MICELLAR DRUG DELIVERY PLATFORM FROM DIBLOCK COPOLYMERS

by

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B.S., Chemistry, Yıldız Technical University, 2016

Submitted to the Institute for Graduate Studies in
Science and Engineering in partial fulfillment of
the requirements for the degree of
Master of Science

Graduate Program in Chemistry
Boğaziçi University

2019

ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude to my thesis supervisor Prof. Dr. Rana SANYAL for her endless patience, attention and scientific guidance throughout this study. I appreciate her support and useful comments throughout my laboratory work.

I would like to express my great thanks to Özgül Gök, for her care during my whole research and laboratory work. I also thank my all labmates, especially Rüveyda Kılıç for being more than a labmate.

I would like to thank my mother Nursel Kavak, my father Fuat Kavak and my brother Mert Kavak for their support, care and endless love.

I wish to thank my dear friends Gülberk Yılmaz and Aykut Akın for their deepest friendship, support and love at every stage of my not only thesis but my life.

I would like to thank dear Anıl Cirav for his endless support.

I want to thank TUBITAK 2210-C for scholarship.

ABSTRACT

MICELLAR DRUG DELIVERY PLATFORM FROM DIBLOCK COPOLYMERS

The main role of chemotherapeutic agents is killing the fast growing cells. Thus, they damage fast growing helathy cells like bone marrow as well. This harm can be prevented by modifying the existing chemotherapeutic agents that are already in clinical use to provide their accumulation into tumor.

In this thesis, preparation of polymers sensitive to two different stimuli and the demonstration of their sensitivity and functionality were investigated. The synthesis of polymers are both redox- and hydrolysis-sensitive. The prepared block copolymers were functionalized by active drug molecules. All obtained structures were characterized via size exclusion chromatography and nuclear magnetic resonance. The formation of micellar structures from these block copolymers were studied via dynamic light scattering.

ÖZET

BLOK KOPOLİMERLERDEN MİSEL TİPİ İLAÇ SALIM PLATFORMU

Kemoterapi ajanları hızlı üreyen hücrelerini öldürmek amaçlı çalıştıtıkları için vücutta hızlı üreyen kemik iliği gibi diğer hücrelere de zarar vermektedirler. Klinikte kullanılmakta olan kemtorapi ajanlarının modifiye edilerek tümör içerisindeki birikiminin sağlanması bu zararın engellenmesini sağlayabilir.

Bu tez çalışmasında, iki farklı uyarana duyarlı polimerlerin hazırlanması ve duyarlılık ve fonksiyonelliklerinin gösterilmesi incelenmiştir. Polimerlerin sentezi hem redoks hem de hidrolize duyarlıdır. Hazırlanan blok kopolimerler aktif ilaç molekülleri ile fonksiyonalize edilmiştir. Elde edilen tüm yapılar, jel geçirgenlik kromatografisi ve nükleer manyetik rezonans spektroskopisi ile karakterize edildi. Bu blok kopolimerlerden elde edilen miselik yapılar ise dinamik ışık saçılması yöntemi ile karakterize edildi.

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1. INTRODUCTION

1.1.Cancer

Cancer is one of the fastest growing problems which effects people all around the World [1]. Cancer formation consists of the division of the cells by developing cancerous tumors in an uncontrolled way and without stopping.

Tumors form new blood vessels to obtain nutritions from human body, this formation is called angiogenesis [2].

Cancerous tumors are able to spread into the body by travelling thorugh the lymphatic system or blood stream. This ability causes different tumor formations into other parts of the body. Consequently, tumor becomes inasive and spreading of tumors is known as metastasis [2].

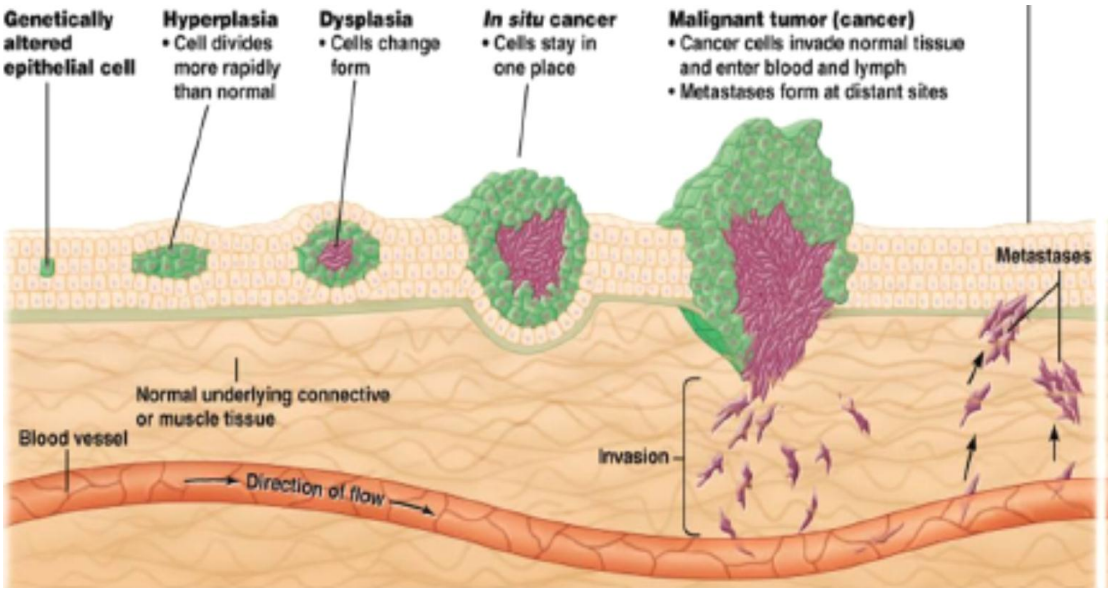

Figure 1.1. Cancer formation [3].

