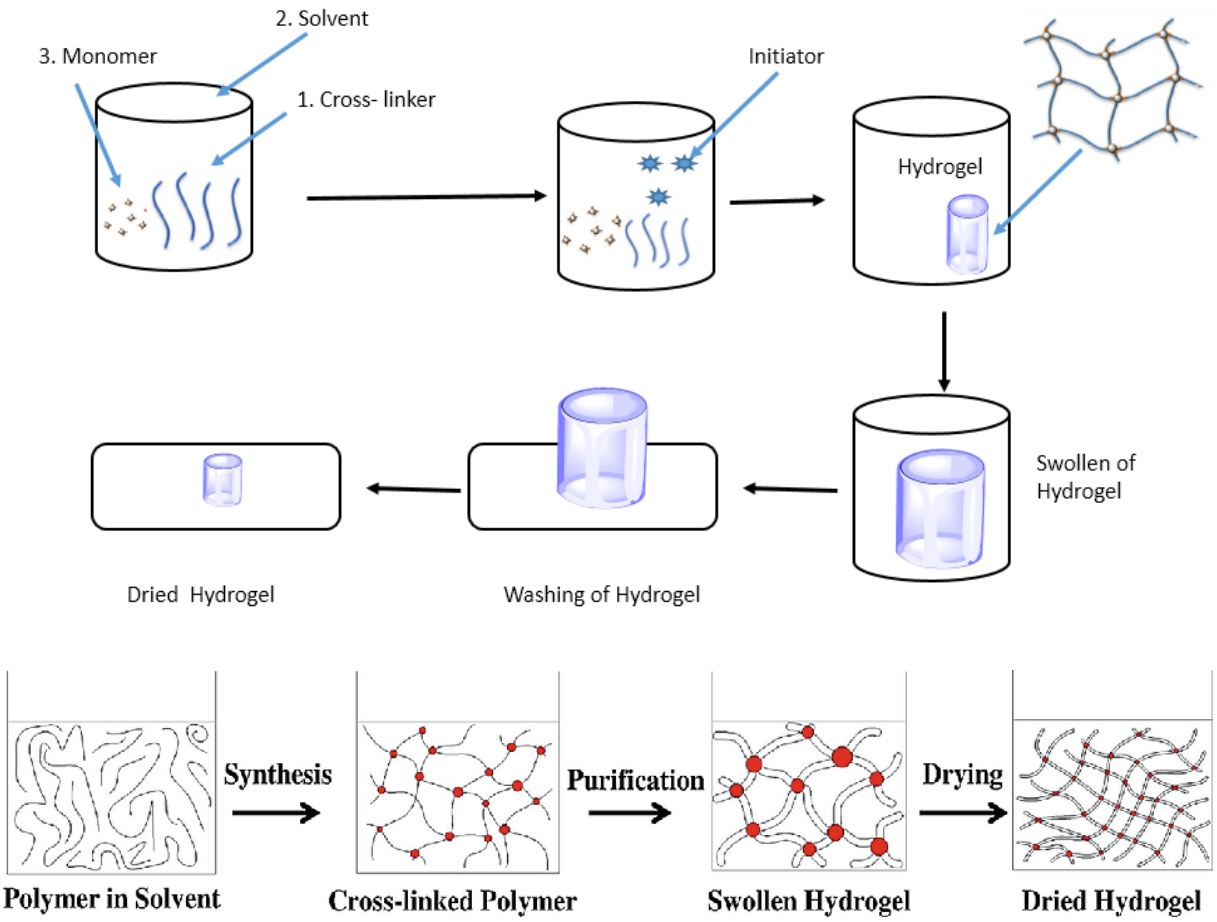


Figure 3. 1. Hydrogel production, swollen, washing, and drying process

The mixed mixture was taken into plastic tubes with one end closed after putting the mixture inside the tubes the second end of the tubes was closed also. The prepared mixture inside the tubes was left in a water bath at 60 °C for about 15 minutes for complete gelling. When gelling was observed, the tubes were taken from the water bath and the gels were removed from the tubes. The hydrogel production, swollen, washing, and drying process shown in Figure 3.1. Moreover, 1.5 ml of ethanol were added after mixing of monomer and other steps were carried out as the same for the synthesis of P($DMA_{0.46}$/$AA_{0.54}$) in water environment (see Table 3.2).

Table 3.2. Preparation of P(DMA/AA) hydrogels in water-ethanol environment at 60 °C, 18 mg APS.

Experiment Code	MBA (mg)	Ethanol (mL)
RM83 P($DMA_{0.75}$/$AA_{0.25}$) RM81 P($DMA_{0.46}$/$AA_{0.54}$) RM85 P($DMA_{0.25}$/$AA_{0.75}$)	10	-
RM84 P($DMA_{0.75}$/$AA_{0.25}$) RM82 P($DMA_{0.46}$/$AA_{0.54}$) RM86 P($DMA_{0.25}$/$AA_{0.75}$)	10	1.5
RM89 P($DMA_{0.75}$/$AA_{0.25}$) RM87 P($DMA_{0.46}$/$AA_{0.54}$) RM91 P($DMA_{0.25}$/$AA_{0.75}$)	5	-
RM90 P($DMA_{0.75}$/$AA_{0.25}$) RM88 P($DMA_{0.46}$/$AA_{0.54}$) RM92 P($DMA_{0.25}$/$AA_{0.75}$)	5	1.5
RM95 P($DMA_{0.75}$/$AA_{0.25}$) RM93 P($DMA_{0.46}$/$AA_{0.54}$) RM97 P($DMA_{0.25}$/$AA_{0.75}$)	1	-
RM96 P($DMA_{0.75}$/$AA_{0.25}$) RM94 P($DMA_{0.46}$/$AA_{0.54}$) RM98 P($DMA_{0.25}$/$AA_{0.75}$)	1	1.5

The hydrogels were washed with double-distilled water for 3 days from unreacted chemicals such as (monomer, cross-linker, etc.). At the end of the third day, some of the gels were dried either at 40 °C in oven or freeze dryer and some were stored in a swollen shape gel. The same method was used in all hydrogel synthesis.

3.2.5. P($DMA_{0.75}/AA_{0.25}$) Hydrogel Preparation

Different amounts of cross-linker (MBA) have been used in order to prepare P($DMA_{0.75}/AA_{0.25}$) hydrogels. First, 0.26 ml of AA and (2 ml or 0.0118 mol) DMA monomers were mixed, then MBA (for RM83, RM89, and RM95 the amount of MBA respectively: 10 mg, 5 mg and 1 mg) were dissolved in 1.5 mL water and added to the monomers. Finally, 0.09 g of APS, based on 1 mol % of DMA monomer, was dissolved in 2.5ml of distilled water for each of the experiments 0.5 ml added to start the gelling process. The mixtures were left in a water bath at a temperature of 60 °C for 15 minutes. For the synthesis of P($DMA_{0.75}/AA_{0.25}$) hydrogels by the addition of ethanol 1.5 ml of ethanol were added after mixing of monomer and other steps were done the same with the synthesis of P($DMA_{0.75}/AA_{0.25}$) hydrogel in water environment.

In order to prepare other P($DMA_{0.46}/AA_{0.54}$) and P($DMA_{0.25}/AA_{0.75}$) hydrogels, with different amounts of cross-linker (MBA) and comonomer ratio the same pathway was followed. The amount of MBA and ethanol in hydrogels were given in Table 3.2.

3.2.6. Synthesis of PDEA microgel (RM-Mi-9)

Synthesis of PDEA microgel via emulsion polymerization were carried out in a 100 ml round bottomed-flask, fitted with N_2 gas inlet and a magnetic stirrer operation at 500 rpm. 50 mg of β-$PDMA_{68}$-*b*-$PDEA_{29}$ diblock copolymer stabilizer (B4, M_n = 25000 g/mol and PDI= 1.86) was dissolved in 45 ml of deionized water and stirred under N_2 gas for 30 minutes then 1.25 ml of DEA was added to the solution and stirred approximately for 20 minutes. In a 10 ml empty bottle, 0.01 g of MBA (1 mol % of DEA monomer) was dissolved in 5 ml of deionized water and added to the round bottomed-flask which was kept mixing for 10 minutes. 10 mg of DL-camphor quinone were dissolved in 5 ml of ethanol and added to the reaction solution. This

process is illustrated in the Figure 3.3. In addition, the reaction started under the light for 3 hours and polymerization process got completed.

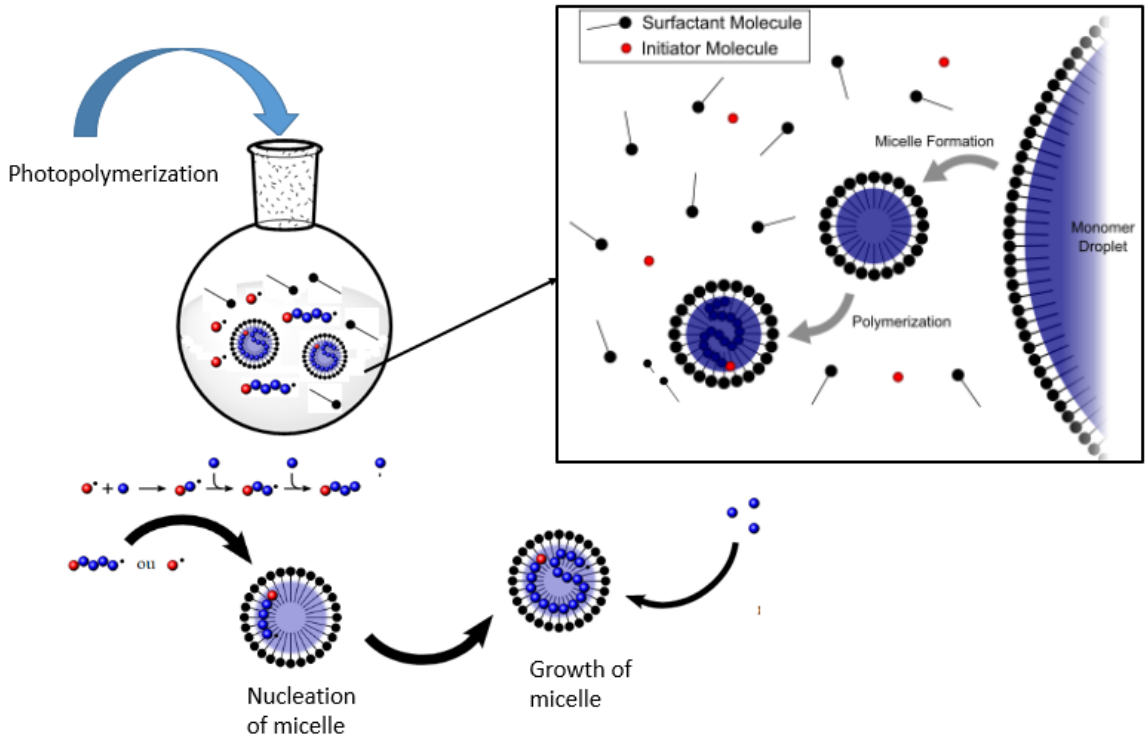


Figure 3.2. Photo emulsion polymerization of PDEA microgel

3.2.7. Synthesis of PDEA microgel (RM-Mi-30) by emulsion polymerization

Polymerization was carried out in a 100 ml round bottomed-flask, fitted with N gas inlet and a magnetic stirrer operation at 500 rpm. 0.025 g of β-$PDMA_{64}$-$PDEA_{36}$ diblock copolymer stabilizer (VB 104, M_n= 25000 g/mol and PDI= 1.86) was dissolved in 60 ml of deionized water and stirred under N_2 gas for 30 minutes then 1,25 ml of DEA was added to the solution and stirred approximately for 20 minutes. In a 10ml empty glass bottle, 10 mg (MBA, 1 mol % of DEA monomer) was dissolved in 5 ml of deionized water and added to the round bottomed-flask which was kept mixing for 10 minutes. 10 mg of APS was dissolved in 5 ml of deionized

water and added to the reaction solution. The reaction occurred in oil bath at 60 ^{o}C and polymerization process completed after 3 hours.

3.2.8. Synthesis of P(NIPAM-*co*-DEA) Microgels

The P(NIPAM-*co*-DEA) microgels were synthesized using surfactant-free emulsion polymerization in a 100 ml round-bottomed balloon, fitted with N_2 gas inlet and a magnetic stirrer operation at 500 rpm. The weight percentage of MBA and APS was 1% of monomers.

Firstly NIPAM (0.03 g or 0.26 mmol), DMA (1.15 g or 6.2 mmol), and 0.01 g of MBA were added to a total volume of 65 mL distilled water. This mixture was then purged with nitrogen gas for 30 min to remove oxygen and homogenized at 500 rpm for 10 min. After homogenization, the polymerization was thermally initiated by placing the reaction flask in a 70 °C oil bath and the instant addition of the APS, which was earlier dissolved in 5 mL of distilled water. After adding the initiator, the mixture became immediately mixed and the polymerization reaction stayed stirred at 500 rpm, for 3 hours.

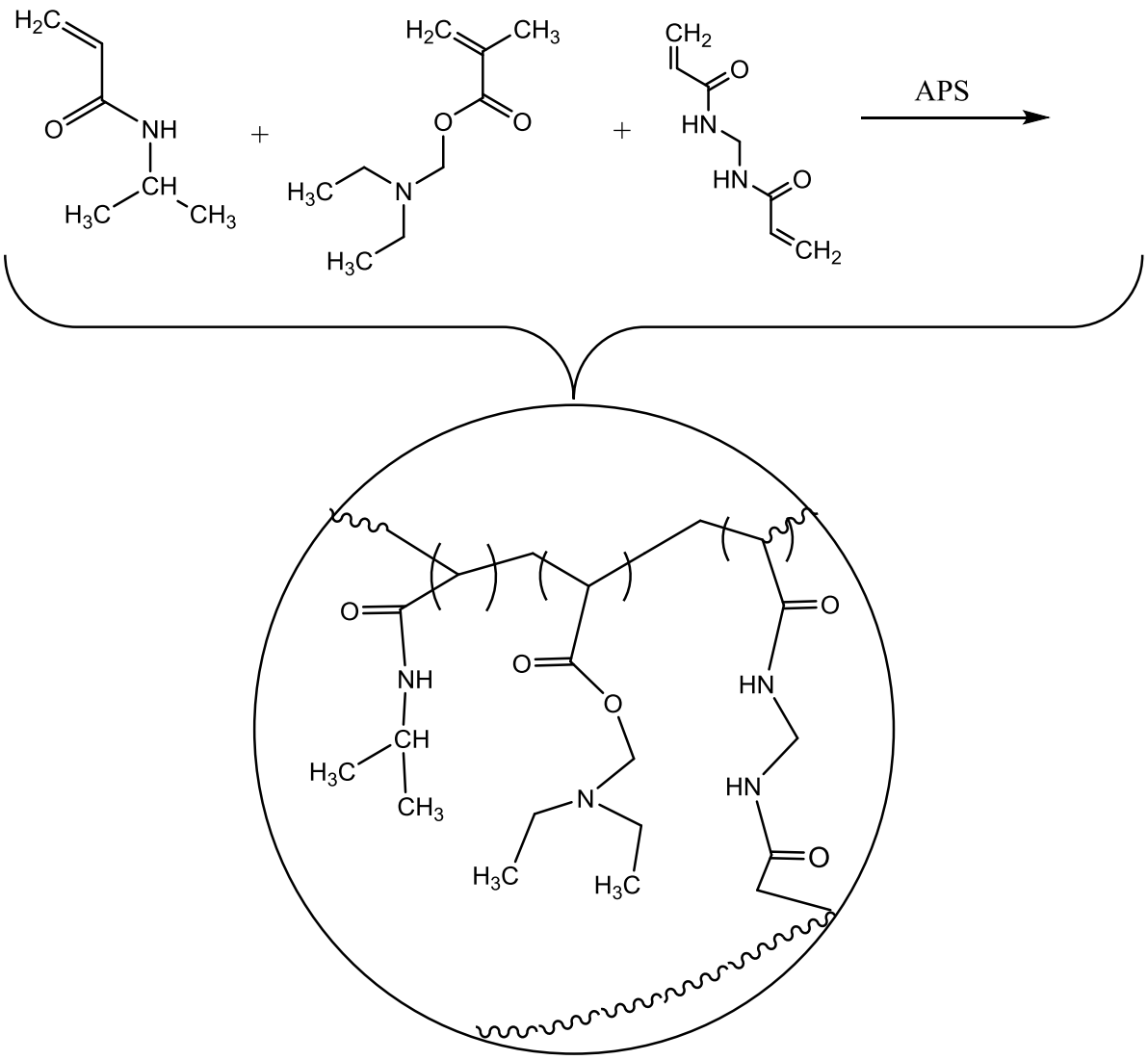


Figure 3.3. Mechanism of P(NIPAM-*co*- DEA) microgel formation

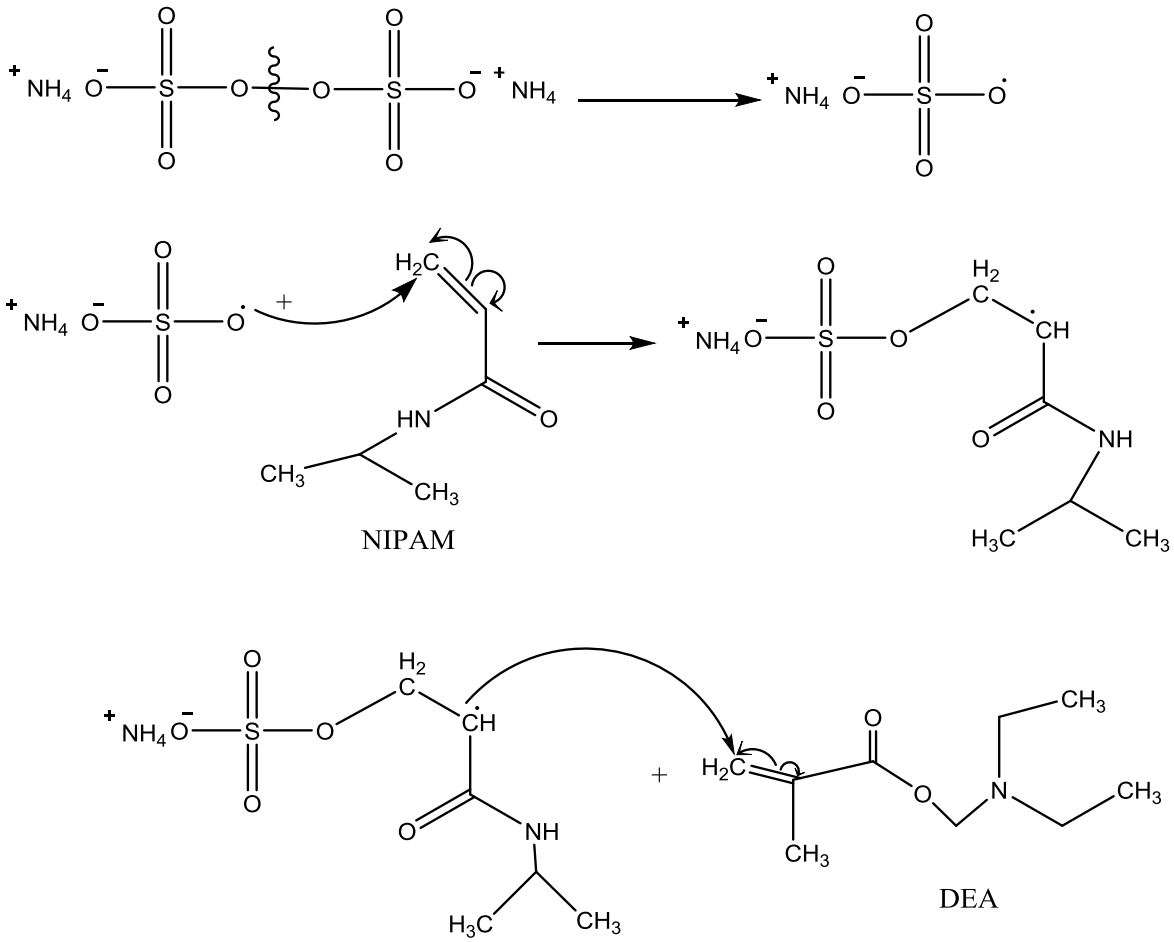


Figure 3.4. The reaction mechanism for P(NIPAM-*co*-DEA) microgel formation

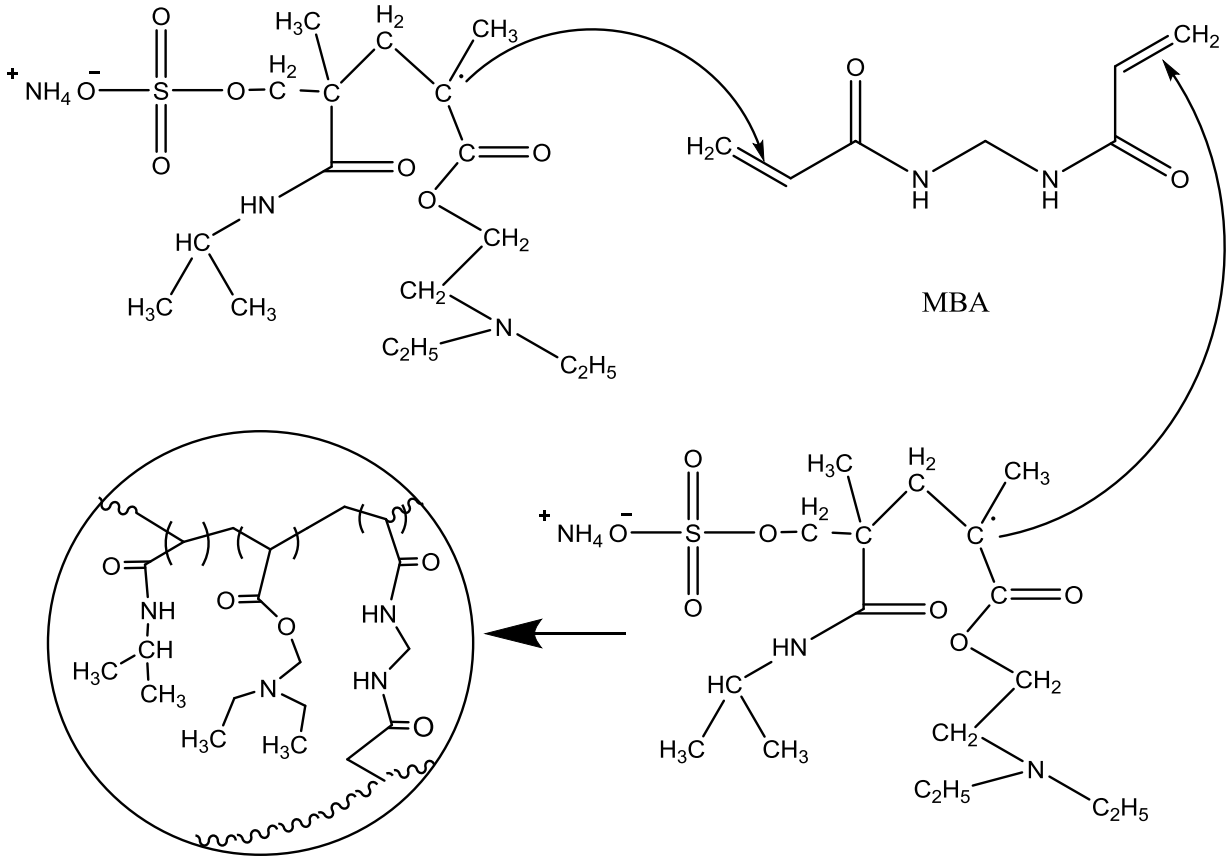


Figure 3.4. The reaction mechanism for P(NIPAM-*co*-DEA) microgel formation (continue)

3.2.9. Preparation of PDEA microgel located P(DMA/AA) hydrogel

P($DMA_{0.46}$/$AA_{0.54}$) hydrogels containing PDEA and P(NIPAM-*co*-DEA) microgels were prepared using APS initiator and MBA cross-linker by free radical polymerization in water/ethanol environment (see Table 3.3). First of all, MBA was dissolved in ethanol-water, secondly, the DMA and AA were added to the MBA solution, thirdly microgel dispersion was transferred before addition of 0.5 ml APS/water solution. The pH of the final mixture was measured around 6.5. The mixture inside the tubes was left in a hot water bath at 60 °C for about 15 minutes to complete gelling. When gelling was observed, the tubes were taken from

the water bath and the gels were removed from the tubes. This procedure is illustrated in Figure 3.5.

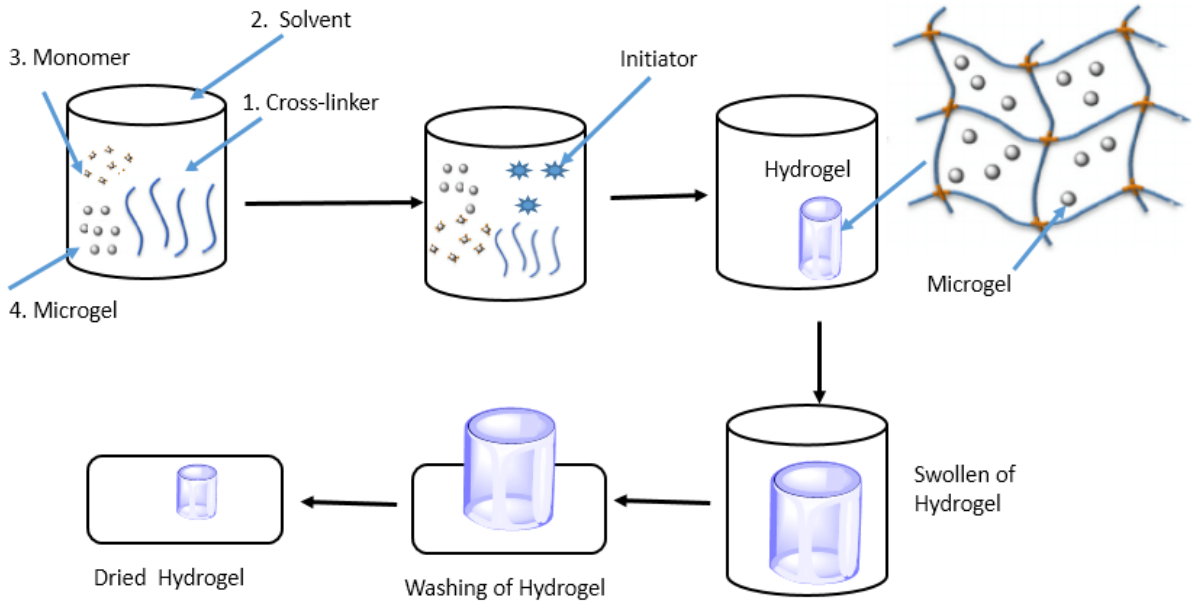


Figure 3.5. Preparation process of microgel embedded hydrogel

Table 3.3. PDEA and P(NIPAM-*co*-DEA) microgel located to P($DMA_{0.46}$/$AA_{0.54}$) hydrogels (1% APS of DMA monomer, 1.5 ml of H_2O).

Experiment Code	MBA (%mol)[a]	Microgel (ml)[b]
RM77 PDEA (RM-Mi-9) RM112 P(NIPAM-*co*-DEA) (RM-Mi-24) RM108 PDEA (RM-Mi-30)	1	1
RM78 PDEA (RM-Mi-9) RM110 P(NIPAM-*co*-DEA) (RM-Mi-24) RM107 PDEA (RM-Mi-30)	0.5	
RM79 PDEA (RM-Mi-9) RM109 P(NIPAM-*co*-DEA) (RM-Mi-24) RM106 PDEA (RM-Mi-30)	0.2	2

[a] (MBA) used % mol basis of DMA monomer

[b] The microgels embedded to hydrogel directly without any washing process

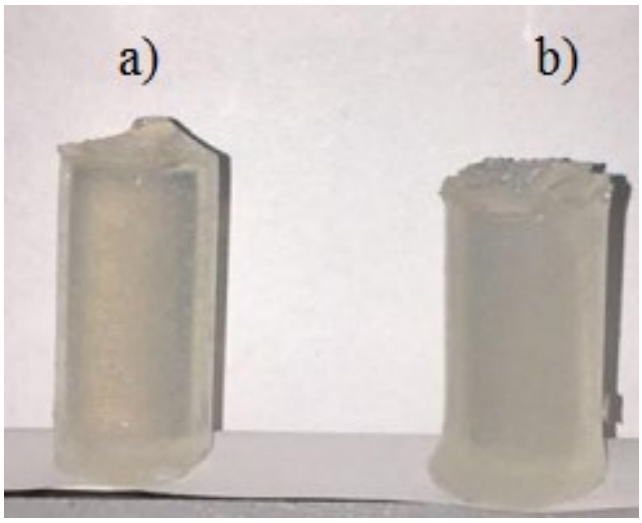


Figure 3.6. P($DMA_{0.46}$/$AA_{0.54}$) containing P(NIPAM-*co*-DEA) microgel, a) RM110 b) RM 112

3.2.10. Synthesis of Au nanoparticle in P(NIPAM-*co*-DEA) microgel

6 ml of P(NIPAM-co-DEA) (RM-Mi-24) microgel was centrifuged at 15000 rpm for 15 minutes. After that, the microgel was separated from the water. The separated microgel was dispersed in 1 ml of $HAuCl_4.3H_2O$ (0.0001 M) (R-AuNPs-3), and stirred using a magnetic stirrer for two hours at 500 rpm. Finally, the reduction agent with the Au: $NaBH_4$ mole ratio of (10:1) was added to the solution.

In synthesis of AuNPs, after loading and reduction the dispersions were separated by a centrifuge. This method is preferred to provide a controlled nanoparticle within the microgel, so it proposed that metal salts should be inside the microgel, not on the surface. The changing of color occurs after reduction with the addition of $NaBH_4$. In AuNPs/ P(NIPAM-*co*-DEA) microgel synthesis, different amounts of $HAuCl_4.3H_2O$ (Table 3.4) were added to 0.1% P(NIPAM-*co*-DEA) microgel solution, and the color change was realized after reduction. The loading of AuNPs to P(NIPAM-*co*-DEA) was done at room temperature and Au locating in P(NIPAM-*co*-DEA) microgel is illustrated in Figure 3.7, also the amount of Au addition is shown in Table 3. 1.

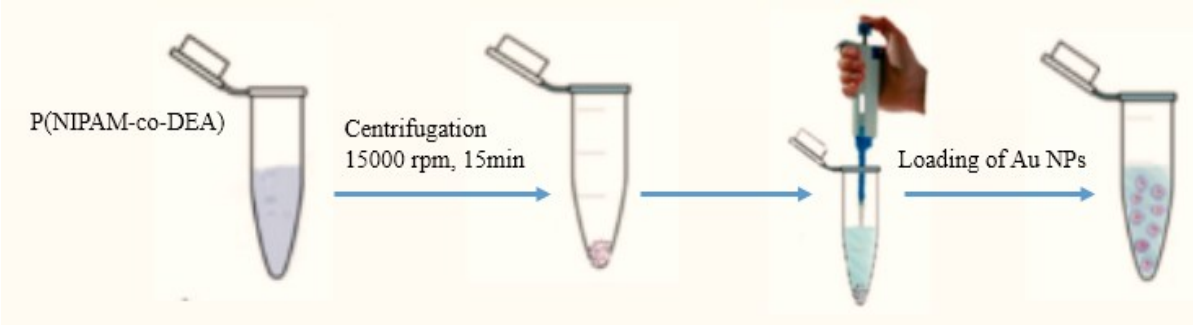


Figure 3.7. The dipping process of AuNPs to P(NIPAM-co-DEA) microgel

Table 3.4. Dipping of AuNPs to P(NIPAM-co-DEA) microgel with different amount of Au

Number	Experiment Code	$HAuCl_4.3H_2O$ mmol	AuNPs(mg)
1	R-AuNPs-1	2.5×10^{-3}	0.5
2	R-AuNPs-2	1.0×10^{-3}	0.2
3	R-AuNPs-3	0.5×10^{-3}	0.1

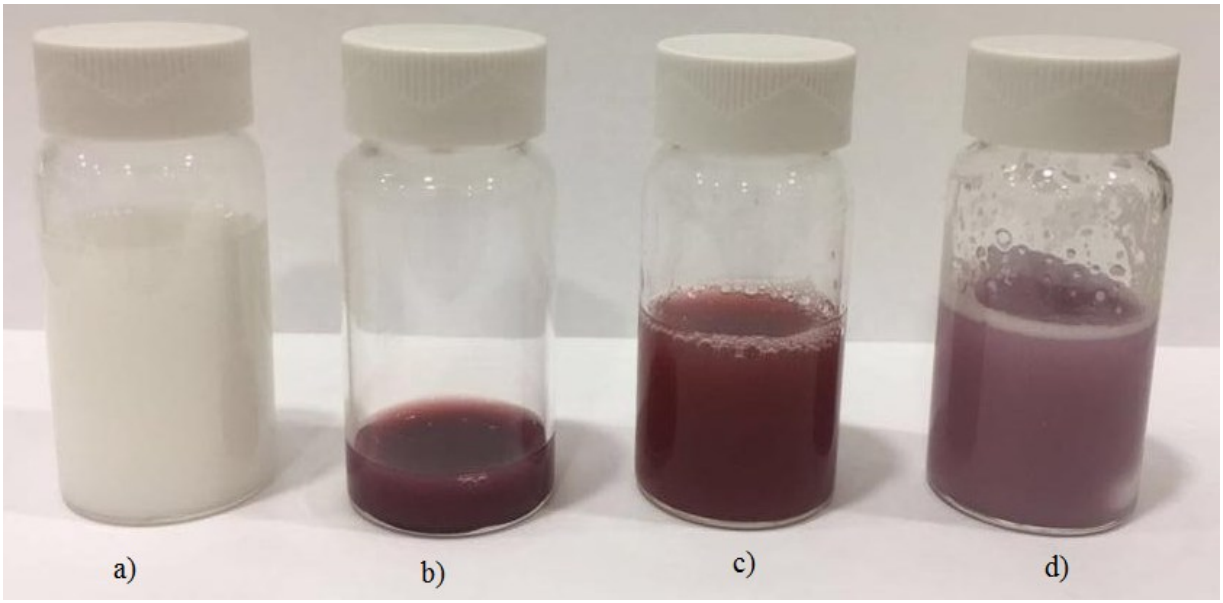


Figure 3.8. Dipping of AuNPs to P(NIPAM-co-DEA) microgel with different amount of Au a) P(NIPAM-co-DEA) microgel, b) R-AuNPs-1, c) R-AuNPs-2, d) R-AuNPs-3

3.2.11. Synthesis of P($DMA_{0.46}/AA_{0.54}$) hydrogel containing AuNPs/P(NIPAM-*co*-DEA) microgel

P($DMA_{0.46}/AA_{0.54}$) hydrogels having AuNPs/P(NIPAM-*co*-DEA) microgel were synthesized using (APS) free radical initiator and (MBA) cross-linker by free radical polymerization method in ethanol-water environment. First of all, P($DMA_{0.46}/AA_{0.54}$) was locked by MBA which was dissolved in ethanol-water, secondly, the AuNPs/ P(NIPAM-*co*-DEA) microgel was added, and finally, APS was added to the solution. The other gelling processes were the same with the preparation of P($DMA_{0.46}/AA_{0.54}$).

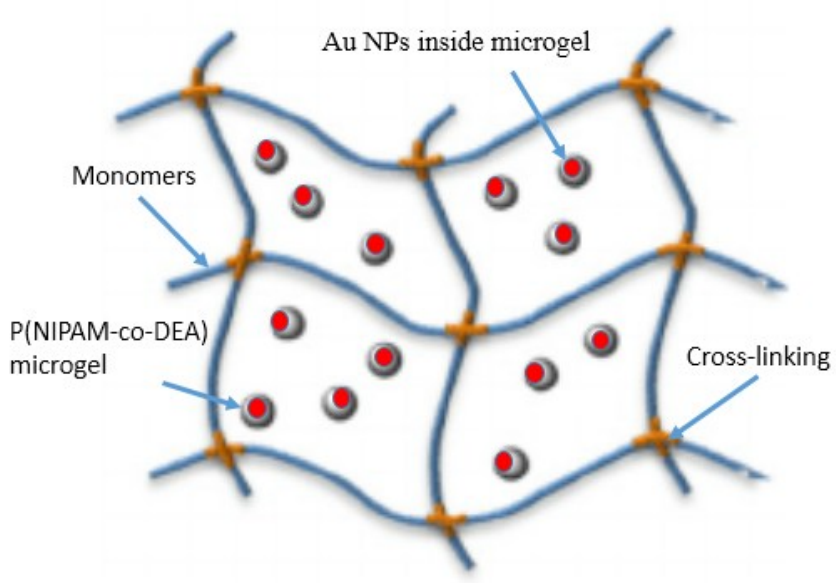


Figure 3.9. Schematic illustration of Au located P(NIPAM-co-DEA) microgel in P($DMA_{0.46}/AA_{0.54}$) hydrogel matrix.

3.2.12. Controlled drug release studies

Fluorouracil (5-FU) is an anti-cancer chemotherapeutic drug. It is used to treat colon, esophagus, stomach, pancreatic, breast, and cervical cancer by injecting it into a vein. Fluorouracil is used as a drug model in this research.

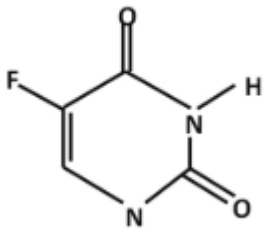


Figure 3. 10. Chemical structure of Fluorouracil (5-FU)

3.2.13. Drug loading

P($DMA_{0.46}$/$AA_{0.54}$) with the experimental code of RM123 was determined to be suited to medicine release systems, according to dynamics and certain physical features (flexibility, stability). 3 ml of Au loaded to microgel, (R-AuNPs-1) centrifuged for 15 minutes at 15000 rpm and dispersed in (10 mg/ml 5-FU methanol solution) solution and mixed with a magnetic stirrer for 3-4 hours at 200 rpm.

For the loading process, firstly 2 ml of DMA and 0.95 ml of AA with the ratio of ($DMA_{0.46}$/$AA_{0.54}$) mixed in a glass bottle by vortex secondly (10 mg, 1% of DMA monomer) of MBA dissolved in 1 ml of distilled water and added to the monomers mixture and vortexed for 1 min. Thirdly, 1 gr/ml of 5-FU loaded P(NIPAM-*co*-DEA) microgel was also added to the mixture. Finally, 1 ml aqueous solution containing 18 mg of APS was added to the mixture.

After putting the mixture inside the syringe the end of the syringe was closed. The prepared mixture inside the syringe was left in a water bath at 60 °C for about 15 minutes for

complete gelling. When gelling was noticed, the tubes were taken from the water bath and the gels were removed from the syringe.

To determine the amount of drug release, the maximum absorption values of 5-FU corresponding to the wavelength was recorded and due to the amount of 5-FU released, the 5-FU calibration curve was calculated and the resulting values were graphed. The calibration curve equation is defined as y = 0.5048 x-0.0687.

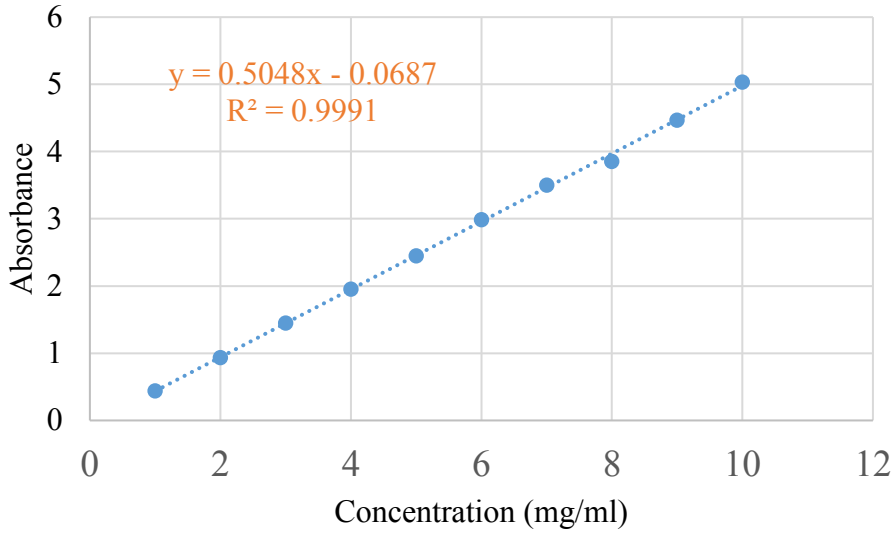


Figure 3.11. Calibration Curve of fluorouracil (5-FU)

4. THEORETICAL KNOWLEDGE

4.1. Hydrogel

For the first time, the combination between the polymer and fluid hydrogels were begun in 1894 in the act of a colloidal gel based on inorganic salt. Hydrogels are three-dimensional hydrophobic polymers that are connected to create a polymer chain by cross-linkers (Jawaid 2017). These types of polymers have the ability to hold biological liquid or water inside their network without changing their primary formation. (O'Sullivan, et al., 1975). Hydrogels are a water-saturated polymeric matrix with various structures that are used for several purposes such as biomedical, pharmacy, biosensors also the separation of biomolecules and cells. Nowadays, hydrogels, have received extensive attention in mass transfer technology because of their stable and flexible structure (Abolfazl, 2016). Hydrogels based on historical perspective are classified into three generations (1^{st}, 2^{nd}, and 3^{rd} generations)(Mantha et al. 2019).