1.2.Nanomedicines in Cancer

Chemotherapy is still one of the most common treatments for cancer. However, poor solubility and lack of targeting of the existing anticancer drugs lead the treatment to the use of nanotechnology. Therefore, nanomedicines have been developed to overcome these obstacles [4]. This technology consists of the drug delivery systems that are based on nanocarriers having improved circulation time and bioavailability including extended half-life and distribution [5].

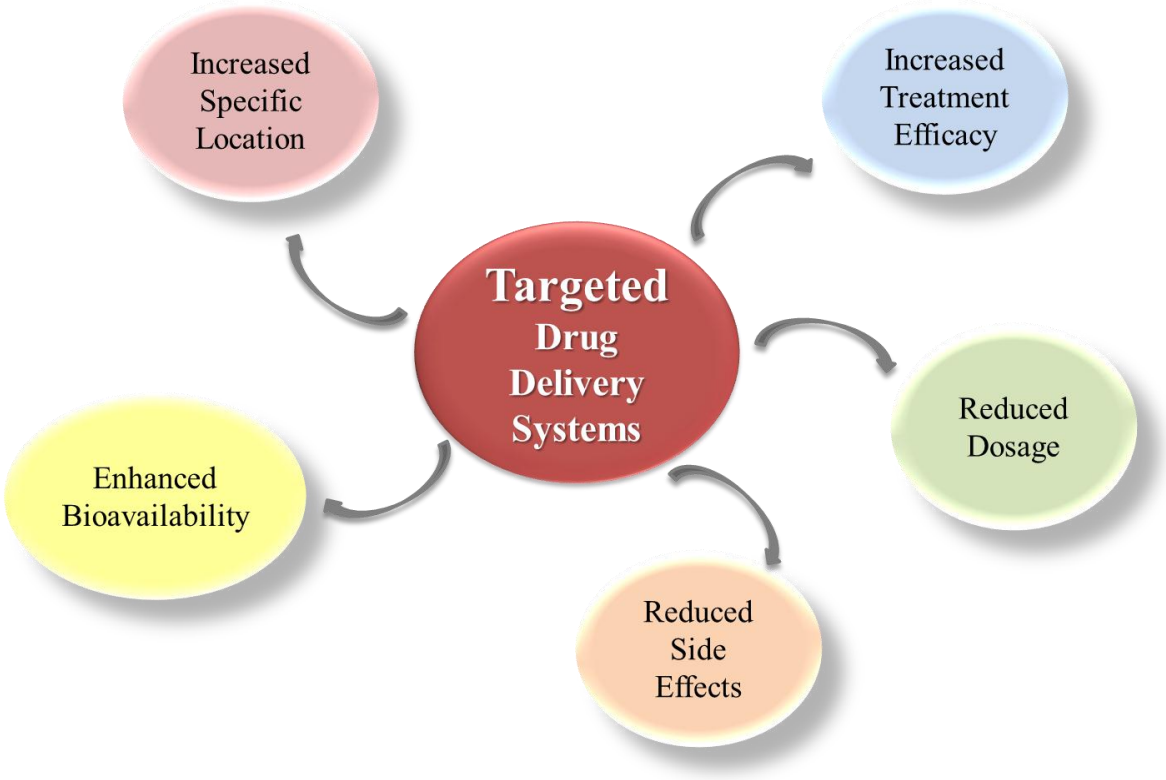

Figure 1.2. Advantages of targeted drug delivery systems.

Nanoparticles are mostly used as drug carriers for cancer treatment and made of biodegradable materials [6]. They have many examples such as gold nanoparticles, polymers, dendrimers, liposomes, polymeric micelles [7].

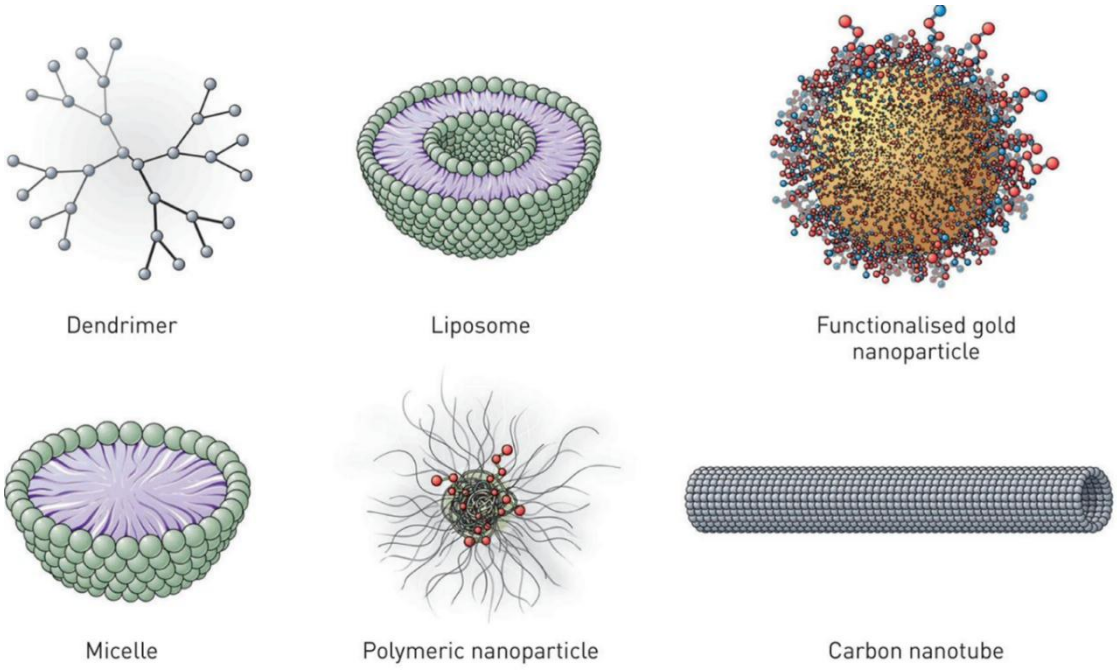

Figure 1.3. Nanostructured drug delivery systems [7].

Passive targeting is another and one of the most remerkable advantages of nanomedicines. There is a concept called EPR (enhanced permeability and retention) effect for passive targeting. Healthy human endothelial cells are elongated perfectly with certain gaps while cancerous endothelial cells are elongated defectively and have bigger gaps than healthy cells. This defection helps the passive targeting of nanoparticles which cannot enter the healthy cells and only get into the cancerous cells because of their bigger size.

Cancerous cells also lack of lymphatic drainage which allows the nanoparticle accumulation into them [9]. Thus, drug becomes more effective on cancerous cells by showing sustained and targeted release, and its side effects reduce thanks to passive targeting [10].

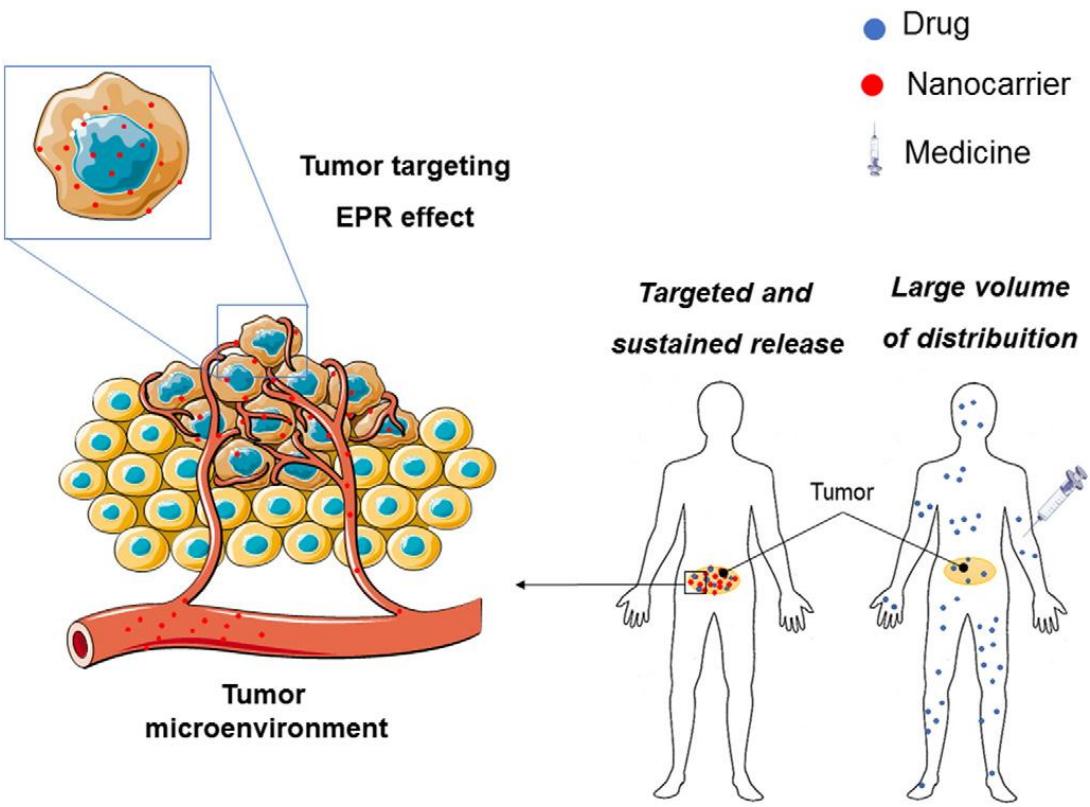

Figure 1.4. Passive targeting by EPR effect [8].

1.2.1. Polymeric Micelles

Polymeric micelles are made of amphiphilic macromolecules which have both hydrophilic and hydrophobic parts. They are self-assembled into aqueous solution while hydrophobic parts gather, hydrophilic domains disperse [11,12].

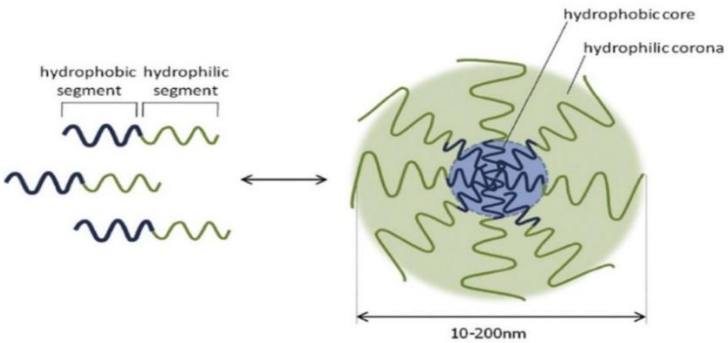

Figure 1.5. Micelle formation [13].