Almost till 1900, the first-generation of hydrogels introduced as a colloidal gel which was made of inorganic salt using multifunctional cross-linkers (Nierzwicki et al., 1975). Main while, the water-soluble monomers got polymerized by cross-linkers (Mantha et al. 2019). At this time, the excellent mechanical characteristic of polymers and their high swelling features were improved.

Second-generation hydrogels: At the beginning of 1970, investigation interests through the synthesis of hydrogels were changed via the use of environment-friendly processes. The factors that affected polymerization incorporated heat, pH, ionic strength, light, and concentration of monomer in the solution. By utilizing environment-stimuli factors we can manage to modify properties for instance drag deliveries systems, gelling, and also biodegradability (Mantha et al. 2019).

Third-generation hydrogels: From 1970 to 1990, characterization of hydrogels was widely considered based on the influence of temperature and pH, nevertheless, in the mid-1990s, the concentration on many projects depended on other physical factors like the self-modeling method, enzymes cross-linkers, and stereo selective cooperation (Yom-Tov et al.,2014). During this period, the focus changed toward studying and promoting composite materials such as polyethylene glycol–polylactide (PEG–PLA) and cyclodextrins, which were stereo chemically participating in nature (Kirakci et al., 2014). Moreover, the exclusive concerns were set on the synthesis of hydrogels depends on their mechanical strength and elasticity (Omidian, et al., 2005). These types of hydrogels are more useful as they offer a condensed opportunity to earlier hydrogel production for the homogeneous admixture of cells and proteins with polymeric solvents (Hiemstra, C. et al., 2006 & 2009). Due to the literature in 1996 poly(hydroxy ethyl methacrylate) hydrogel has been reported as the first cross-linked material, particularly its high water bond (Cheon.S.et al., 2012).

Hydrogels experienced verities of changes and were used in a plenitude of applications after 1970 for more than 45 years form on their characteristics. According to the sources, hydrogel polymers are classified into three major classes, synthetic, natural, and the combination of both natural and synthetic characters polymers (hybrid materials).

Gels, are interesting materials similar to other solid and liquid. Because the liquid content of gels is very high, about 97% of gels are considered liquid. Protection of shear modulus during deformation of gel, provided by cross-linking in the network structure. In addition, gels due to their liquid and solid unique aspects they can remain widely stable by showing thousands of times the volume change (Dusek, 1993). A perfect gel should provide the following three remarkable appearances (Parhi 2017).

i. They must own at least two elements especially the gelling agent and a fluid element.

ii. Individual components should be connected throughout the system.

iii. They should present mechanical characteristics of the solid-state.

4.2. Classification of Hydrogel

The hydrogel can be classified based on their preparation, responsiveness nature, biodegradable properties, polymer type, and also their effective applications (Figure 4.1). The most common class is environmental sensitive or stimuli-responsive hydrogels (Prinsy, et al., 2015). Such hydrogels respond environmental stimuli well. Hydrogel also could be classified as cationic, anionic, and neutral based on their ionic charges which are existed in the bond group of polymers. The types of cross-linking agents also can be the standards for classification. Classification of hydrogels based on their characteristics could be reported as chemical, physical, and finally biochemical properties (Bahram, et al., 2016). Classification according to polymeric composition can be represented as homo polymeric hydrogels, co-polymeric hydrogels, and multi polymeric hydrogels (Sweta, et al., 26).

Based on the monomer/polymer sources, hydrogels are classified as natural (Table 4.1), synthetic (Table 4.2), and a combination of natural and synthetic (hybrid) (Ullah et al. 2015). Moreover, synthetic hydrogel in contrast to natural hydrogel is more useful due to its enormous application based on its mechanical and chemical characteristics. Besides that**,** the combined result of natural biopolymers and synthetic polymers a kind of hybrid hydrogels. For instance, the combination of collagen and/or chitosan with P(NIPAM), and PVA result with such a hybrid hydrogel (Endo and Sugiyama 2013).

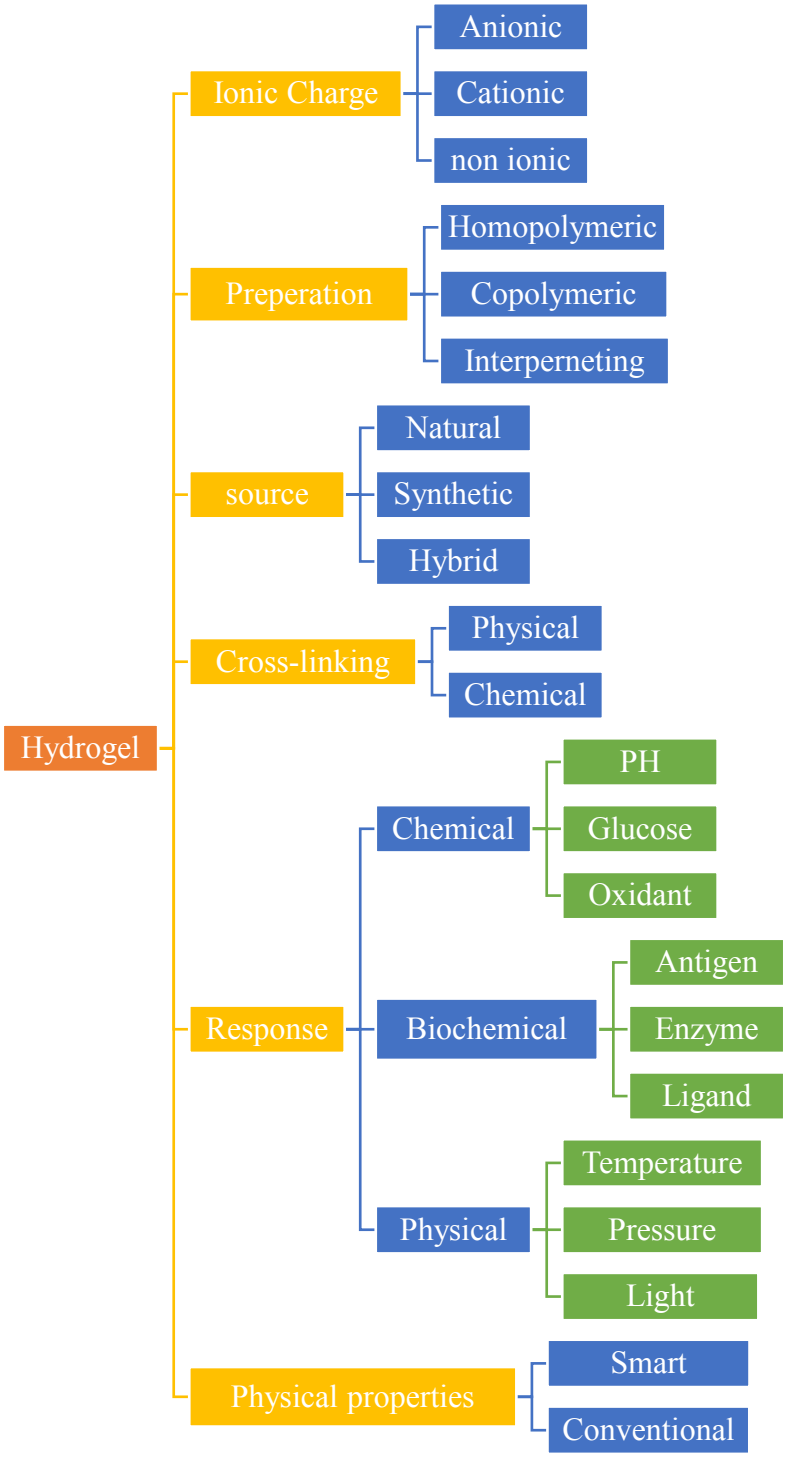


Figure 4.1. Classification of hydrogels according to their features.

Table 4.1. Application of natural monomer/polymers in hydrogel system

Natural polymers	References
Gelatin	(Jaipan et al., 2017, Petros et al., 2020)
Hyaluronic acid	(Borzacchiello et al. 2015)
Alginate	(Houghton et al. 2015, Wasikiewicz et al. 2005)
Fibrin	(Schneider-Barthold et al. 2016)
Chitosan	(Domalik-pyzik and Pielichowska 2019)

According to the character of cross-link combination, physical and chemical are given as classifications of hydrogels (Figure 4.2). molecular complexities and ionic interaction in the structure of hydrogel which is based on the physical interaction define physical hydrogels, considering the arrangement of covalent bonds of cross-linkers in the hydrogel structure are defined as chemical hydrogels (Garg, et al., 2016).

Targeting specific characteristics of the hydrogel can be controlled by managing the degree of cross-linkers. Based on the existence or nonexistence of electrical charge in the structure of cross-linkers hydrogels are classified into 4 classes which are showed in Figure 4.3 Moreover, the amphoteric electrolyte can be explained as the charges which are carrying both acidic and basic groups, while zwitterionics are carrying in each structure of the cross-linker chain both of anionic and cationic groups.

Table 4.2. Synthetic monomers applied in hydrogel production

Synthetic polymers	References
Hydroxyethyl methacryate (HEMA)	(Moghadam & Pioletti ,2016)
Vinyl acetate (VAc)	(Lee, Tan, and Cooper 2007)
Acrylic acid (AA)	(Sennakesavan et al. 2020)
N-(2-Hydroxy propyl) methacrylate (HPMA)	(Brahim, et al., 2003)
N-Vinyl-2-pyrrolidone (NVP)	(Wei et al. 2010), (Timaeva et al. 2020)
N-Isopropylacrylamide (NIPAM)	(Urosevic et al. 2018), (Wasikiewicz et al. 2005)
2-(2'-hydroxyethoxy)ethyl methacrylate) (HEEMA)	(Pilar et al. 2009)
2-(2-methoxyethoxy)ethyl methacrylate (MEEMA)	(Garcia-García et al. 2011)
Poly(ethylene glycol methacrylate) (PEGMA)	(París and Quijada-Garrido 2009)(Lei et al. 2013)
Poly(ethylene glycol)diacrylate (PEGDA)	(Stillman et al. 2020)
Poly(ethylene glycol) dimethacrylate (PEGDMA)	(Zhou et al. 2009)

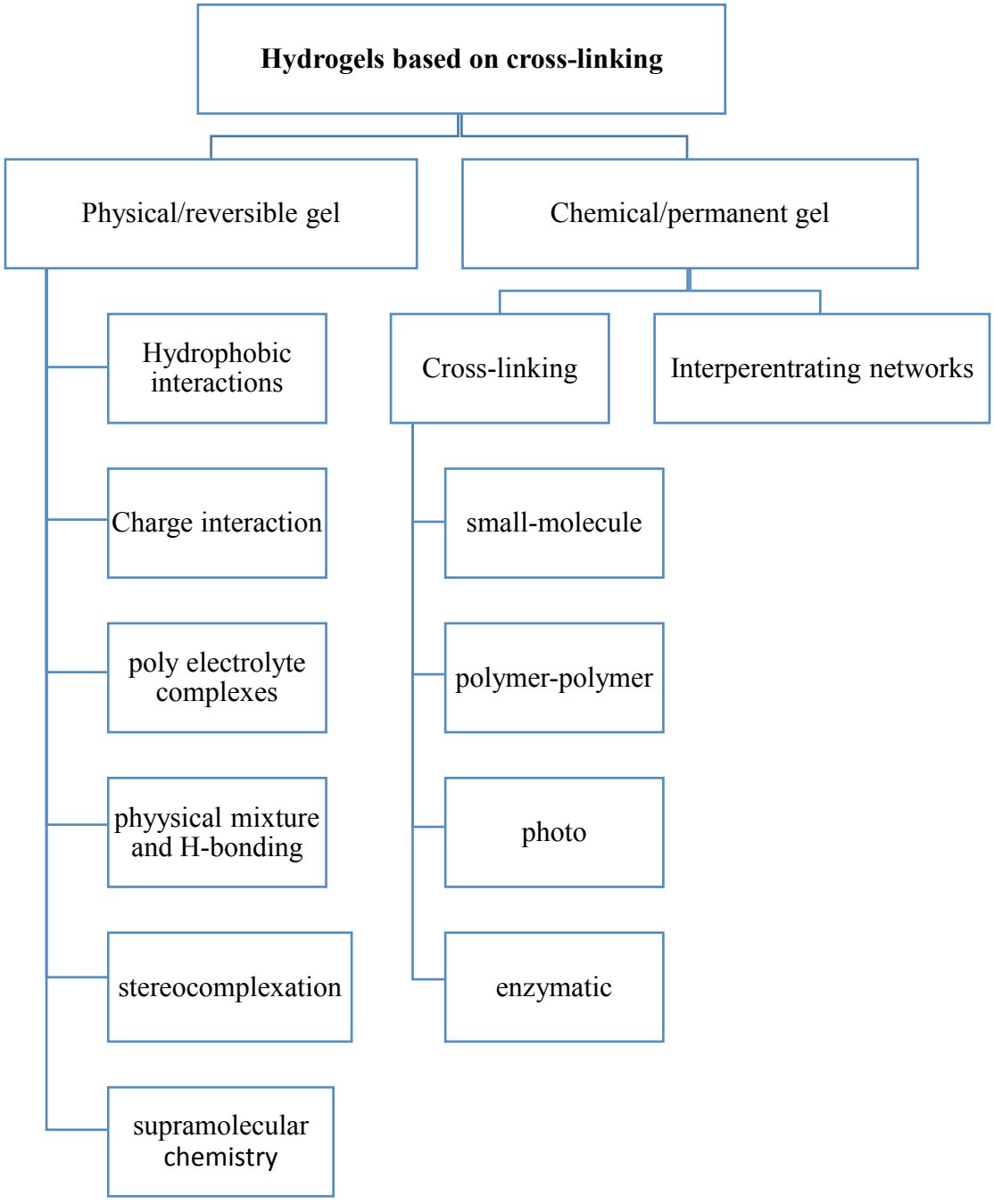


Figure 4.2. Classification of hydrogels according to cross-linkers.

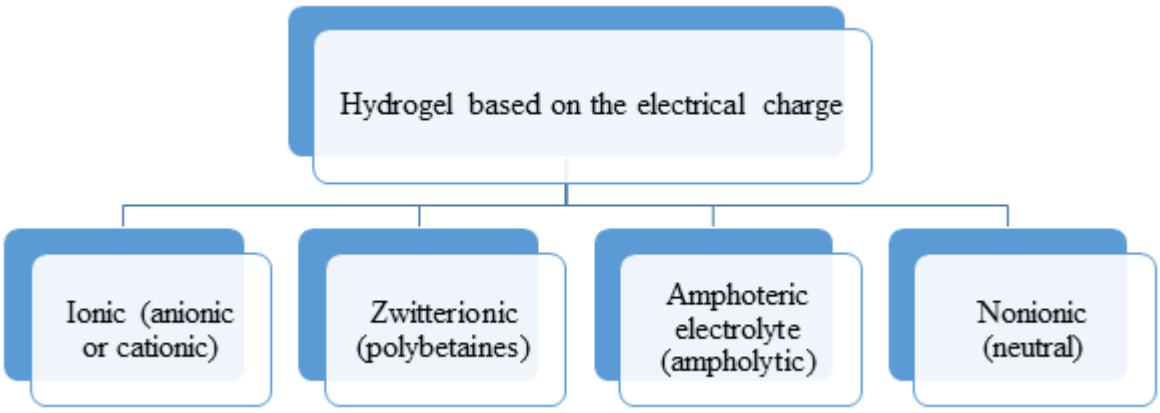


Figure 4.3. Classification of hydrogel based on the existence or nonexistence of electrical charge on the crosslinked chains

According to the physical structure and chemical combination hydrogels are classified into three classes as shown in Figure 4.4. Furthermore, semi-crystalline hydrogels are the heterogeneous composite of amorphous and crystalline forms (Ahmed 2015).

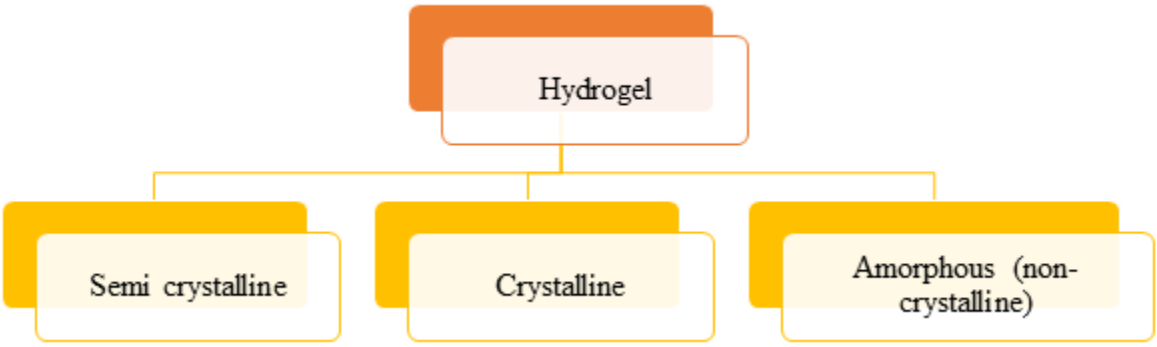


Figure 4.4. Classification of hydrogel according to their physical and chemical structure

4.3. Classification According to Polymeric Composition

a) *Homopolymeric hydrogels*: According to the type of monomer and polymerization techniques, homopolymers may own cross-linked on main chain compositions. For instance, polymer poly (vinyl pyrolidone) is a homopolymer that has been prepared by irradiation technique using a gamma source. Moreover, poly acrylic acid can be another example of homopolymer hydrogel; which has stable and elastic properties.

b) *Copolymeric hydrogels*: The random production of hydrogel which is based on the combination of two or more different monomer/polymer with at least one hydrophilic characteristic refers to copolymer hydrogels. Poly (ethylene glycol)-(α-caprolactone)- (ethylene glycol) is an example of the copolymer. Moreover, In another works, poly[((2-dimethyamino)ethyl methacrylate)-*co*-(acrylic acid)] has been studied to controlled drug release hydrogel application (Betül, 2012).

C) *Multi-polymer interpenetrating polymeric hydrogel (IPN)*: This type of polymers is familiar for the incorporation of two polymers which is produced by immersing a pre-polymerized hydrogel in the same of different monomers and initiator solution. Interpenetrating polymeric hydrogel is responsive to pH and temperature because of the lack of a binding interpenetrating flexible chain while it can modify pore size and paused drug release. The linear cationic poly(ally ammonium chloride) in acrylamide/ acrylic acid copolymer hydrogel can be presented as a great example of the interpenetrating polymeric hydrogel with both mechanical strength and pH-sensitivity (Elsayed , 2019).

4.4. Environmental Sensitive Hydrogel

The environment-sensitive hydrogels or smart hydrogels are presently the topic of essential scientific research in several fields. Changes in the external environment such as temperature (Hoffman, AS, 1987), light (Suzuki. A and Tanaka, 1990), pH (George and Abraham, 2007), chemical environment (Torres-Lugo and Peppas, 1999), solvent composition

(Kokufuta et al., 1998), ionic strength (Xu et al., 2008), electric field (Kim et al., 1999 and Tanaka et al., 1982), affect the compression and expansion of the hydrogel.