Micelle formation occurs after the concentration of block copolymers increase a specific concentration which is called critical micelle concentration (CMC) [13]. If a drug is loaded physically inside the micelle, CMC is important in terms of stability of micelle to prevent burst release while the drug is travelling into the blood stream. When micelle formation occurs, if concentration of polymers are lower than CMC, it can cause the fragmentation of micelles which result in dispersion of monomers in the body.

Moreover, CMC of the micelle is remarkable in terms of thermodynamic stability. Additionally, small CMC is wanted to be obtained because it means that when CMC is small, amount of polymer needed for micelle formation becomes less. [13,14].

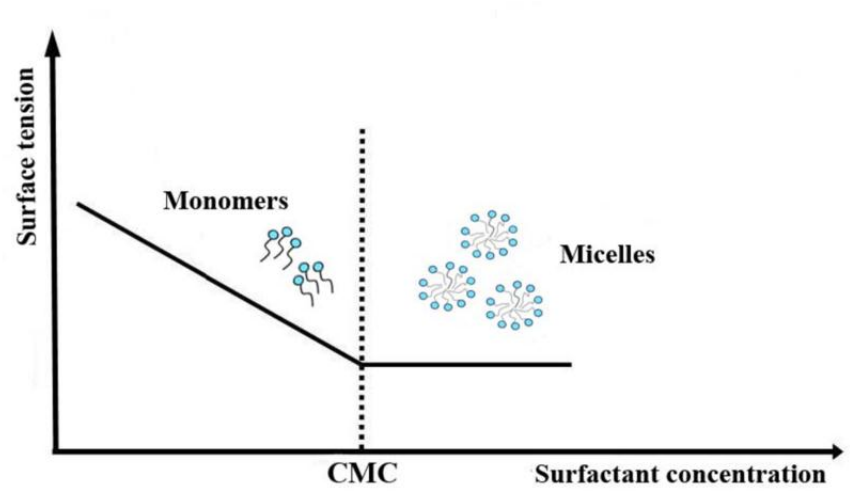

Figure 1.6. Graphical demonstration of micelle formation after reaching CMC.

CMC determination can be done by using Nile Red. Nile red is a pink colored hydrophobic compound and when encapsulated into micelles, it does not change its color.

However, when it is not entrapped into the micelle, it becomes colorless because of dissolution by the solvent. So that we can understand from its color change if the micelle formation is successful or not.

Polymeric micelles are promising agents for water insoluble drug deliveries. Hydrophobic drug molecules can be either covalently linked to the hydrophilic end of the polymer or physically entrapped inside the micelle. They provide specific targeting to the cancer cells and do not show cytotoxicity against healthy cells because of EPR effect. Moreover, polymeric micelles are made of polymers that only cleave the bonds to release the drug under specific conditions. They can be consisted of pH sensitive bonds, redox responsive bonds, enzyme sensitive bonds and so on [15,16].

Thus, drug delivery with polymeric micelles increase the bioavailability of the drugs by them only in the tumor site. Polymeric micelles also give the advantage of administering high doses without serious adverse effects. They, moreover, reduce the frequency of treatment thanks to sustained release [10,13].

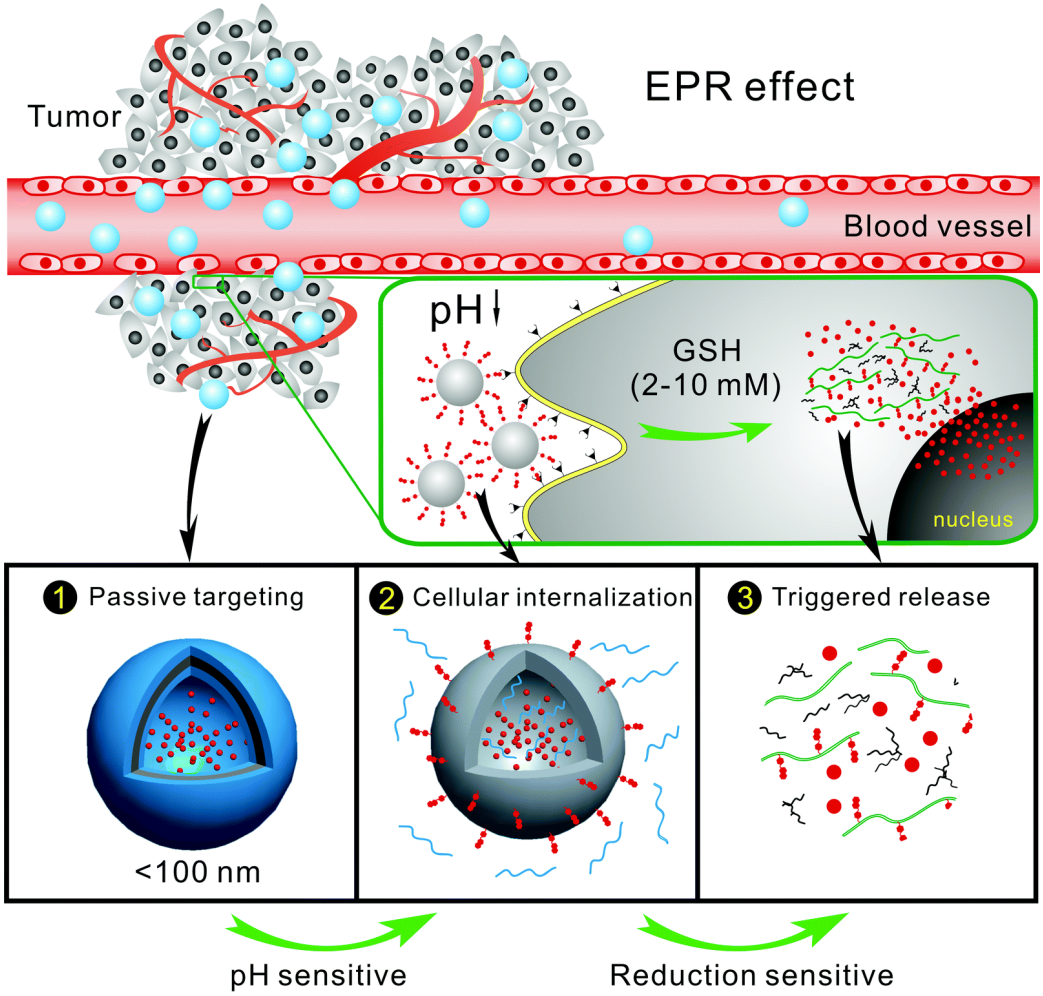

Figure 1.7. Triggered drug release from polymeric micelles [16].

Polymeric micelles have different types of structures which depend on the nature of copolymers and the environment that micelle is formed. Depending on the length of the hydrophilic and hydrophobic parts, micelles may be spherical, rod or lamelle. Polymeric micelles with various architectures are shown in Figure 1.8. [17].

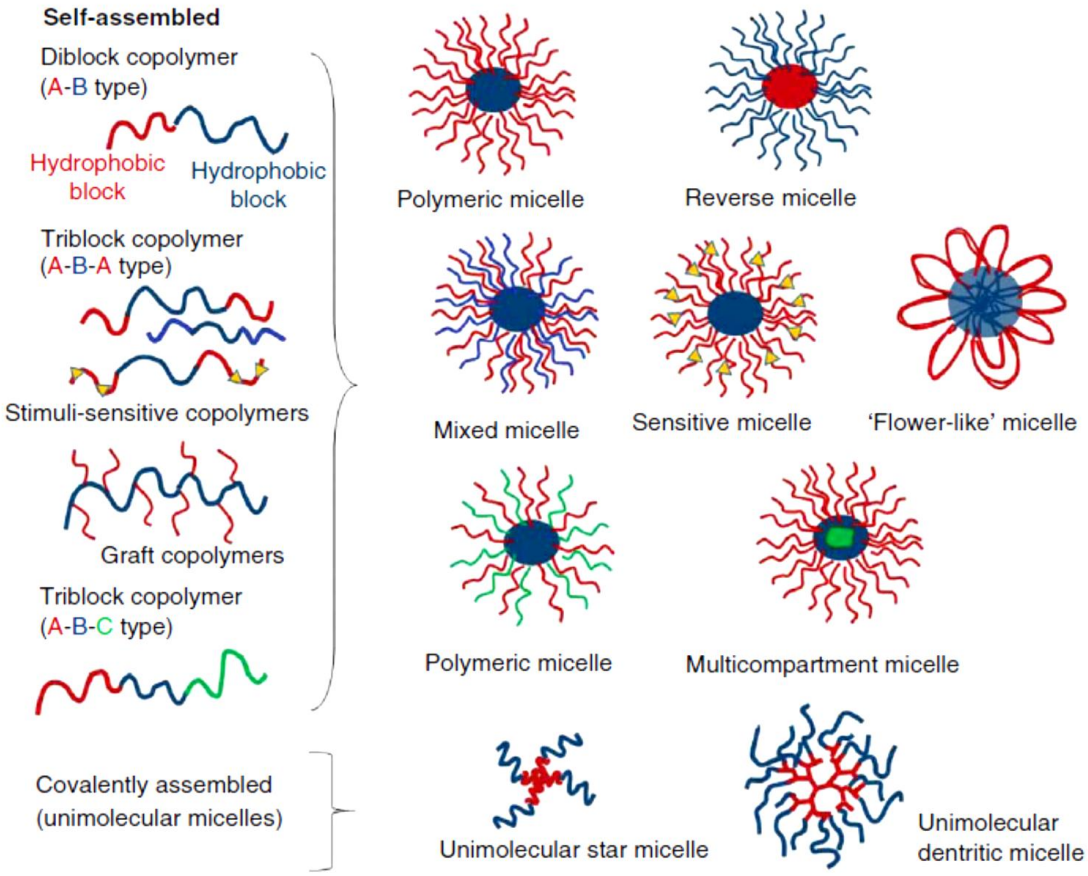

Figure 1.8. Types of polymeric micelles [17].

When polymers are put into nonaqueous media, reverse micelle formation is obtained. They are preferred when physiological conditions are not appropriate for micelles which are formed in aqueous media [18].

Another type of micelle is based on the mix of more than two copolymers which have the same blocks with different lengths or different blocks with the same molecular weights. Mixed micelles are favored because of having more thermodynamic stability than single copolymer micelles. Thus, this higher thermodynamic stability leads to the prevention of premature leakage of encapsulated drug [19].

Triblock copolymer micelles are composed of a long hydrophilic part and two small hydrophobic domains at the both ends of the hydrophilic part. Triblock copolymers form flower-like micelles in aqueous media. Hydrophobic drugs are dissolved in the core of these type of micelles resulting in sustained release. In contrast to other micelle forming methods, flower-like micelles do not require organic solvent dissolution [20].

Unimolecular micelles are formed from a single molecule that consists of several hydrophobic and hydrophilic parts. Unimolecular micelles Show a sustained release profile. However, CMC value of unimolecular micelles cannot be analyzed clearly [17].