4.4.1. pH-sensitive hydrogel

Polymers that can respond to changes in pH values with groups that can be ionized in their structure are called pH-sensitive polymers (Table 4.3). These polymers, which contain ionizable groups in the main polymer chain, form polyelectrolyte in aqueous systems (Figure 4.5). An increase in the hydrodynamic diameter of the polymer occurs due to the effect of the electrostatic repulsion force resulting from the formation of a charge in the polymer backbone (Yalçın, 2006).

pH-sensitive polymer structurally includes donor or accepter groups such as carboxylic and sulfonic acids or basic groups (ammonium salts) which is environmental-stimulus according to the pH changes. The polyelectrolytes polymers are sensitive to pH that have plenty of such ionizable groups.

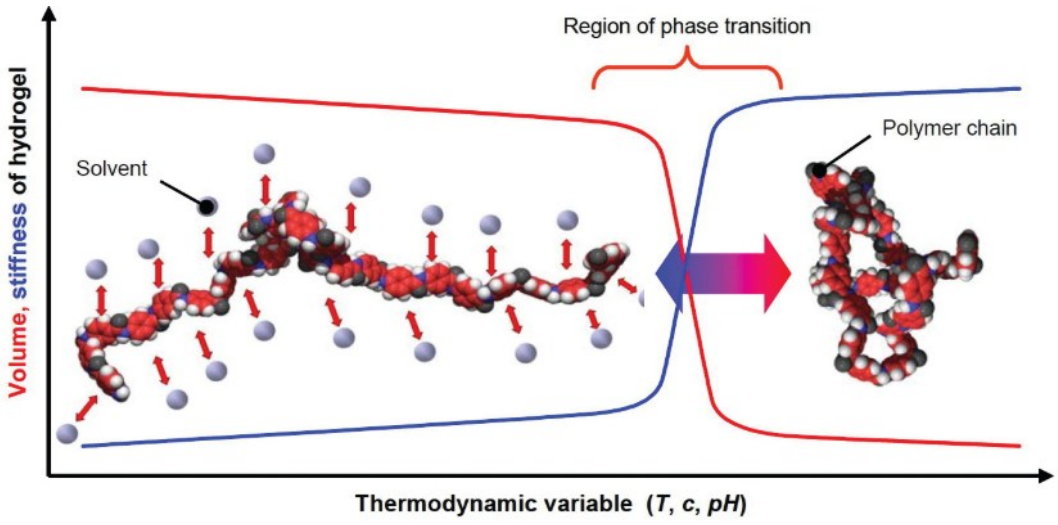


Figure 4.5. Phase transition behavior of stimuli-responsive hydrogels.

The deprotonation of anionic polyelectrolytes happens basic environment which caused the enhancement of electrostatic repulsions and allows water molecules to penetrate stimulate the extreme swelling of the hydrogel. On contrary, by ionization of cationic polyelectrolytes in an acidic environment caused the swollen of hydrogels (e.g. PDMA). Amphiphilic hydrogels include both acidic and basic groups; hence, in contrast to the neutral statement amphiphilic hydrogels present two-phase transitions in both acidic and basic media. These polymers at low pH accept hydrogen but replace it for other cations above the pKa value, therefore the ionization occurred at higher pH. The hydrodynamic quantity and expanding capability of these polymers rise distinctly while carboxylic groups become ionized and the highest elevation approaches near pH 7.

Table 4.3. pH-sensitive hydrogel and their polymer system (Rana, et al., 2015)

No	Polymer system	Comment
1	poly(methacrylic acid-co-methyl methacrylate)	Hydrogel, 22/78 molar %, with two cross-linking degrees (0.3 and 0.5%)
2	Poly (acrylamide-co- acrylic acid) (White, et al., 2015)	Super porous hydrogels with fast responsive properties of system
3	chitosan-alginate, chitosan-carboxymethylcellulose sodium and chitosan-carbopo	Polyelectrolyte complex hydrogels with prolong drug release systems using Diltiazem HCl
4	Chitosan–Poly(vinyl alcohol)	Modified pH sensitive swelling

For different monomers can be achieved, different pH-sensitive behaviors and degrees of swelling. Poly(acrylic acid), poly[(2-dimethyamino)ethyl methacrylate] and poly[(2-diethyamino)ethyl methacrylate] weak acidic/basic polymers for pH-responsive (Figure 4.6) also phosphoric acid derivatives polymers have been listed in these groups (Bahram, et al.,2016).

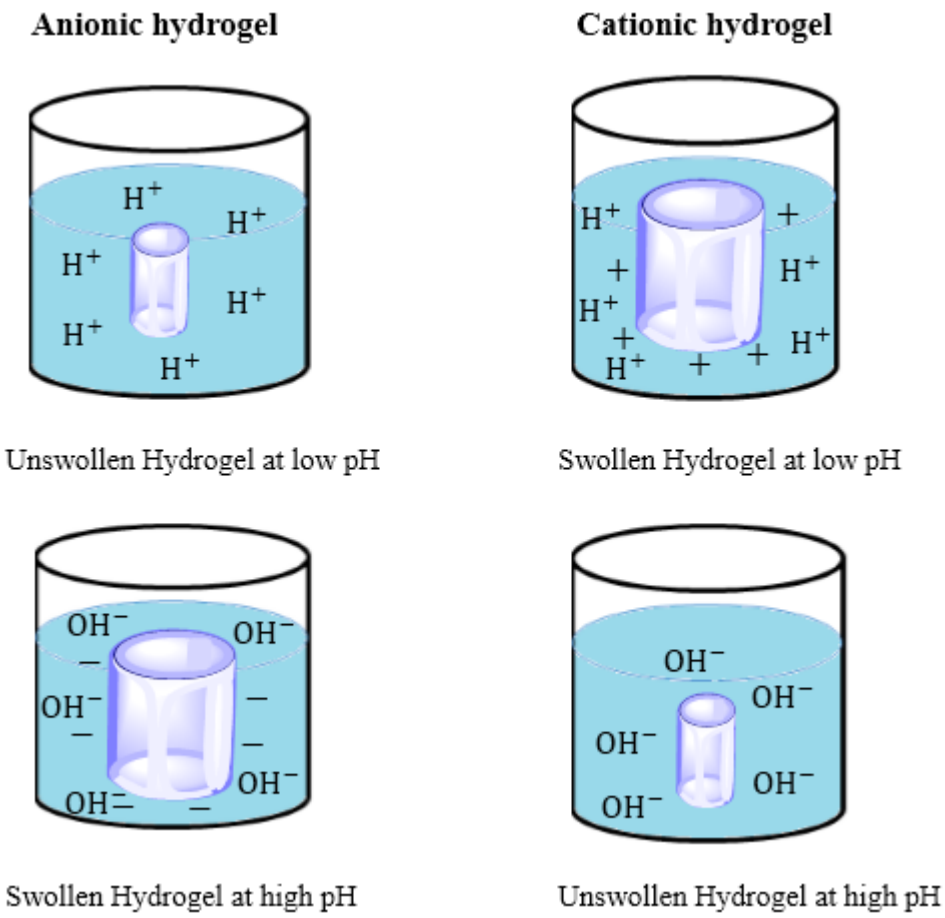


Figure 4.6. pH-responsive swelling: Anionic and cationic hydrogels (Usta and Asmatulu 1845).

4.4.2. Temperature-sensitive hydrogel

Temperature-sensitive polymers are extensively examined in advanced drug delivery proposes. Hydrogels that respond to temperature (Table 4.4) change by the effect of the external atmosphere tend to exchange their structure, balance of hydrophilic or lipophilic, and solubility. (Alexander C. 2006). In most common polymers as the temperature enhance directly the water solubility of the polymers also increased. Despite this, in some cases (negative temperature), the water solubility dramatically shows reduction while raising the temperature (Y. Qiu, et al., 2001).

The elements of polymers are unsolved above a specific temperature named the upper critical solution temperature (UCST) while the elements of polymers that are unsolved below a specific temperature named as a lower critical solution temperature (LCST) (Figure 4.7). In general, polymers with LCST are applied in drug delivery systems compared to the polymers with Upper critical solution temperature (UCST). At LCST interaction forces present decreases in hydrogen bonds linking water molecules and polymer, compared to the interaction forces between polymer-polymer and water–water bonds which caused weakness of the interaction and finally a phase separation exhibited in the shape of the gel (Fogueri LR.et al., 2009).

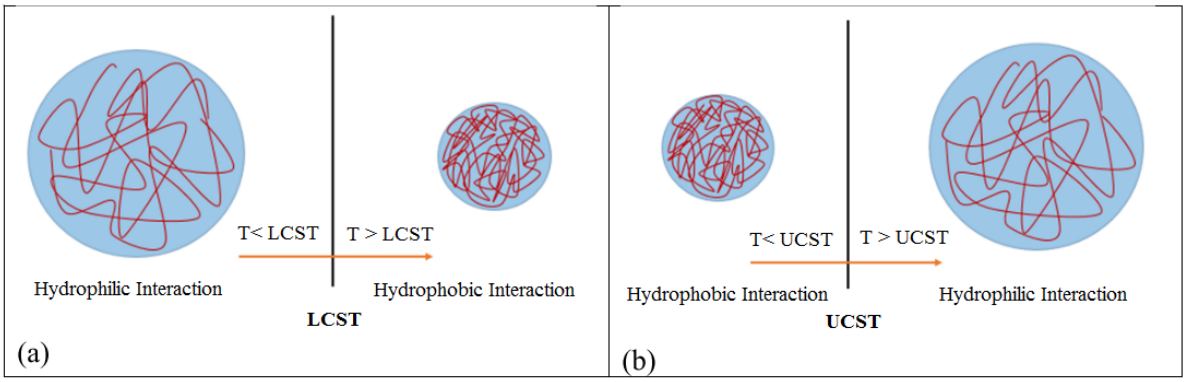


Figure 4.7. Sol-gel transition of thermosensitive hydrogels in response to temperature change: (a) (LCST), (b) (UCST) (Usta and Asmatulu 1845).

Table 4.4. Temperature-sensitive hydrogel and their polymer system (Rana et al.,2015).

No	Sensitivity type	Polymer system	Comment
1	pH-thermo	poly-N-isopropyl acrylamide Poly	Formed by Electrochemically induced polymerization
2	Thermo	poly(N-t butyl acryl amide-co-acrylamide)	Prepared by free-radical cross- linking copolymerization
3	Thermo	poly(N-isopropyl acrylamide) (PNIPAAm)-poly(ethylene glycol) diacrylate	A fast and reversible phase change with alteration in temperature and able to encapsulate and release various proteins

When the temperature is under the LCST, the water molecules make hydrogen bonds with the polar groups of the polymer and shape kind of hydrophobic groups as iceberg water. When the temperature rises higher the LCST, these hydrogen bonds are delivered to the bulk including a high accumulation in entropy destroying the polymer network (Rana et al., 2015).

4.4.3. Photosensitive hydrogels

Light is the other stimulus used in the drug delivery application of hydrogels (Usta and Asmatulu 1845). Light-sensitive hydrogels have many interests as compared to others because light stimulus can be directly affected in particular expenses with great efficiency (Schiphorst 2018). The rate-limiting standard for pH-sensitive hydrogels is the diffusion of hydrogen ions, while for temperature-responsive hydrogels, the rate-limiting measure is thermal diffusion. (Khan et al. 2016). By the way, the properties of photo-responsive hydrogels change while irradiated with light of the appropriate wavelength.

Light sensitive hydrogel characteristics caused to change their shape, size, which commences changing in diffraction, darkness, and besides that change the color of the reaction statement. Lately, gels sensitive to ultraviolet light were also attracting attention. The temperature of the hydrogels is raised by molecules of thermal-active hydrogels that absorbed light, which caused to change in the swelling capacity of the hydrogels. (Meng and Hu 2010). The "local" temperature of the hydrogel is raised by chromophore (photo-responsive functional group)which absorb the light as heat, underexposure to a definite wavelength (Tyagi et al. 2019). Light-stimulus polymers are expressly valuable as it is effortlessly obtainable in the environment (friendly to the environment) and does not cover the use of secondary sources of energy such as heat, electricity, or magnetic field. These types of polymers have been intended to be utilized in bio separation spring-lock reinforcement elements for large noise structures, solar sails, morphing surfaces of aircraft, drug delivery system (DDS), medical stents, recordable and erasable memories (Meng and Hu 2010).

Light-sensitive hydrogels can increase and contract in contact with ultraviolet (UV) or visible light. Visible light in contrast to UV light grants many advantages included large-scale availability, low cost, efficiency of use, and reliable performance. The UV- sensitive hydrogels are synthesized by combining a Leuco derivative molecule (usually neutral only separate into ion pairs at the time of UV exposure) into the polymer network. In response to UV irradiation at a set temperature, the hydrogels discontinuously swelled, though, contracted when the UV light was removed (Priya James et al. 2014).

4.4.4. Electro-sensitive hydrogels

Sensitivity to the electric or magnetic field has a significant role in biological applications such as drug delivery systems and artificial innovations (Shiga, 1997). Electro-sensitive hydrogels caused shriveling or swelling in the appearance of an employed electric

field. As mentioned before gels that are sensitive to electric current are usually polyelectrolyte-based. These are also known as pH-sensitive gels.

Electro-responsiveness has extensive utilization in the scope of drug delivery system (DDS), artificial muscle actuation, and energy transductions, and sound dampening due to the conversion of electrical energy into mechanical energy (Khan et al. 2016).

4.4.5. Pressure-sensitive hydrogels thermodynamic

According to the thermodynamic evaluations which are based on uncharged hydrogel theory pressure, can influence the phase transition. Due to this theory while the same hydrogels shot in low-pressure caused the rising of the pressure. Pressure effects on the donation of free energy to the gel-water system can be the reason to increase the swelling rate of gels depends on pressure. Furthermore, the destruction of entropy at high pressure leads to a decrease in the entire volume of the system. Due to the condensation of the system, the internal energy would increase because of the gel-gel and water-water interactions in case the gel remained in the dropped state. (Huang et al., 2008).

Under the 120 atm pressure the volume change of temperature-sensitive gels such as PNIPAM gel, poly (N-N-propyl acrylamide) gel, and poly(N, N-dimethyl acrylamide) gel were studied by the impact of hydrostatic pressure on the swelling temperature-responsive gels.

During the volume phase-transition of the gels, the excess enthalpy and volume of the gel-water systems were monitored in order to note that the pressure sensitivity of these gels begins with their temperature sensitivity and is the consequence of the increase of their lower critical solution temperature with pressure. Thermodynamically, an equalization characterizing the lower critical solution temperature of the temperature-sensitive gel to the pressure was

determined. These tests will demonstrate the link between hydrogel pressure and temperature sensitivity (Zhong et al.,1996).

4.4.6. Solvent-sensitive hydrogels

Although solvent-sensitive hydrogels are not common today; hydrogels that undergo phase transition are synthesized by changing the solvent compound. The sensitivity of the gel to the solvent is based on the similarity between the groups in the gel structure and the solvent molecules. The fact that the groups are similar increases the sensitivity of the gel to the solvent. In addition, a gel sensitive to solvent composition if it provides the necessary conditions, other stimulants (pH, temperature, ion, etc.) can be also sensitive.

4.5. Properties of Water Bound in Hydrogels

The insolubility of hydrogels in water is significant for the protection of the system's purity. One of the common techniques to determine this task is the administrate both chemical type and the ratio of cross-linked polymers or macromolecules, while the ratio of cross-linking has greatly influenced the behavior of hydrogel in the water environment in contrast to the performance of water bound in the hydrogel. The hydrogel hydrophilicity is owed to several water-solubilizing groups: Carboxylic acid, hydroxyl, carbonyl, amide (carboxamide) , sulfonic acid, $–COO^-$, $>CHNH_2$, $–CONH_2$, etc., in the polymer system (Gun'ko et al., 2017) . Water performs an essential function in hydrogels by preserving their purity, solubilization, and diffusion of materials, which is critical for biomedical, biotechnological, and environmental utilization (Buchmann and Schaumann 2017).

One of the issues considered common controversialist is the presence or absence of "different states/types" of water inhomogeneous compounds of water and amorphous polymer, expressly, after effects climbing from the presence of different phases (crystalline vs amorphous,

many compositions) have been estimated. This concept indicates that water molecules settle into various categories with different performance thermodynamically. A commonly accepted standard is that water molecules near to the hydrophilic chains are in some way “bound” to the polymer and, consequently, act individually from “free” water particles (Müller-Plathe 1998)

Hydrogels according to their water content can be classified to:

- Low swollen degree (20-50 %) hydrogels

- Medium swollen degree (50-90 %) hydrogels

- High swollen degree (90-99. 5 %) hydrogels

- Super-absorbent (>99.5 %) hydrogels

Increasing the hydrophilic groups in the gel structure will also increase the swelling of the gel, as it will increase the impression of hydrogen bonding. Three types of water are mentioned in a swollen gel (Figure 4.8).

a. Connected water: it is water that can bond hydrogen with polar groups such as - OH, - NH_2, -COOH, -NR_2, -SO_3H, etc.
b. Interface water: this is water collected around hydrophobic (water-decoherent) groups of the polymer. Although there is no mention of a bond such as a hydrogen bond, it is hydrophobic thought that there may be a weaker binding around 8 groups than hydrogen bonding.
c. Free or Mass Water: This type of water that fills the pores of cross-linked polymers acts like normal water. In other words, without a bond, physically water molecules are located in the pores and do not interact with the polymer (Betül, 2012).

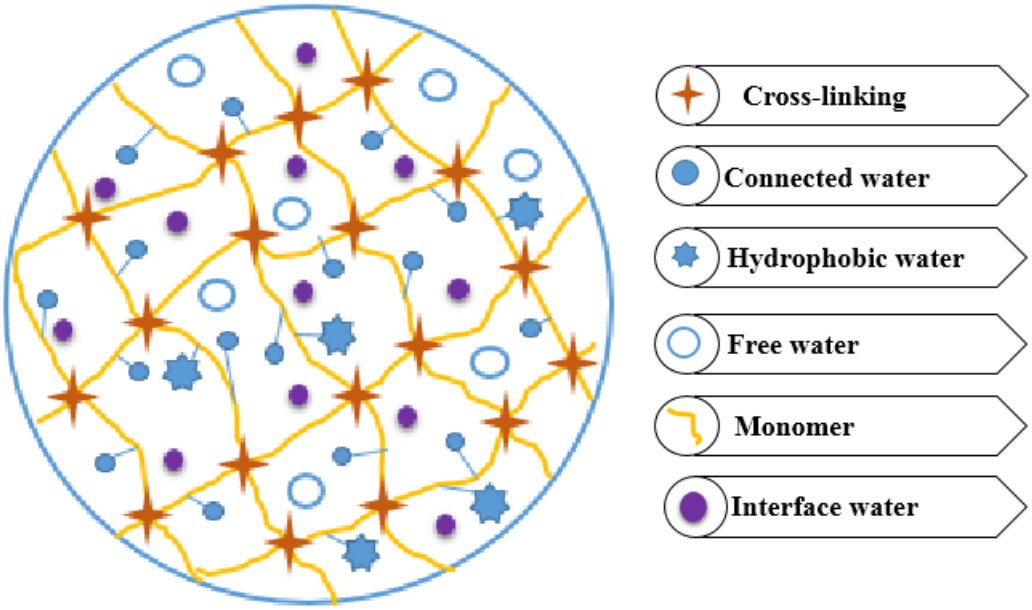


Figure 4.8. Schematic illustration of the hydrogel system.

4.6. Hydrogen Bonds of Hydrogel System

PAA and PMAA produce networks with polyethylene glycol. These networks have hydrogen bonds between the oxygen of the polyethylene glycol and the carboxylic combination of PAA/PMAA. Hydrogen bonds is seen both between PMA and poly(ethylene glycol), and poly(methacrylic acid-*graft*-ethylene glycol). If the protonation of carboxylic acid groups occurs caused to hydrogen bonds are formed which shows pH-sensitive swelling of the gels.

4.7. Characterization of Hydrogels

A simple technique to measure the existence of hydrogel in a system is to disperse the polymer in water using a cylindrical vial and visually observe the structure of the unsolvable substance.

The differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) are the main techniques applied to characterize and measure the mass of free and bound water inside the hydrogels system (Phillips et al., 2003).