Micelles can be formed as pH or temperature sensitive. Some types of copolymer linkages serve to make micelles vulnerable to, for example, pH changes. For instance, ester linkage is used in the structure of copolymer to make the micelle pH sensitive. Hydrazone or disulfide bond is used to make reduction responsive linkers [21].

Shuai *et al*. have developed a micelle whose polymers have dual sensitive to pH and reduction. When the micelle reaches to the tumor area, because of its acidity and high level of glutothione presence, drug release observed [22].

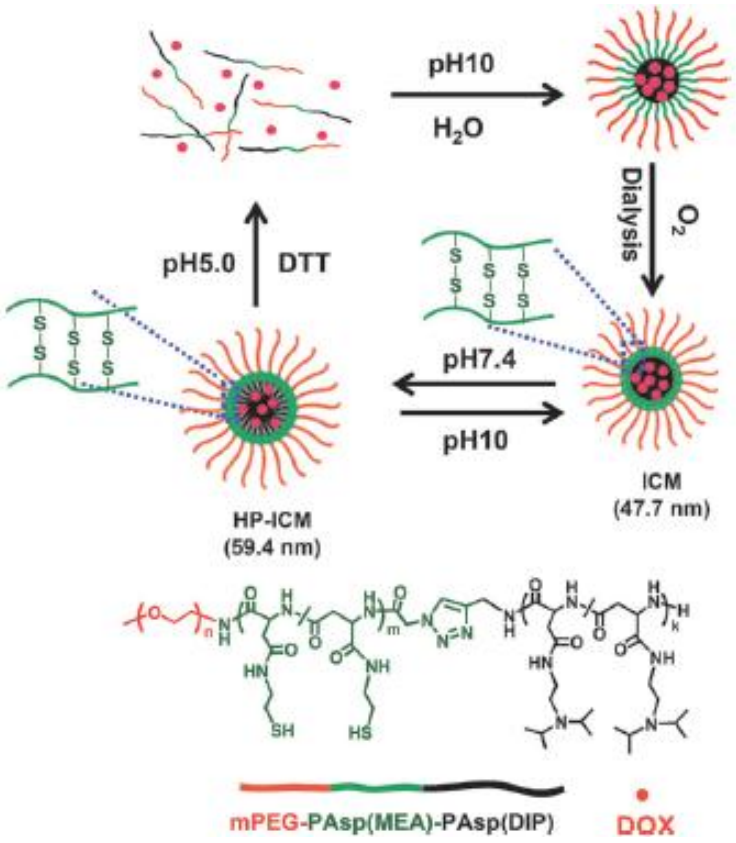

Figure 1.9. Formation and drug release from dual responsive micelle [22].

Synthesis of FA-PMgDP] block GAL-based copolymers by Zhao *et al.* is another example to the dual pH-redox sensitive micelles [23].

The poly(pyridyl disulfide methylacrylate) (PDEMA) units were redox-sensitive while poly[2-(diisopropylamino) ethylmethacrylate units were pH-sensitive.

pH-sensitiveness made the micelle show release in the tumor site and disulfide crosslinking prevented the drug leakage through the body and showed a perfect stability.

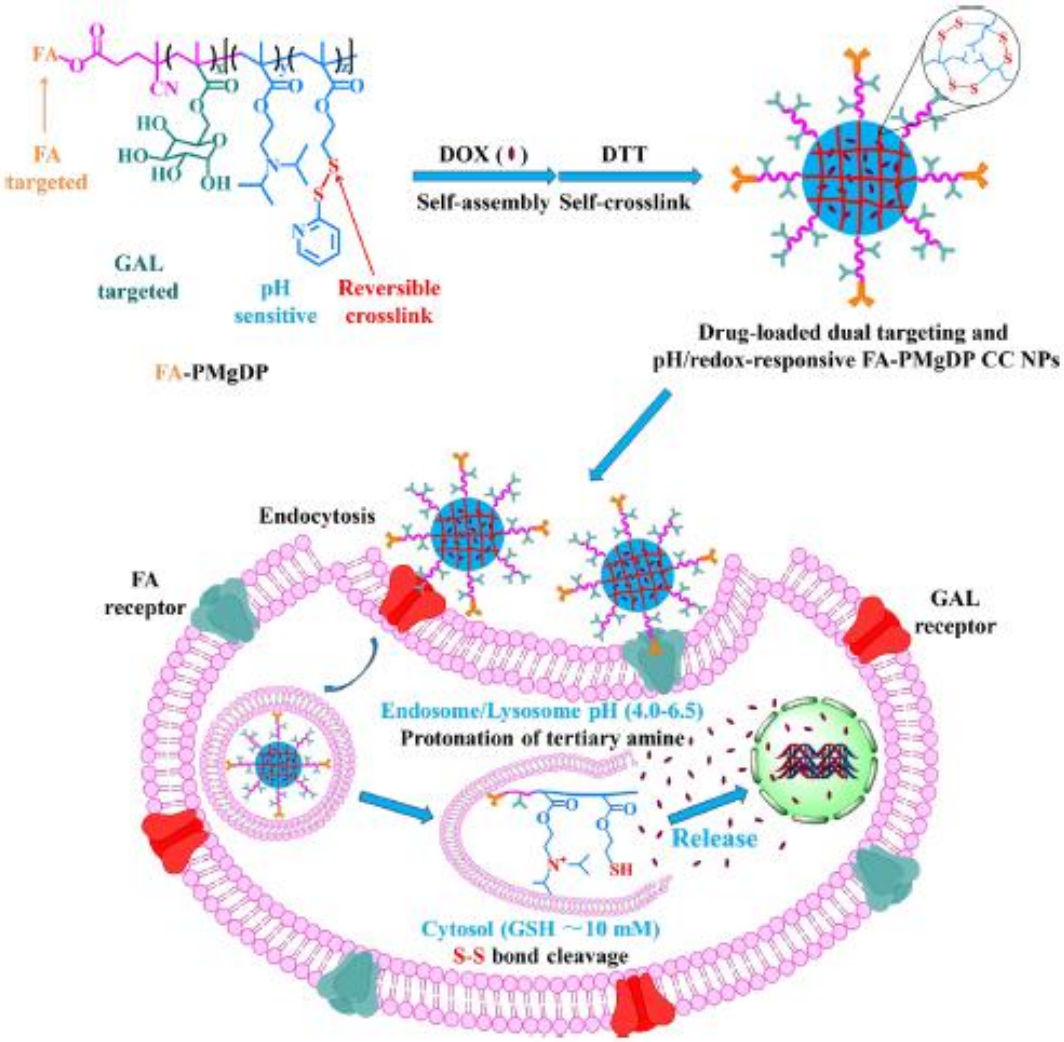

Figure 1.10. Site of action of pH-redox responsive DOX loaded micelles [23].

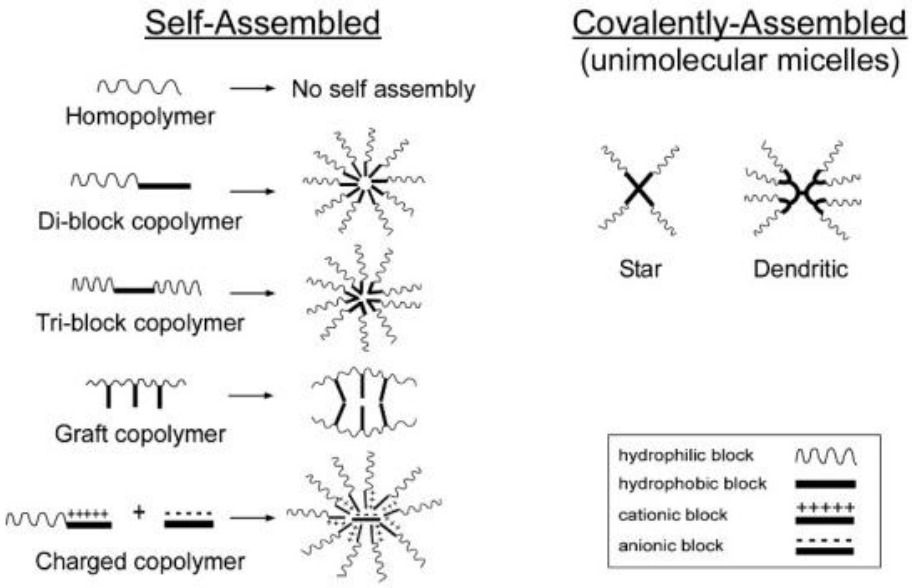

Figure 1.11. Copolymer micelle structures.

Diblock copolymers are used to form micelles for drug delivery and provide sustained drug release by using the hydrophobic interactions between drug and core of micelle. Graft copolymers have one polymer chain and another as grafted. They also have micelle forming property. Dendrimers are the another way to form micelles since they have certain number of end groups.

1.3.RAFT Polymerization

Among the many polymerization techniques like reversible deactivation radical polymerization (RDRP), atom transfer radical polymerization (ATRP), nitroxide-mediated radical polymerization (NMRP), reversible addition-fragmentation chain transfer polymerization (RAFT) is mostly used for the polymerization of acrylates, acrylamides, and methacrylates and requires mild conditions [24].

Chain transfer agents (CTA) are used as a form of thiocarbonylthio compound to control the polydispersity and molecular weight of the polymer.

RAFT polymerization consists of five steps; (I) Initiation, (II) Reversible chain transfer/propagation, (III) Reinitaition, (IV) Main equilibrium, and (V) Termination.