Solubility**:** Commonly the solubility of hydrogel is determined by measuring its insoluble particle in the dehydrated sample after dipping in deionized water at room temperature for approximately 16-48 h. The sample needs to be produced at a dissolve concentration to ensure that the hydrogel element is fully spread in water (Sennakesavan et al. 2020).

Swelling behavior of hydrogels: The swelling degree of hydrogels can be determined via various parameters. Among all parameters in a hydrogel is the character of porosity is the critical one. Also according to this character hydrogel can be classified into four different groups in particular: non-porous, microporous, macro porous, and super porous hydrogels (Sennakesavan et al. 2020).

Besides that, hydrogels have a swelling quality (Figure 4.9), which is the most important one in their occurrence. The swelling of hydrogels can be observed in three steps: (a) Diffusion of water into the hydrogel system (water transferring in is termed primary bound water), (b) relaxation of polymer networks (more water transferring in is named secondary bound water), accompanied by (c) development of the hydrogel chains (extra water transferring in is called as free water) (Bashir et al. 2020).

The other characteristic of hydrogel is the potential to swell while keeping in contact with a solvent that is thermodynamically fit to the hydrogel. While a hydrogel in its primary nature, by association with solvent molecules, first it attacks the hydrogel surface and then enters the polymeric chain. In this case, the unsolved glassy phase is separated from the rubbery hydrogel area with a changing frame (Ganji, et al., 2010).

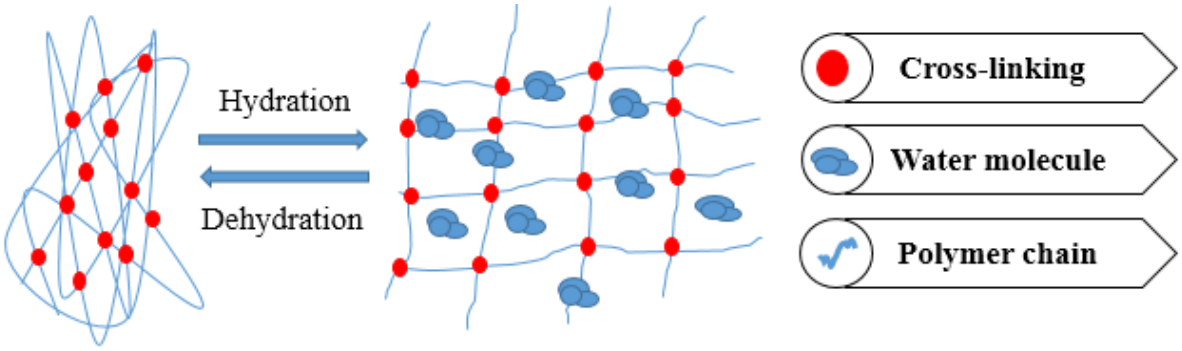


Figure 4.9. Scheme of hydration and dehydration in a hydrogel.

The swelling ratio, Rs of hydrogels can be determined by the following formula (Sennakesavan et al. 2020);

$$R_S = \frac{W_S - W_d}{W_d} \times 100 \qquad (4.1)$$

Herein Ws, and Wd are respectively the weight of swollen hydrogel, and the initial weight of hydrogel before immersion in water.

Scanning Electron Microscopy (SEM): SEM was used to provide data about the hydrogel surface topography, structure, and other properties.

4.8. Advantage and Disadvantages of Hydrogels

The most significant feature of the hydrogel is its water content which maintains a standard of flexibility that is quite comparable to natural tissue. Moreover, hydrogels own high-grade transportation characteristics that can simple to modify. Hydrogel also have the ability to respond to external changes such as pH, temperature, or the assembly of metabolite and deliver their load as a consequence of a similar transition (Das and Ram., 2014). Hydrogels have some technical feature which becomes more applicable in contrast to other chemical products.

Hydrogels have the highest strength and stability in swelling conditions while storage. Also, they are colorlessness, odorless, and non-toxic with photo stability. Moreover, they have the highest absorption capacity (maximum equilibrium swelling) in normal saline solution and pH-neutrality after swelling in water.

The primary limitation of hydrogels is that they are non-adherent and probably require to be protected by a secondary dressing and additionally creates an impression considered by the movement of the grubs. Besides that, hydrogels costly while having low mechanical strength and challengeable to regulate (Das and Ram., 2014).

4.9. Application of Hydrogels

Hydrogels are recognized as a harmless drug delivery system for the oral route of treatment and also have the mucoadhesive capacity which could increase drug release and absorption. Hydrogels as drug delivery systems have many benefits including biocompatibility, low toxicity, and excellent swelling response but depending on chemical moieties of the gel-forming polymers and route of treatment some limitations would seem in the delivery of active pharmaceuticals. These limitations could be slow responsiveness of stimuli-sensitive hydrogels, the possibility of rapid burst drug release, the possibility of drug reactivation, limited hydrophobic drug delivery, low mechanical strength, etc. (Ghasemiyeh and Mohammadi-Samani 2019).

The main cause for universal applications of hydrogels is their features namely absorption, swelling and shrinkage performance, hydrophilicity, and biocompatibility. Hydrogels have unique utilization in the pharmaceuticals area including diagnostic aid, curative, and implantable instruments like catheters, biosensors, synthetic skin, and tissue engineering.

Biocompatibility of hydrogels commonly occurs when hydrogels in the completely swollen mood are nearly viscoelastic, soft, rubbery, and low in interfacial angle with biological fluids, which reduces the risks of a negative immune response (Bashir et al. 2020).

The elastic quality (as things go to the existence of nets of the chain) of entirely swollen or hydrated hydrogels is determined to be decreasing irritation to the encircling tissues after implantation (Parhi 2017).

4.10.Microgel

Microgels are three-dimensional crosslinked smooth chains which have the ability to swollen in a suitable dissolvent. According to the size, hydrogels usually can be categorized into macrogels and microgels (Dai and Ngai 2013). The first microgel particles were synthesized 70 years ago by Staudinger and Husemann. These synthesized microgel particles were prepared using the divinylenzene monomer in a highly diluted organic solvent. In 1949, Baker used the term microgel for the first time.

The last three decades have witnessed tremendous importance in microgels because of their potential for utilization over a large-scale range of manufacturers. Various attractive and wide-ranging reviews of many perspectives of microgel development, characteristics, and applications previously exist in the literature, which incorporates the many distinct particle types that can be contemplated to fall within the broad sphere of nano-/ micro-particles (Thorne, Vine, and Snowden 2011).

Microgels have a larger surface area than macrogels and greater flow capability within the continuous phase in which they disperse. Macrogels can be synthesized homogeneously in the working solution at +4 ºC in the presence of an accelerator. In the synthesis of microgels,

stable monodispersed particles are formed by working at high temperatures and high mixing speed (Saunders et al., 1996).

4.10.1. Stimuli-responsive microgels

Stimuli-responsive polymers can react physically and/or chemically to changes in their media (Gao, Li, and Serpe 2015). Regarding the dimension, flexibility and interaction forces of microgels have been also called "smart materials" which can respond to external stimuli for instance temperature, pH, light, ionic strength, magnetic fields, and also electricity (Ballauff and Lu 2007). Since the invention of microgels (polymeric nanoscale substances) as a delightful class, individually the consolidation of stimuli-responsive characteristics into gel nanoparticles has obtained expanding attention. Individually, concerning their usage in loading and release purposes, microgels, in contrast to other polymeric compositions, show outstanding properties (Klinger and Landfester 2012).

Individual attention is centered on the microgels which are close to body temperature with the characteristics of lower critical solution temperature (LCST). For instance, one of the most common polymers which have been extensively considered is PNIPAM with a lower critical solution temperature of 34 °C. We can obtain several biological applicability due to the temperature stimulus characteristics of these polymers guide a variety of biological applicability. Microgels can be examined as drug delivery devices, supplies for tissue engineering, and materials for inhibiting medical adhesion (Balaceanu, Demco, and Pich 2011).

Microgels can be classified in three main categories: They are (i) pH sensitive, (ii) ionic strength responsive microgels, (iii) thermo-responsive microgels.

4.11. Metal Nanoparticles

Nanoparticles (NPs) are dimensional materials with at least 100 nm size. This comprehensive group of substances covers particulate substances. Moreover, according to their shape, these substances can be zero, one, two, or three dimensional. By changing the size and shape, NPs can be shown unique colors and features that is able to be used for bio imaging designs. Hence, NP's are formed of three layers expressly:

(a) Functionalization of the surface layer with many invisible molecules, alloy ions, surface-active agents, and polymers.

(b) Shell layer, with different chemical materials properties in contrast to the core in all perspectives.

(c) The core, the center of NPS (Khan, et al., 2019).

Nowadays nanotechnology has a fundamental perspective to synthesize and produce nano-size material/structures. In order to produced nanomaterials or nanostructures with the desired size, morphology, and chemical form should pay attention to physical features and nanostructure applications. Despite this, for some decades valuable nanoparticles of metals have received too much attention (Norsuzila Yaacobi et al. 1989). Metallic nanoparticles are prepared by the synthesis of metal characteristics of small.

NP designs, it is important to identify the effect of physical, chemical features of metals and finally decide the techniques for synthesis. Metallic nanoparticles are prepared by the synthesis of metal characteristics of small. Last but not least, NPs features based on their structure, size, and configuration could define the plasmonic, catalytical, and also magnetical properties of metals (Huynh et al. 2020).

In recent decades, industrialized sectors covered by nanotechnology due to its applicability in electronic accommodation systems, magnetic interdigitating and pre concentration of object analytes, biotechnology, and controlled drug loading. Furthermore, the particle size of nanoparticles that are used in the field of biotechnology is between 10 and 500 nm, rarely passing 700 nm intervals. The nanosize of these particles provide several interactions with biomolecules on the cell surfaces and within the cells in the process that can be decoded and designated to many biochemical and physicochemical features of these cells (Mody et al. 2010).

4.11.1. Classification of nanoparticles

According to the morphology, dimension, and chemical properties of NPs are widely divided into several classes. Depending on physical, biochemical, and chemical features, some of the common types of NP's are presented here (Khan, et al., 2019).

a) Carbon-based NPs
b) Metal NPs
c) Ceramics NPs
d) Semiconductor NPs
e) Polymeric NPs
f) Lipid-based NPs

Metal NPs: By progressing of nanotechnology, noble elements such as Ag, Pd, Au (Figure 4.10), and Fe nanostructures are extensively considered in recent researches to produce bioengineering substances that have the ability to be applied in the process of new symptomatic devices to resist severe illnesses**.**

Based on Au-thiol chemistry, Shi and coworkers reported AuNPs with 3.8 nm in diameter in thiol-functionalized PNIPAM microgels by in situ reductions of $HAuCl_4$ aqueous

solution. By applying this technique, the authors were able to obtain and manage the distribution of AuNPs inside the microgel. On the other side, the authors used AuNPs joined within a PNIPAM-based copolymer PNIPAM-*co*-allylamine, copolymer which is sensitive to both temperature and pH. By this hybrid method, the authors were able to modulate both the catalytic activity of AuNPs and provide label-free in situ localized surface plasmon resonance (LSPR) controlling of the catalyzed chemical reaction".

While the monitoring is done through stimuli-sensitive volume phase changes of microgels, the inflection is presented with changing the solution temperature, therefore replacing the critical physico-chemical micro media of gold nanoparticles, which can be caused by a variation of the localized surface plasmon resonance. Furthermore, Rehman and coworkers reported a temperature and pH sensitivity microgels based on the crosslinked NIPAM and DMA in water media. The P(NIPAM-*co*-DMA) microgels were examined as host a nanoreactor for the synthesis of AuNPs.

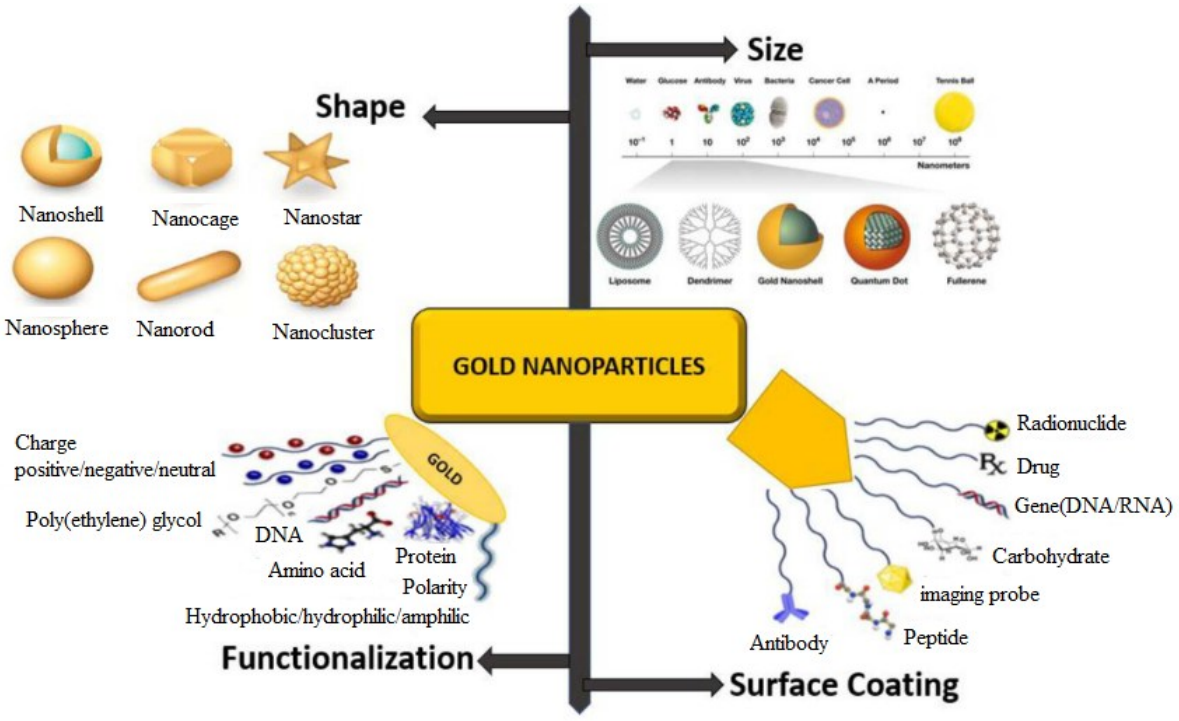


Figure 4.10. Utilization of Au-NPs according to their shape, size, functionalization, and surface coating

4.11.2. Characterization of metal nanoparticles

Based on physicochemical features of metal nanoparticles various characterization methods have been recognized. The following methods can be used as the most common methods for the characterization of NPs. In this thesis, we used the SEM and TEM methods for the characterization of Au nanoparticles.

a) X-ray diffraction (XRD)

b) X-ray photoelectron spectroscopy (XPS)

c) Infrared (IR) spectroscopy

d) Scanning electron microscope (SEM)

e) Transmission electron microscopy(TEM)

f) Brunauer–Emmett–Teller (BET)

g) Dynamic light scattering (DLS)

4.12. Drug Release

The objective of medication formulation and delivery is to deliver a therapeutically effective dose of a medicine to a specific site of action for a certain amount of time. Several factors influence the design of the final formed product for medication delivery. The controlled release systems were initially used in the 1950s and utilized for the administration of non-medical chemicals such as insecticides and antifouling materials. They were originally employed in medical research in the 1960s, and methods for gradual release of big compounds were created in the 1970s (Vogelson, 2001). Controlled release systems seek to provide a blood-based distribution profile of the medication over a lengthy time period (Figure 4.11).

The loading of hydrogels as drug carriers is based upon two main techniques. The hydrogel monomer is combined with the drug with or without the linker in one approach, and is let to polymerize, and the drug is trapped within the matrix. In the second technique, a prefabricated hydrogel can expand to equilibrium in an appropriate drug solution. The hydrogel filled with drugs is dried and the device is developed (Song et al., 1981).

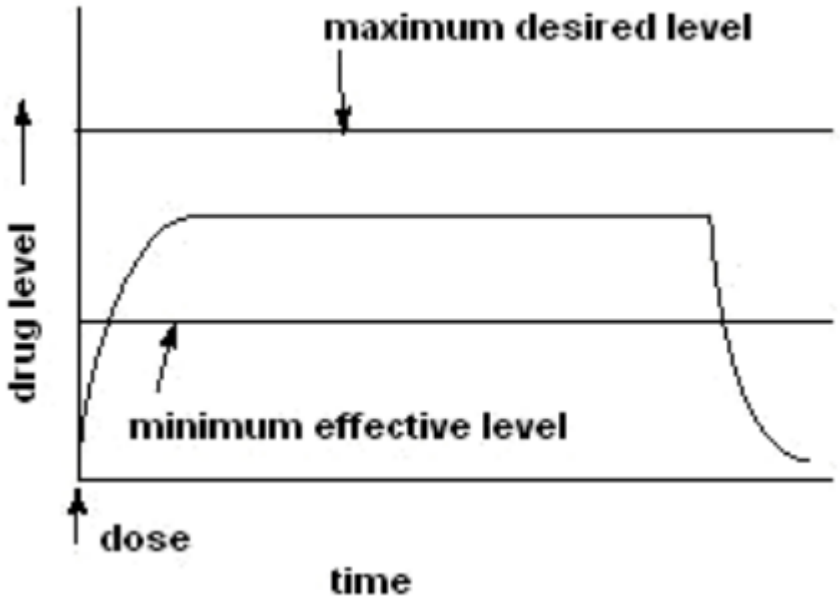


Figure 4.11. Controlled-delivery dosing (Brannon-Peppas,. 1997).

4.12.1. Duration of release

The objective of medication administration is to maintain the medicine for a specific amount of time at the proper therapeutic dosage (Figure 4.12). To reach this objective, there are various approaches. The first is to take a single dose and release the medication immediately to the site of action. This technique is effective for a brief time of activity for acute therapeutic

therapy. The objective is to keep the medicine at the therapeutic level for a lasting length of time with chronic conditions. Administration of multiple doses is one way of delivering sustainable therapeutic concentrations (Dewey et al., 2005).

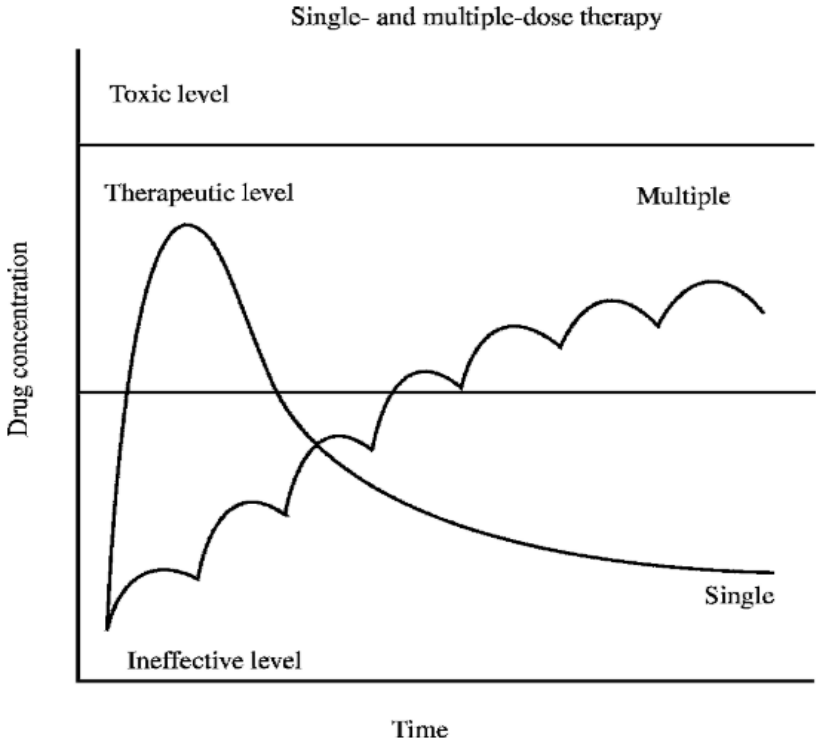


Figure 4.12. Plot of medication concentration against time for single and multiple dosages (Dewey et al., 2005).

Stimuli responsive hydrogel are the most common hydrophilic carrier material utilized in the manufacture of oral controlled drug delivery systems and have emerged as a viable medicinal alternative. One of its most essential properties is its high swell ability, which has a significant impact on the release kinetics of an integrated medication. When the device comes into contact with water or biological fluid, it causes polymer chain relaxation and volume expansion. The integrated medication then diffuses from the system (Brannon-Peppas, 1990). Furthermore, the most important rate control methods of commercially accessible controlled release products are diffusion, swelling and erosion (Langer, 1983).

5. RESULT AND DISCUSSION

5.1. Characterization of Hydrogels

Characterization of hydrogels have been carried out based on its physical properties such as softness/hardness, flexibility, split, stability, swollen of hydrogels in acid/base, and distillate water (Table 5.1).

By increasing the degree of cross-linking the flexibility and softness of the P(DMA/AA) decreases, on the contrary by decreasing the degree of MBA the hydrogels are getting soft and flexible at room temperature. Besides that, in the ethanol environment in contrast to water media, a high percentage of swelling represented (Figure 5.1).

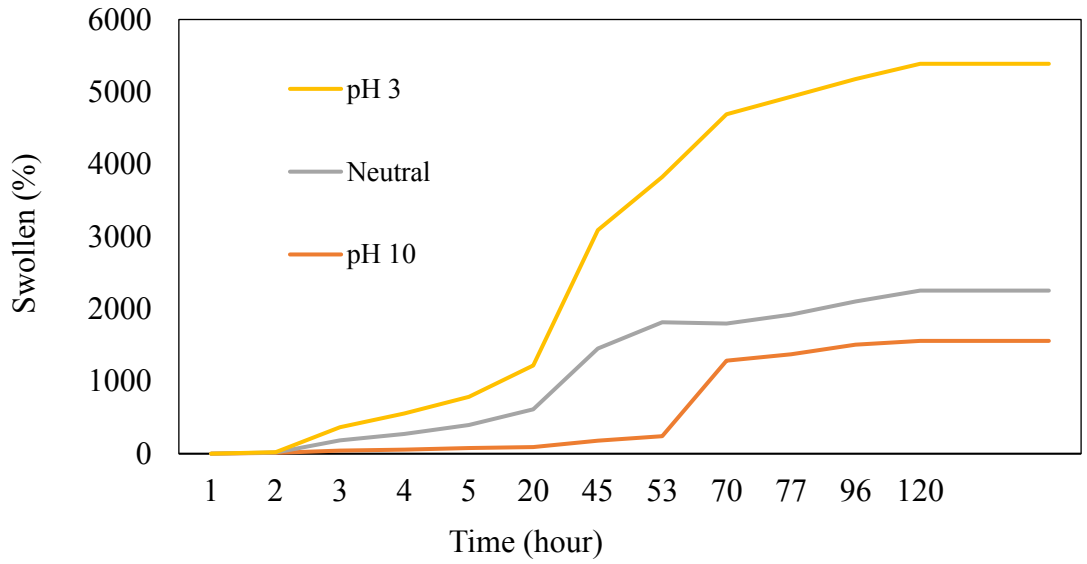


Figure 5.1. Swelling of P ($DMA_{0.46}/AA_{0.54}$) hydrogel (RM88) in water with different pH

Table 5.1 Characterization of hydrogels.

Experiment code	Flexibility	Softness/hardness	Split	Swollen	Stable/unstable
RM81	No	Soft	yes	good	Unstable
RM82	Yes	little hard	No	great	Stable
RM83	little	Hard		A little good	
RM84	little				
RM85	little				
RM86	little				
RM87	No	Soft	yes		Unstable
RM88	Yes	Soft	No	great	Stable
RM89	Little	Hard		little	
RM90				little	
RM91				Good	
RM92	Yes				
RM93	No	Soft	Yes		Unstable
RM94	No	Soft	Yes		
RM95	little	Hard	No	little	Stable
RM96	Yes	little hard		good	
RM97	Little	little hard		little	
RM106	No	Soft	Yes	little	Unstable
RM107	No			Good	
RM108	Yes				Stable
RM109					
RM110			No	Great	
RM111			Yes	Good	Unstable
RM77	No				Stable
RM78	Yes		No		
RM79			No	Little	

By examining the swelling rate of P($DMA_{0.46}$/$AA_{0.54}$) hydrogel at acidic, neutral, and basic pH values, the great rate of the swelling has been recognized at acidic pH, which shows that P($DMA_{0.46}$/$AA_{0.54}$) is a pH-responsive hydrogel.

5.2. Characterization of Microgel

According to the dimensional analysis of PDEA and P(NIPAM-*co*-DEA) (Figure 5.2),(RM-Mi(9,24,30)) microgel and DLS for size distribution analysis results, the hydrodynamic radius of the microgel was recorded respectively as 52,82 and 126 nm in aqueous dispersion and the PDI value with particle size distribution was recorded as 0.07, 0.0512, and 0.366 (Figure 5.3) . The swollen of microgel in acid, based and neutral is acid > neutral > based respectively.

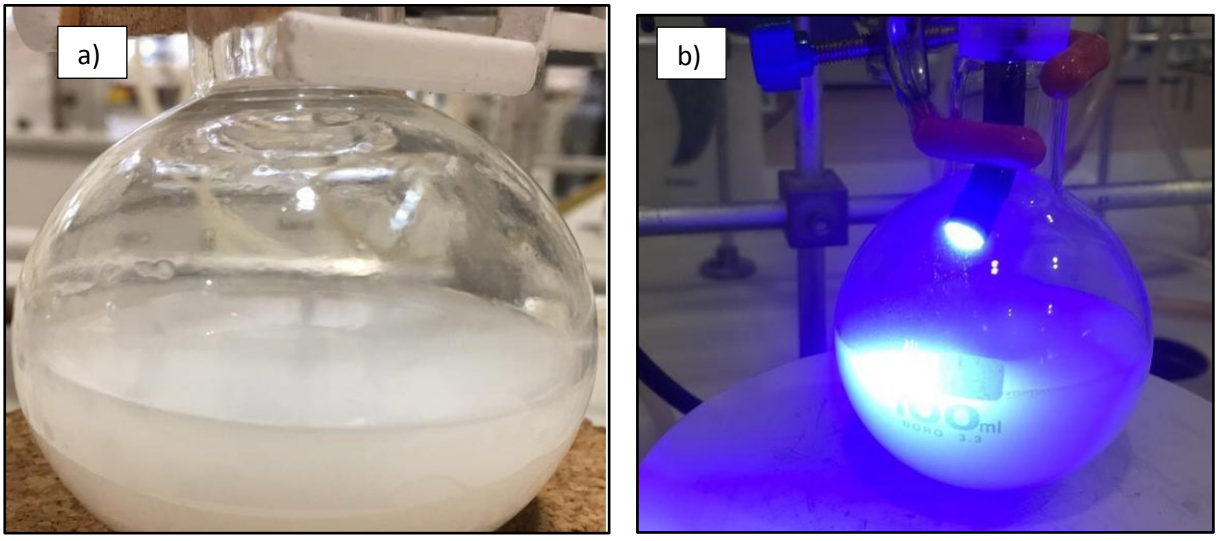


Figure 5.2. Image of emulsion polymerization of a) P (NIPAM-*co*-DEA) b) PDEA microgels

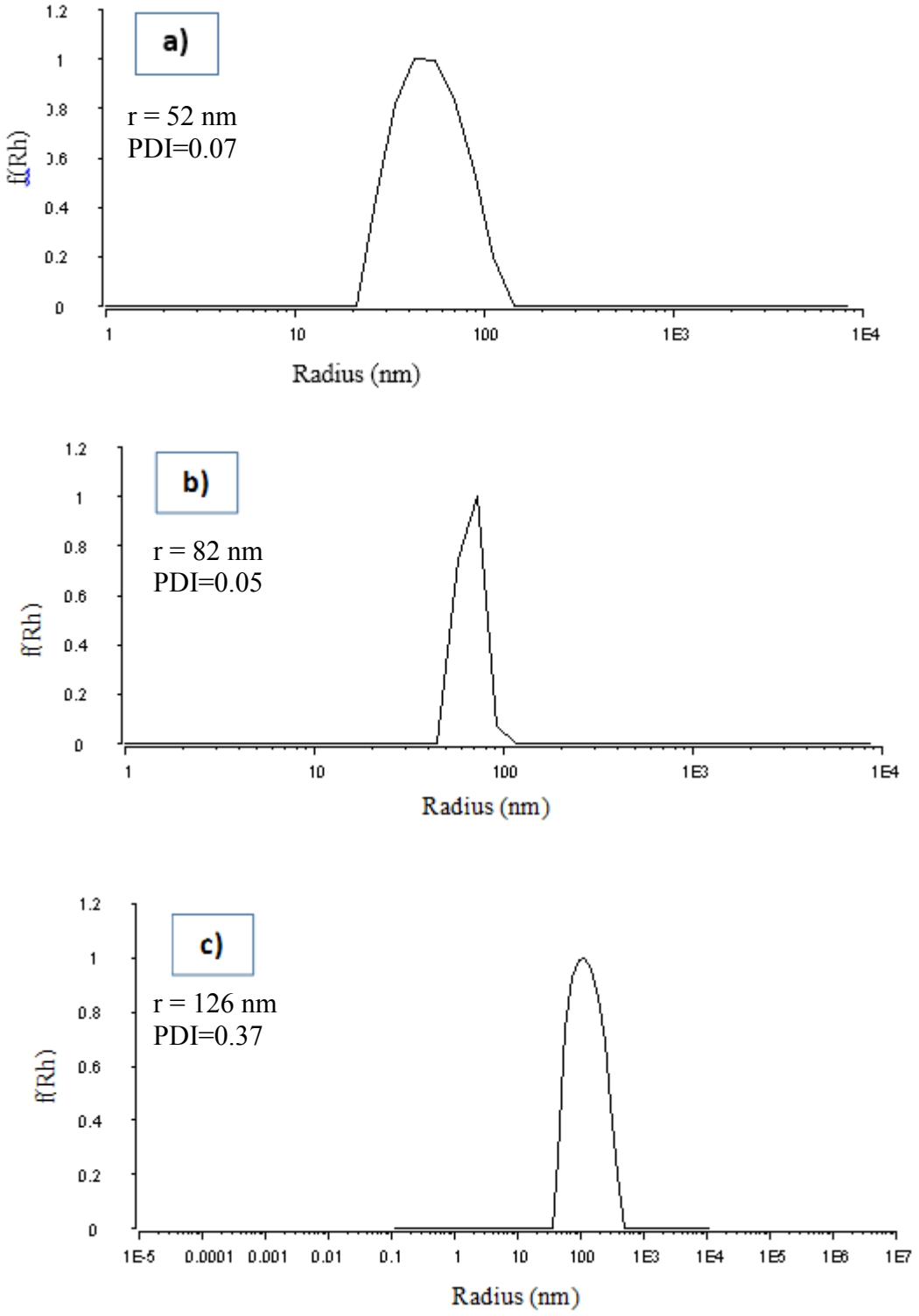


Figure 5.3. Hydrodynamic radius distribution of PDEA microgel respectively a) RM-Mi-9, b) RM-Mi- 30, c) RM-Mi-24 (DLS) 24 °C, pH 7.0

5.2.1 pH studies of microgels

Hydrodynamic radius changes of PDEA microgels at pH=3, neutral, and pH=9 were determined by DLS measurements. It was observed that pH increase causes a decrease on the size of microgel due to deprotonation of tertiary amine residues of PDA as expected (Figure 5.4).

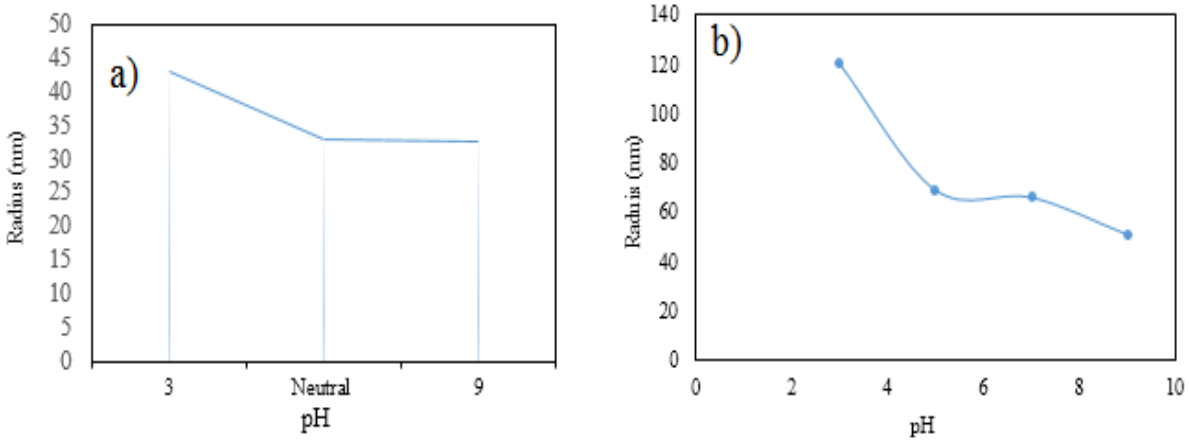


Figure 5.4. pH sensitivity study of PDEA microgel respectively a) RM-Mi-9, b)RM-Mi- 30 by DLS

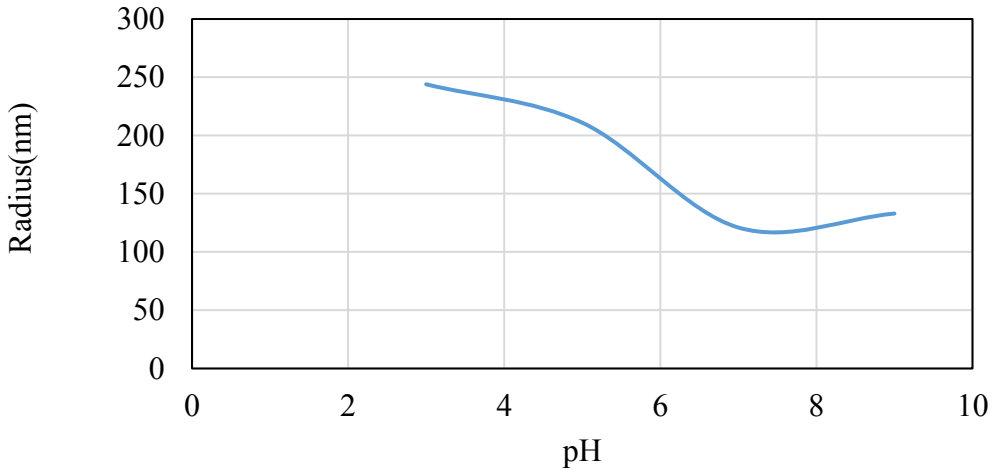


Figure 5.5. pH-responsiveness of P(NIPAM-*co*-DEA) microgel as measured with DLS studies.

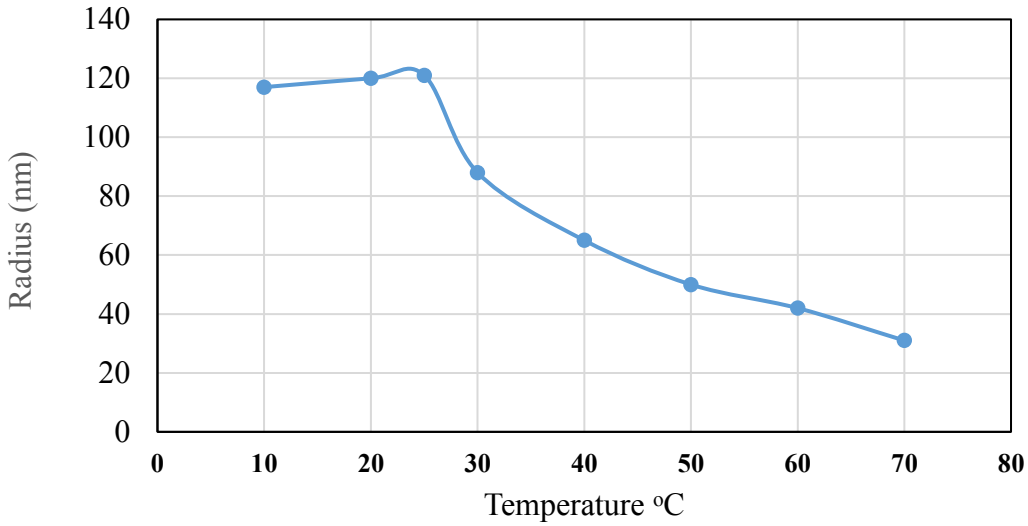


Figure 5.6. Temperature-responsiveness of P(NIPAM-*co*-DEA) microgel as measured with DLS studies.

The water molecules create hydrogen links with the polar polymer groups, and they form hydrophobic like iceberg water when they are below the LCST. As the LCST temperature rises, such hydrogen bonds are supplied to bulk and the network is destroyed by a large accumulation of entropy (Figure 5.6).

In pure water, PNIPAM has a lower critical solution temperature (LCST) of around 32 °C, which is near to human body temperature. The cross-linked PNIPAM is hydrophilic and absorbs water to a swelled form at temperatures below its LCST. The inflated state is caused by strong hydrogen bonds between PNIPAM and water below LCST, but the shrunken state is caused by interactions with PNIPAM that are stronger than the hydrogen bonds above LCST. For NIPAM temperature studies, as the temperature of the medium rises, these hydrogen bonds with water molecules break, and the cross-linked polymer chains gradually collapse.

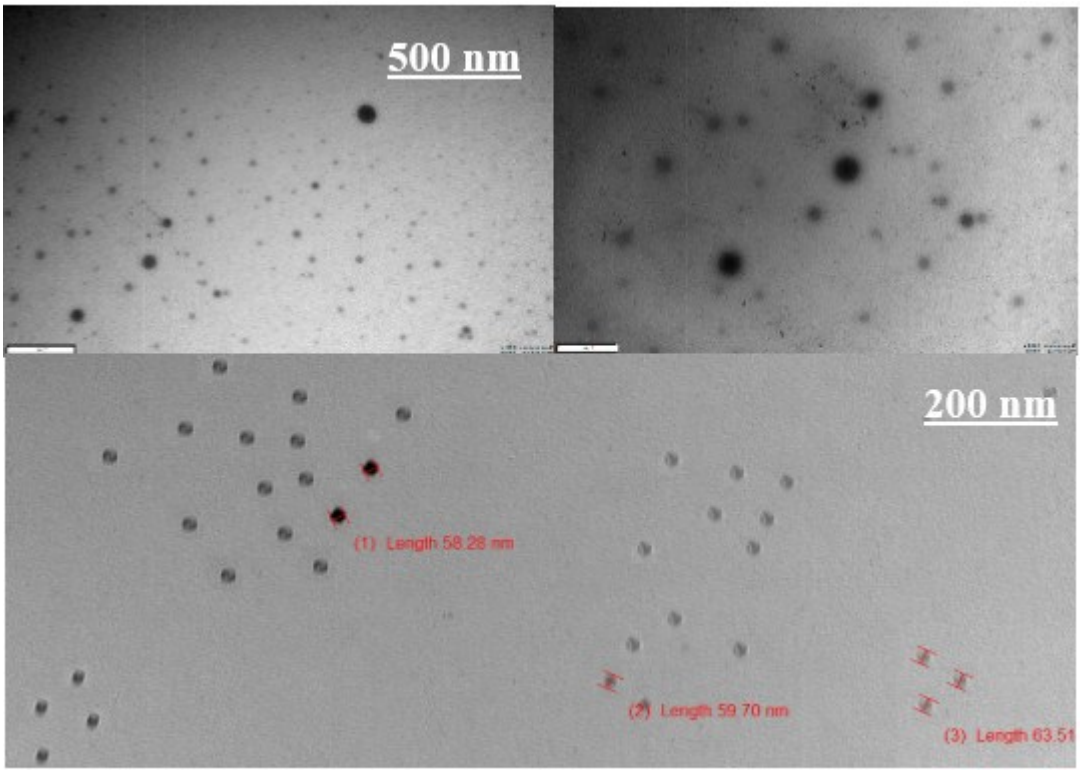


Figure 5.7. TEM images of PDEA microgel RM-Mi-9

For RM-Mi (5,6,9) compherchinon was dissolved in 5 ml of ethanol and used as initiator for photo emulsion polymerization. For the RM-Mi-5 the time of polymerization was about 30 minutes under the light which was not a stable microgel, while the time increased to 90 minutes the result became better but still, there were unreacted monomer/stabilizers which removed by the dialysis process. For the RM-Mi-9 experimental code, the reaction time increased to 3 hours, as the results show the RM-Mi-9 microgel exhibited is a great feature for loading to the hydrogel (Figure 5.6). However, it was not a perfect sample for the drug delivery system.

By the time, using (β-$PDMA_{75}$-$PDEA_{25,}$ Mn= 25000 g/mol, PDI=1.86) diblock copolymer as stabilizer for RM-Mi-(25, 26, 27, 28) experimental code. In addition, the size of the microgel directly changes according to the amount of stabilizer, the amount of monomer, cross-linker, initiator, and stabilizer presented in Table 5.2.

Table 5.2. Amount of monomer, solvent, initiator, stabilizer and cross-linker for producing DEA microgel

Experiment code	Monomer DEA(ml)	Solvent H_2O(ml)	Cross-linker MBA(gr)	Initiator APS(gr)	Stabilizer (gr)
Rm-Mi-(5,6,9)	1.25	50	0.01	0.01	β- DMA68-DEA29 =(0.05)
RM-Mi-26	2.5	50	0.025	0.025	β- $PDMA_{75}$-$PDEA_{25}$ =(0.1)
RM-Mi-26	2	50			β- $PDMA_{75}$-$PDEA_{25}$ =(0.1)
RM-Mi-27	1.25	50	0.01	0.01	β- $PDMA_{75}$-$PDEA_{25}$ =(0.05)
RM-Mi-28	1.25	60	0.01	0.01	β-$PDMA_{75}$-$PDEA_{25}$ =(0.025)
RM-Mi-29	1.25	60	0.01	0.01	β-$PDMA_{64}$-$PDEA_{36}$ =(0.025)
RM-Mi-30	1.25	70	0.01	0.01	β- $PDMA_{64}$-$PDEA_{36}$ =(0.025)

For the RM-Mi-25 the amount of stabilizer was more than the monomer and there were too many micelles in the solution which caused the sedimentation of the microgel, while the amount of monomer decreased to 2 ml in RM-Mi-26, but the sedimentation of microgel was observed. However, the color was still milky.

The RM-Mi-27 microgel produced with the 300 nm radius which was not the expectable size of the microgel for future studies. To control the size of the microgel the amounts of water and stabilizer were decreased (RM-Mi-28) but the result was not as we wanted for this work.

Using of (β-DMA_{64}-DEA_{36}) copolymer as stabilizer present as an ideal stabilizer for the synthesis of PDEA microgel as in RM-Mi-29, DLS result shows the size of microgels were nearly the same (r=113 nm, PDI= 0.09). As we know changing even one of the parameter can affect the result of the microgel, in a similar way the amount of water were increased to 70 ml (Rm-Mi-30) (Figure 5.7). Last but not least, the size of PDEA microgel decreased to (r= 67 nm, P.D.I=0.04) which were measured by DLS. The PDEA (RM-Mi-30) microgel was also characterized by Transmission Electron Microscopy (TEM). For better and more specific viewing of the PDEA microgel TEM image, the sample was colored by (1 mmol CsCl).

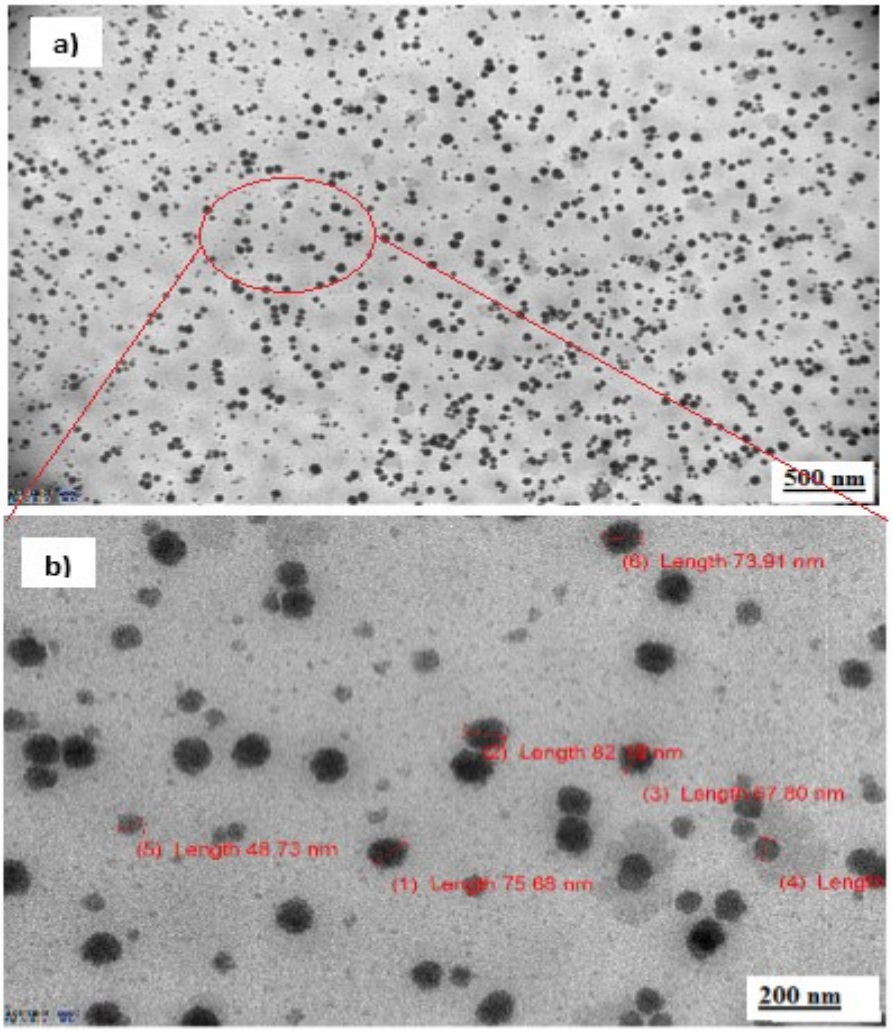


Figure 5.8. The TEM image of PDEA microgel (RM-Mi-30)

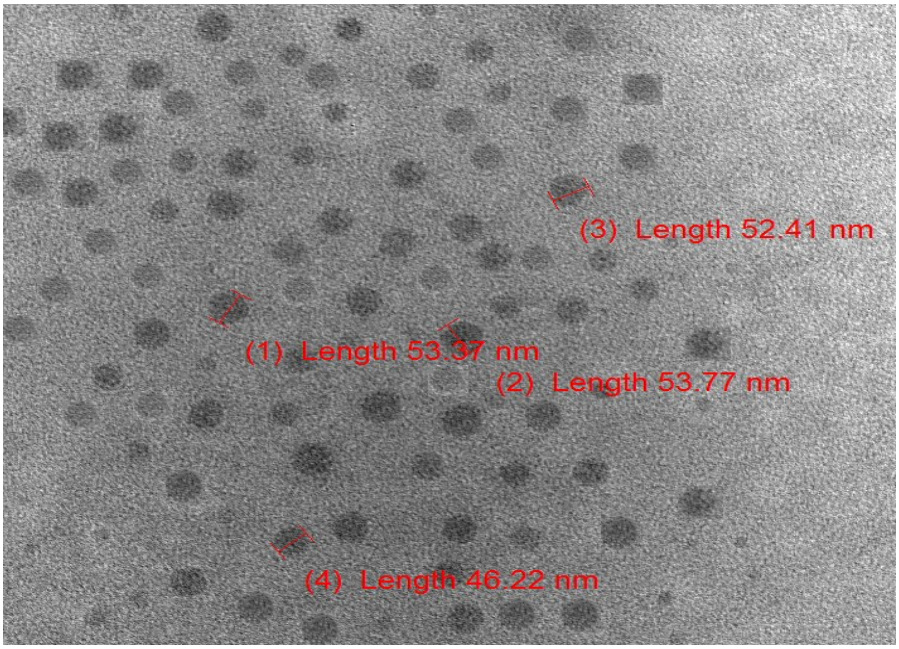


Figure 5.9. The TEM image of P(NIPAM-*co*-DEA) microgel

5.3. Characterization of P(DMA/AA) Hydrogel Containing PDEA Microgel

When microgel is located into a hydrogel, it is expected to cause significant changes in some chemical and physical properties of the hydrogel (Figure 5.8), such as swelling, flexibility, softness-hardness and stability. For this purpose, the synthesis of PDEA microgel doped P(DMA/AA) hydrogel (Figure 5.11 and Figure 5.12) was successfully carried out by simply adding the PDEA microgel to the medium before gel formation.

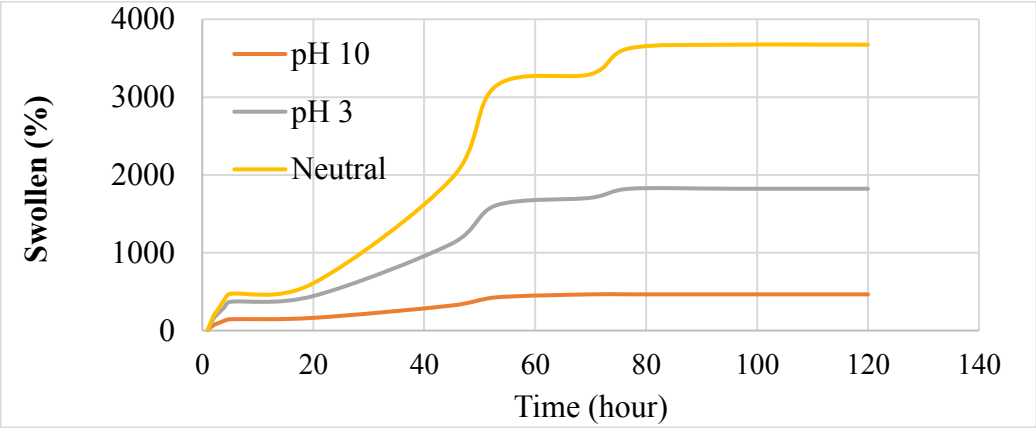


Figure 5. 10 Swelling of PDEA microgel containing P($DMA_{0.46}$/$AA_{0.54}$) hydrogel (RM101) at different pHs.

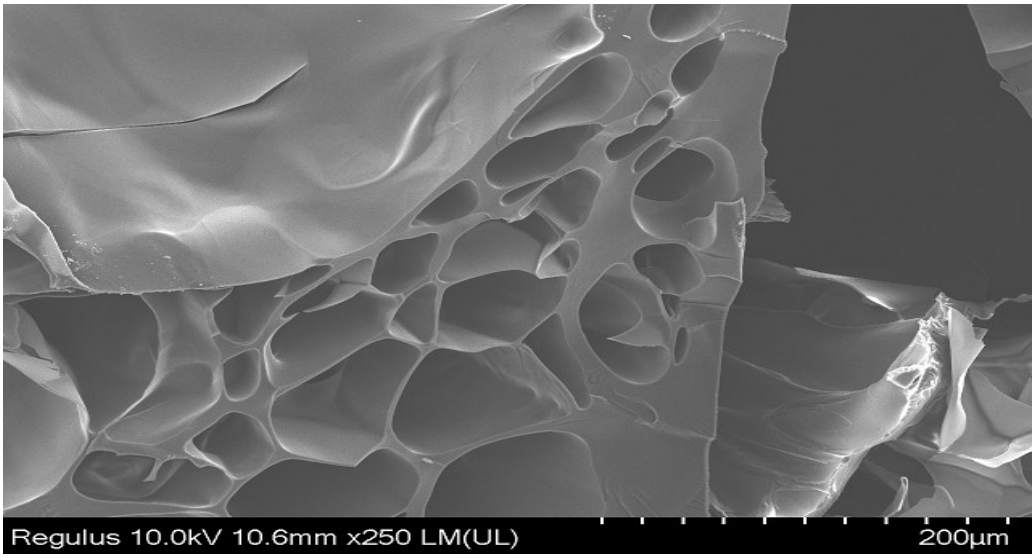


Figure 5.11. SEM image of P($DMA_{0.46}$/$AA_{0.54}$) hydrogel.

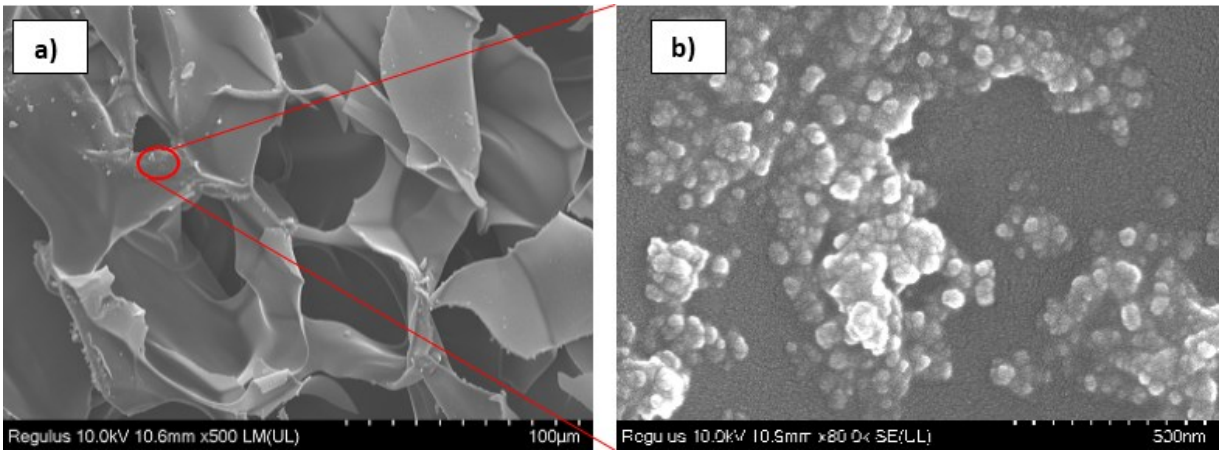


Figure 5.12. SEM images: a) P($DMA_{0.46}$/$AA_{0.54}$) hydrogel, b) PDEA (RM-Mi-9) micro particles in hydrogel sample.

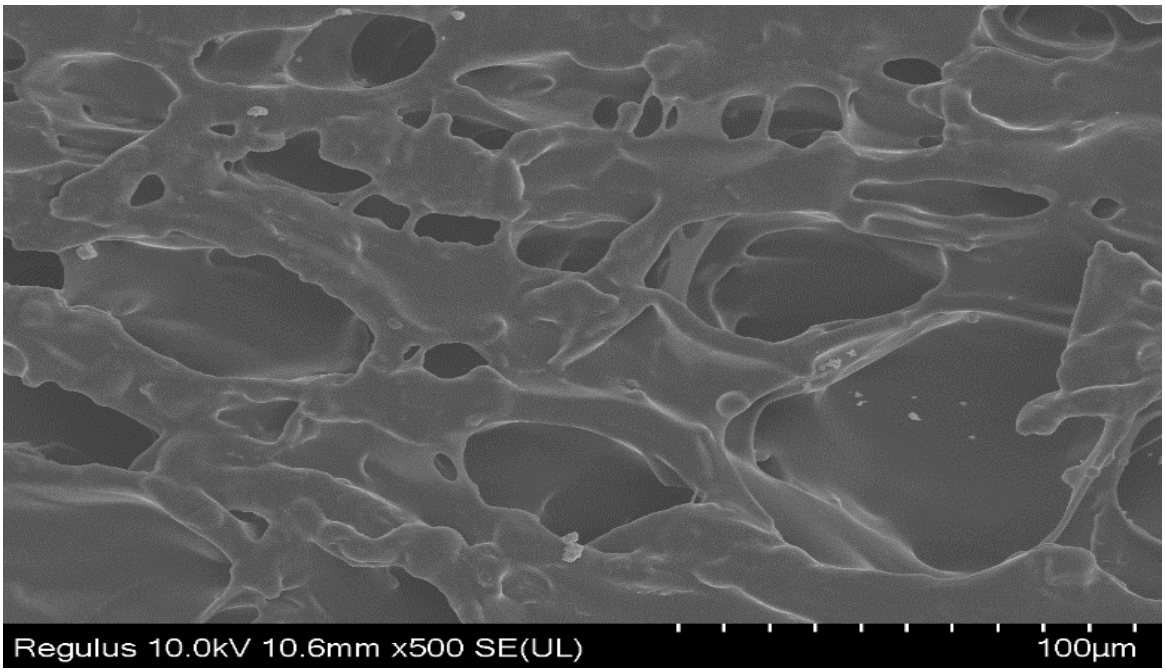


Figure 5.13. SEM image of P($DMA_{0.46}$/$AA_{0.54}$) RM120, hydrogel containing AuNPs/ P(NIPAM-*co*-DEA) (RM-Mi-24) microgel.

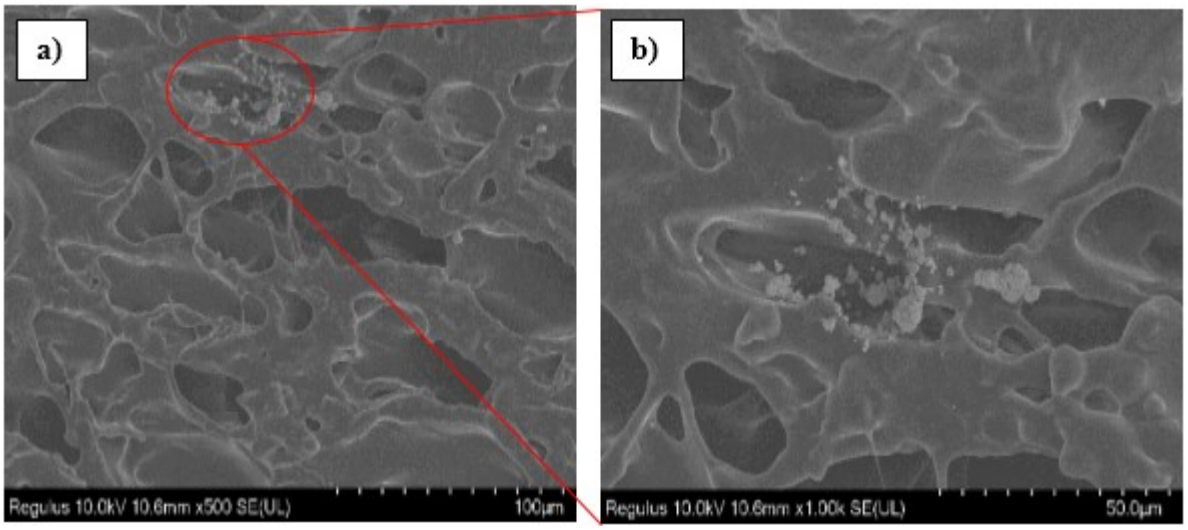


Figure 5.14. SEM images a) P($DMA_{0.46}$/$AA_{0.54}$) hydrogel containing AuNPs/P(NIPAM-*co*-DEA) (RM-Mi-24) at 100 µm, b) AuNPs/P(NIPAM-*co*-DEA) micro particles in hydrogel sample at 50 µm.

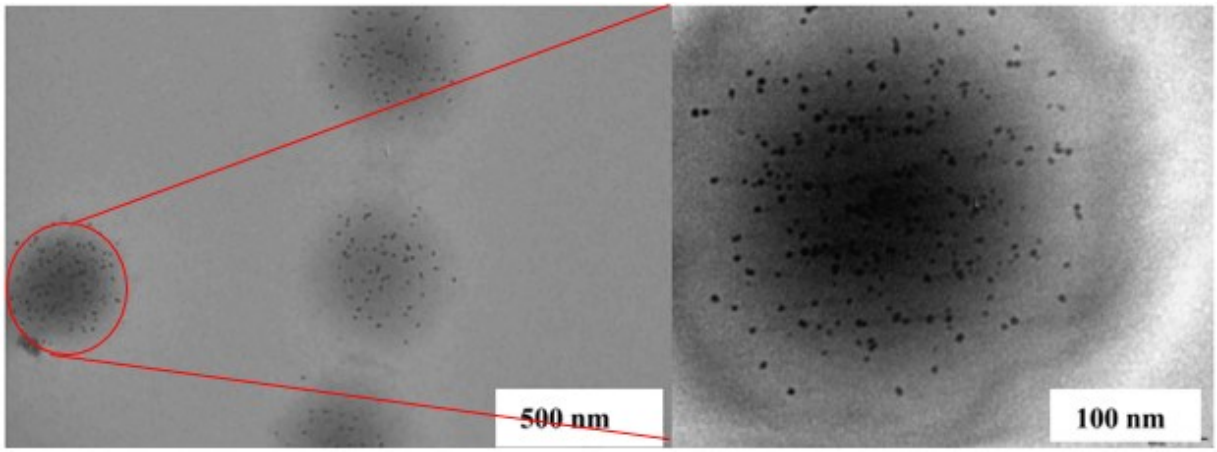


Figure 5.15. TEM image of AuNPs/ P(NIPAM-*co*-DEA) microgel (R-AuNPs-2).

AuNPs were successfully produced inside the microgel, as shown in the Figures 5.15. By managing the amount of $HAuCl_4.3H_2O$ used during synthesis, maximum AuNPs loading was achieved within the microgel. The diameter of the microgel increased when the amount of $HAuCl_4.3H_2O$ was increased. The average size of AuNPs was found to be between 10-15 nm (Figure 5. 16, and Figure 5.18).

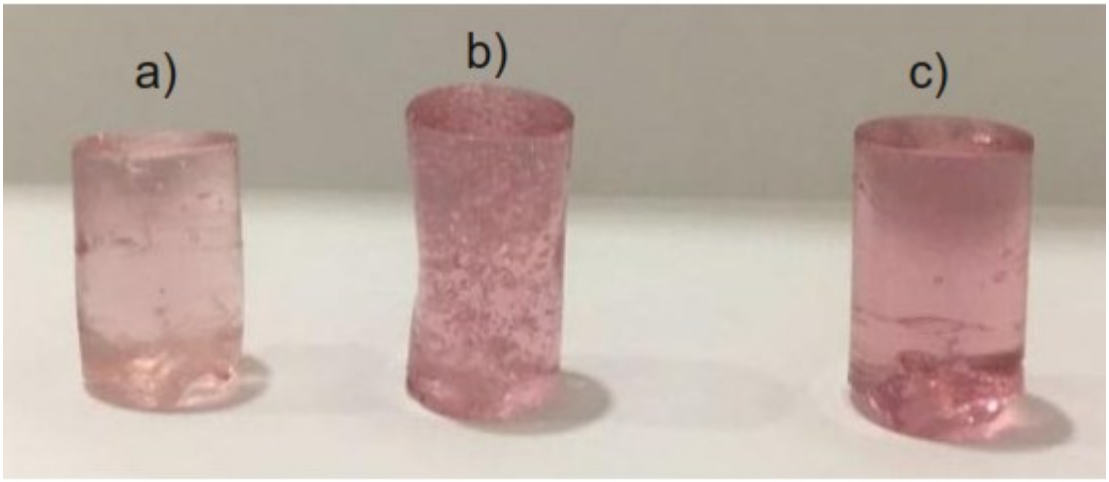


Figure 5. 16. Image of Au located P(NIPAM-co-DEA) microgel in $P(DMA_{0.46}/AA_{0.54})$ hydrogel matrix. a) R-AuNPs-3, b) R-AuNPs-2, c) R-AuNPs-1.

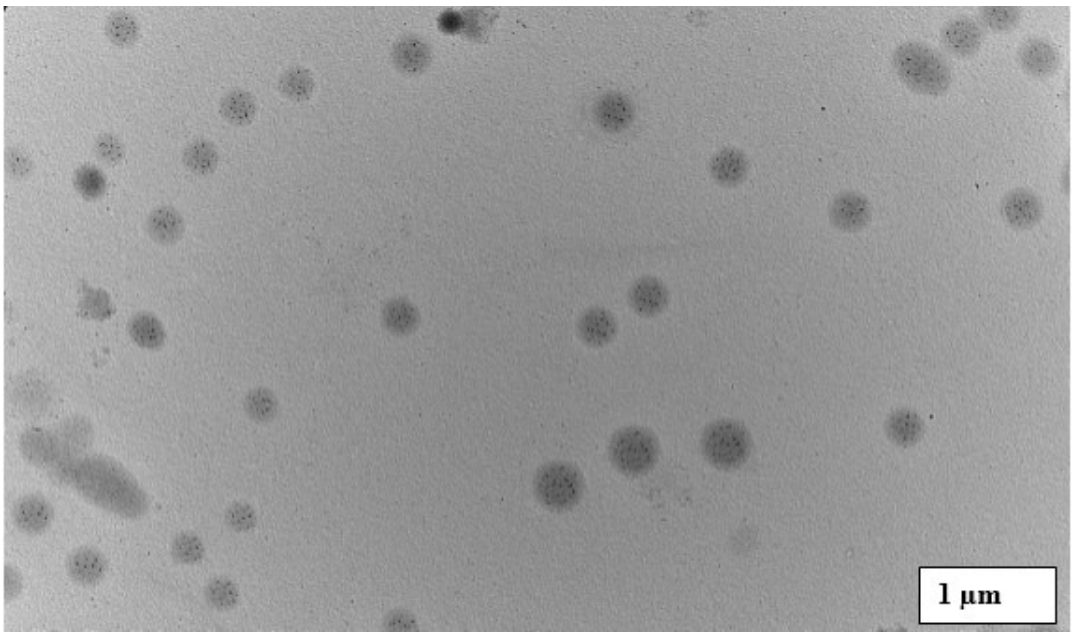


Figure 5.17. TEM image of AuNPs/ P(NIPAM-*co*-DEA) microgel (R-AuNPs-3)

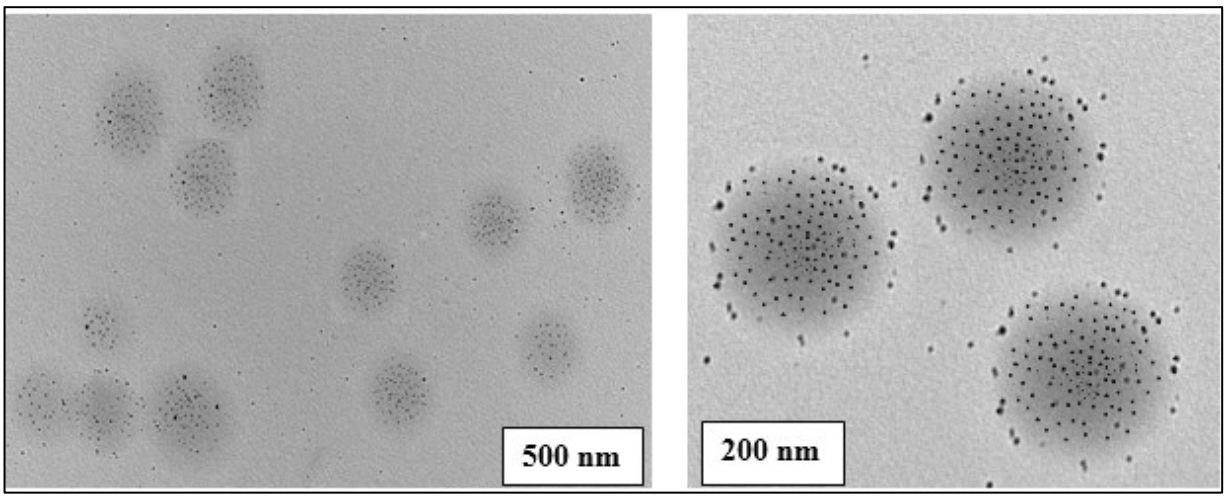


Figure 5.18. TEM image of AuNPs/ P(NIPAM-*co*-DEA) microgel (AuNPs-3)

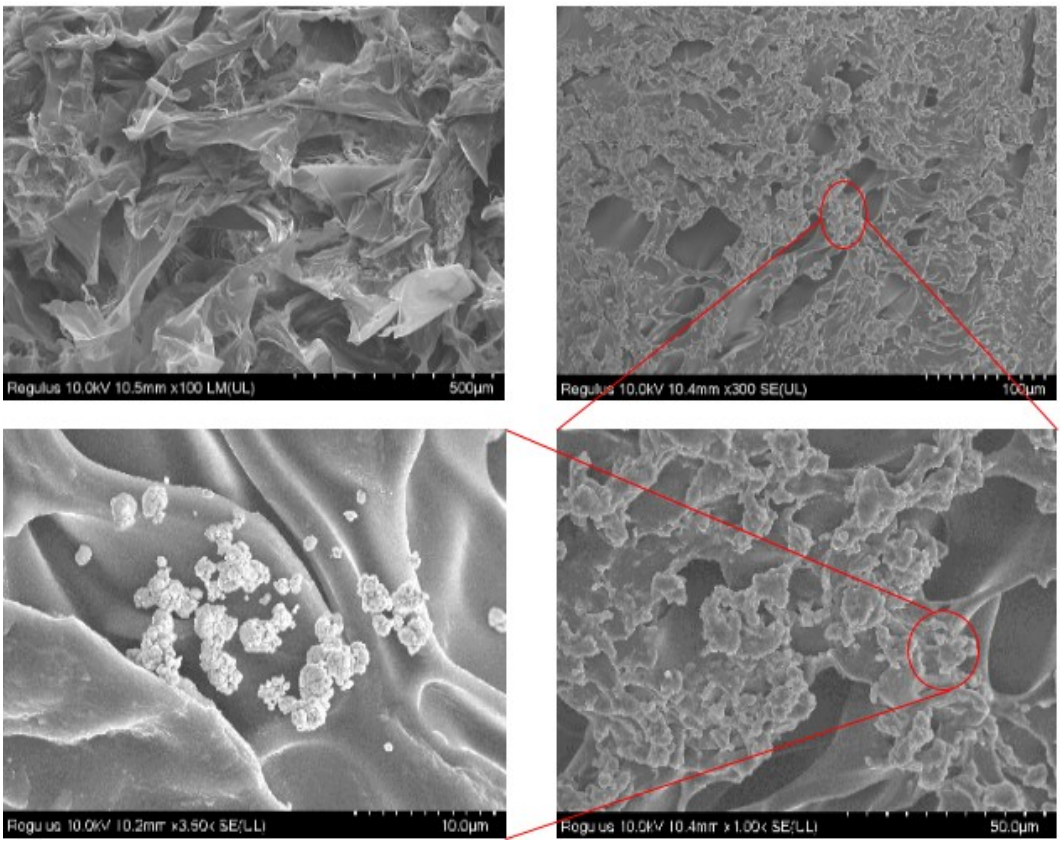


Figure 5.19. SEM image of P($DMA_{0.46}$/$AA_{0.54}$) hydrogel containing P(NIPAM-*co*-DEA) microgel having AuNPs

5.4. Calculation of Controlled Drug Release Studies

To determine the amount of drug release, the maximum absorption values of 5-FU corresponding to the wavelength was recorded and due to the amount of 5-FU released the 5-FU calibration curve was calculated and the resulting values were graphed. The calibration curve equation is defined as y = 0.5048 x-0.0687.

Table 5.3. Essential properties for calculation of drug release system

Number	Parameter	Amount
1	Wavelength	272 nm
2	Drug	5-FU
3	Dissolution Medium	Distillated water
4	Slope	0.5048
5	Intercept	-0.0687
6	Dilution Factor	0.04
7	Volume	50 ml

Loading efficiency of 5-FU into of ($DMA_{0.46}/AA_{0.54}$) hydrogel containing P(NIPAM-*co*-DEA) microgel having AuNPs was calculated as follows:

Concentration of drug (COD) (µg/ml)= [(Absorbance) ± intercept]/slope

COD. (x) µg/ml = [y (Absorbance)+ 0.0687]/0.5048

Dissolution basket volume = 50ml

Drug loaded to the hydrogel = 0.02mg and the weight of hydrogel = 0.089 mg

The result of drug release calculation is shown in Table 5.3**.**

Dilution factor = Drug load / Dissolution basket volume = 0.02/50 = 0.0004

Amount of drug released mg/ ml = Concentration × Dissolution bath volume × dilution factor/1000.

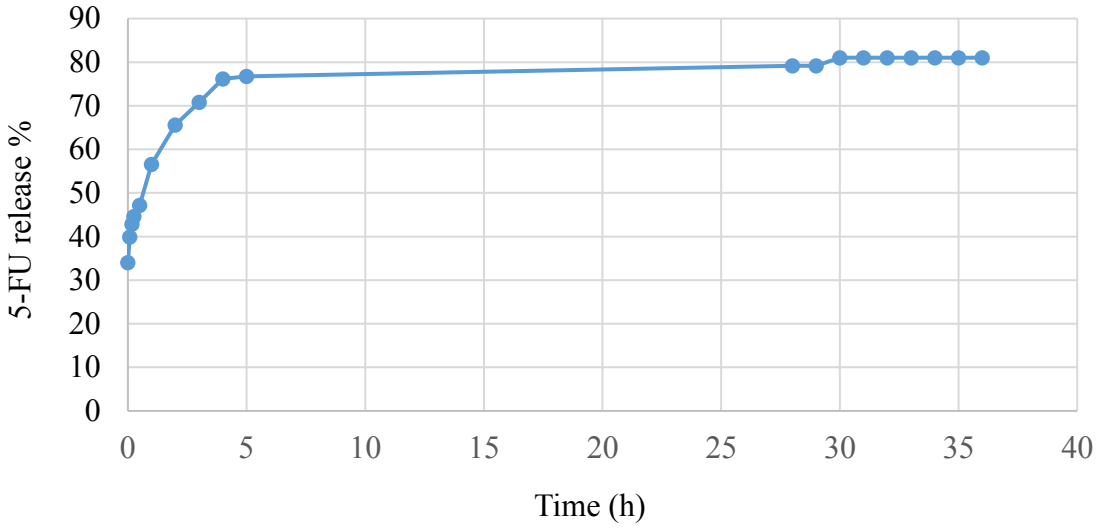


Figure 5.20. Releasing 5-FU drug over time of P($DMA_{0.46}$/$AA_{0.54}$) gel in H_2O environment

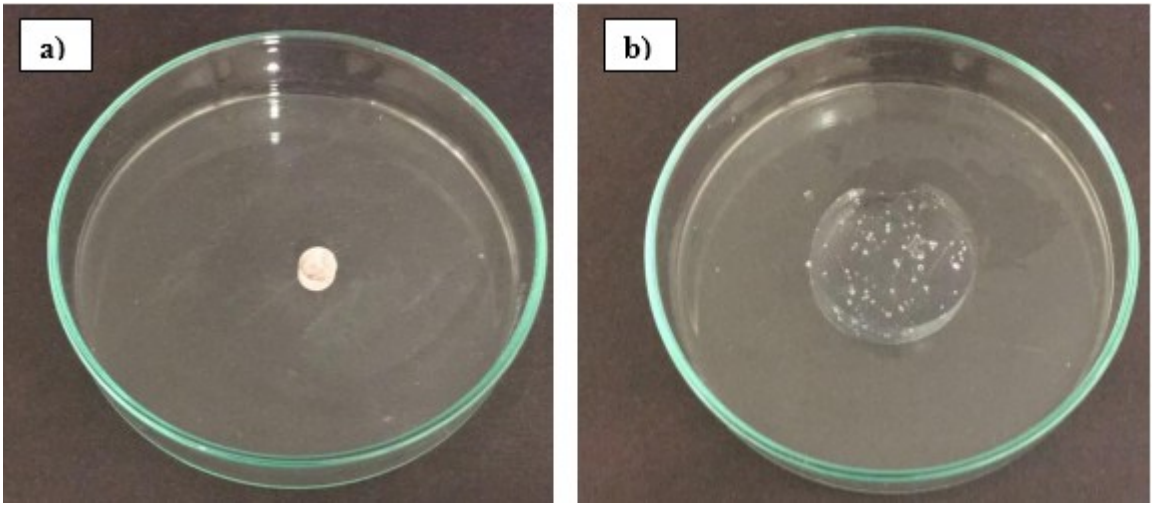


Figure 5.21. Gel image of P(DMA/AA) hydrogel (RM123) a) hydrogel containing 5-FU before release; b) hydrogel containing 5-FU after release

Table 5.4. Amounts of 5-FU released and % drug release values

Number	Time (h)	Absorbance (A)	Concentration (μg/ml)	Amount of drug release (mg)	Amount of drug release (%)
1	0	0	0	0	0
2	0.0833	0.0119	0.15967	0.00798	39.9168
3	0.1666	0.0178	0.17135	0.00857	42.8387
4	0.25	0.0213	0.17829	0.00891	44.5721
5	0.5	0.0266	0.18879	0.00944	47.1969
6	1	0.0455	0.22623	0.01131	56.5571
7	2	0.0638	0.26248	0.01312	65.62
8	3	0.0743	0.28328	0.01416	70.8201
9	4	0.085	0.30448	0.01522	76.1193
10	5	0.0862	0.30685	0.01534	76.7135
11	28	0.0912	0.31676	0.01584	79.1898
12	29	0.0912	0.31676	0.01584	79.1898
13	30	0.0949	0.32409	0.0162	81.0222
14	31	0.0949	0.32409	0.0162	81.0222
15	32	0.0949	0.32409	0.0162	81.0222
16	33	0.0949	0.32409	0.0162	81.0222
17	34	0.0949	0.32409	0.0162	81.0222
18	35	0.0949	0.32409	0.0162	81.0222
19	36	0.0949	0.32409	0.0162	81.0222

6. CONCLUSION AND RECOMMENDATION

Hydrogels are three-dimensional interconnected matrices that have recently gained popularity as a biomedical material. The gel has capacity to absorb biological fluids explains its biocompatibility and usage in a variety of therapeutic purposes, including medication administration and encapsulation. One of the most critical fields of investigation nowadays is drug delivery, and one of the primary issues is the creation of biocompatible, flexible, and durable materials. As a result, hydrogels are an excellent choice for various applications. Moreover the result of this research could briefly listed below.

1. Both (PDEA and P(NIPAM-*co*-DEA)) microgel and (PDEA-P(DMA/AA), P(NIPAM-*co*-DEA)-P(DMA/AA)) hybrid hydrogels system having pH responsive natural were synthesized. Characterizations of microgels were carried out with DLS and SEM techniques. The hydrogel system was also characterized with SEM studies. The pH and temperature responses of PDEA and P(NIPAM-*co*-DEA) microgel were also studied with DLS. Swelling and deswelling behaviors of hydrogels were investigated depending on pH change.

2. Characterization of hydrogel was done based on its physical properties such as softness/hardness, flexibility, split, stability, swollen which showed in Table 5.1. Among P($DMA_{0.75}$/$AA_{0.25}$), P($DMA_{0.46}$/$AA_{0.54}$), and P($DMA_{0.25}$/$AA_{0.75}$) the P($DMA_{0.46}$/$AA_{0.54}$) hydrogel were selected for loading of microgel, AuNPs/microgel and drugs.

3. PDEA microgels have been prepared mostly by emulsion polymerization, effected in the presence of various stabilizers showed in Table 5.2.

4. In P(NIPAM-*co*-DEA) as the temperature of the medium rises, these hydrogen bonds with water molecules break, and the cross-linked polymer chains gradually collapse.

5. PDEA and P(NIPAM-*co*-DEA) microgel with (RM-Mi(9,24,30)) experimental code were successfully loaded to the P($DMA_{0.46}$/$AA_{0.54}$) hydrogel.

6. Swelling of PDEA microgel containing P($DMA_{0.46}$/$AA_{0.54}$) hydrogel (RM101) at pH 7 higher in contrast to basic and acidic environment (pH 3 and pH 10).

7. TEM images of microgels were obtained and these images were found to support PDI values obtained with DLS.

8. Locating AuNPs inside P(NIPAM-*co*-DEA) microgel were successfully done with the amount of 0.2 and 0.1 mg AuNPs and the result were proved by TEM images.

9. AuNPs/P(NIPAM-*co*-DEA) microgel were successfully added to P($DMA_{0.46}$/$AA_{0.54}$) hydrogel by using free radical polymerization and the result was proven by SEM images.

10. Taking into account the data in the Table 5.4 and Figure 5.19 **:**
    - In the loading study, it was found that 0.089 g P($DMA_{0.46}$/$AA_{0.54}$) gel containing 0.1% MBA cross-linker (RM123), with exact 0.02 mg 5-FU was loaded. It was observed that 76% of loaded 5-FU was released into the water solution medium within the first 5 hours.
    - The amount of emission increased during the first 5 hours is almost constant over the next 8 hours.
    - The absorbance value after 25 hours was found to be the same as the measurement after 36 hours and the exact release rate was confirmed to be 81%.

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