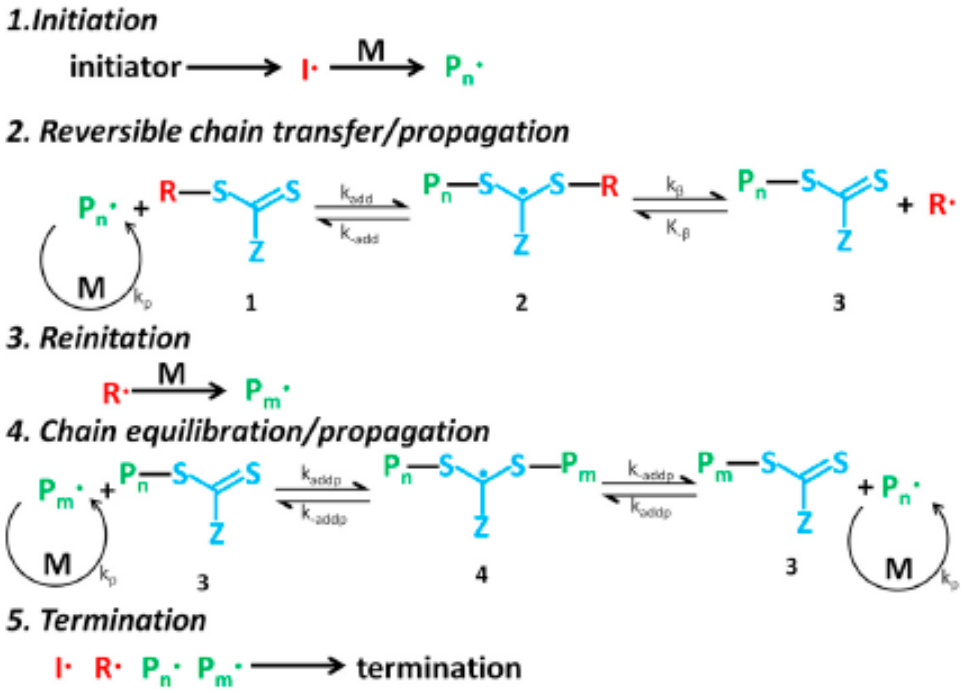

Figure 1.12. Mechanism of RAFT polymerization [24].

RAFT polymerization starts with the activation of the initiator (I) (i.e. AIBN) which propagates with the monomer (P_n) as can be seen in the first step.

In the second step, n monomer bearing radical polymer (P_n) combines with RAFT agent to give macro RAFT agent (macro-initiator).

Third and forth steps are the re-initiation of the monomers with the radical group coming from second step (R) to give a new polymeric radical (P_m) and propagation of the polymer chains equally thanks to equilibrium between the macro-initiator and

radical polymers. Polymerization ends with radical-radical termination which leads to the dead polymer.

RAFT polymerization is a versatile polymerization method to synthesize block copolymers. It provides control over the molecular weight of the generating polymers and leads to the low polydispersity. Moreover, RAFT polymerization is compatible with a wide range of polymers and gives control on the end group functionality [24].

Some RAFT agents are shown in the figure 1.13..

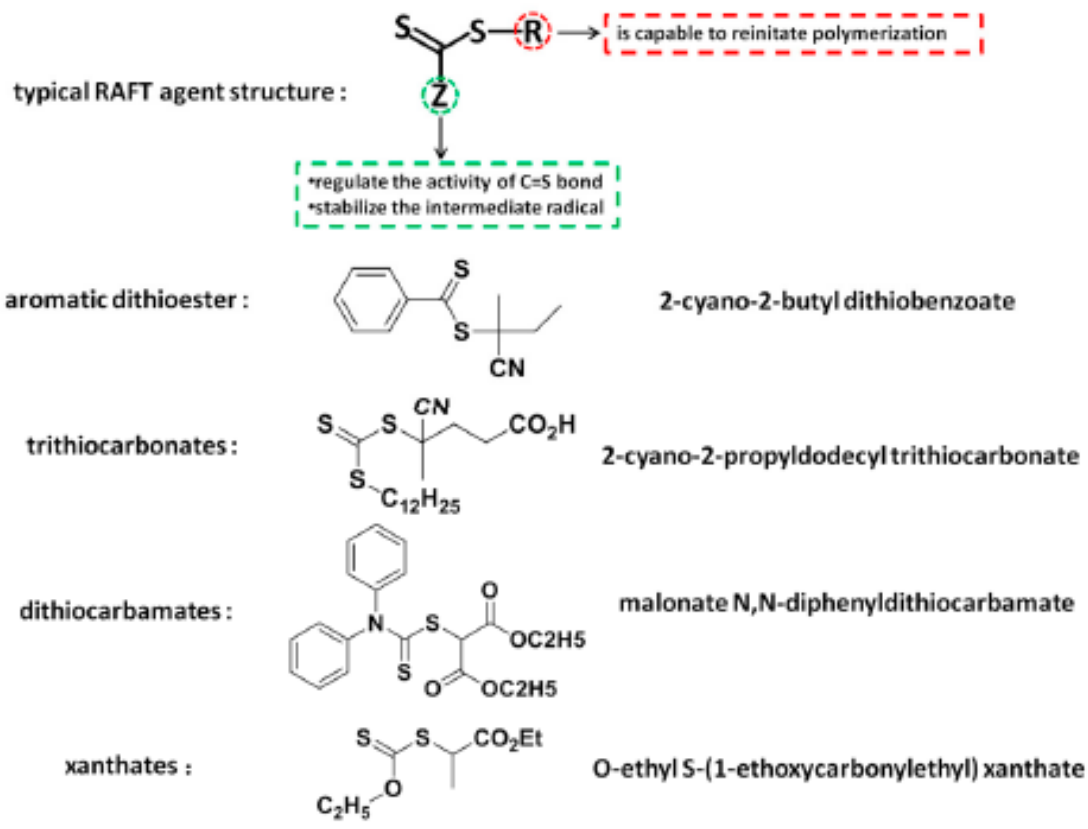

Figure 1.13. Some types of RAFT agents [24].

2. AIM OF THE STUDY

Since conventional chemotherapy agents have many drawbcaks, novel drug delivery systems have become important. Chemotherapy drugs are mostly water insoluble and while they have to be carried into human body, it becomes a struggle for delivering the drug. Since micellar structures are composed of hydrophobic drug attached hydrophilic biocompatible and biodegradable polymers which have well defined architectures, they become the best candidate for the drug delivery.

In this study, a diblock copolymer containing a chemotherapy drug attached hydrophobic block and oligoethylene glycol side chain containing hydrophilic blockwas synthesized (Figure 2.2.). This block copolymer was used to obtain a micellar drug delivery platform (Figure 2.3). The drug is attached to the polymer via a linker that is both redox-responsive and hydrolyzable (Figure 2.1). Thus, the drug release is due to the response against two different environments: reductive and acidic.

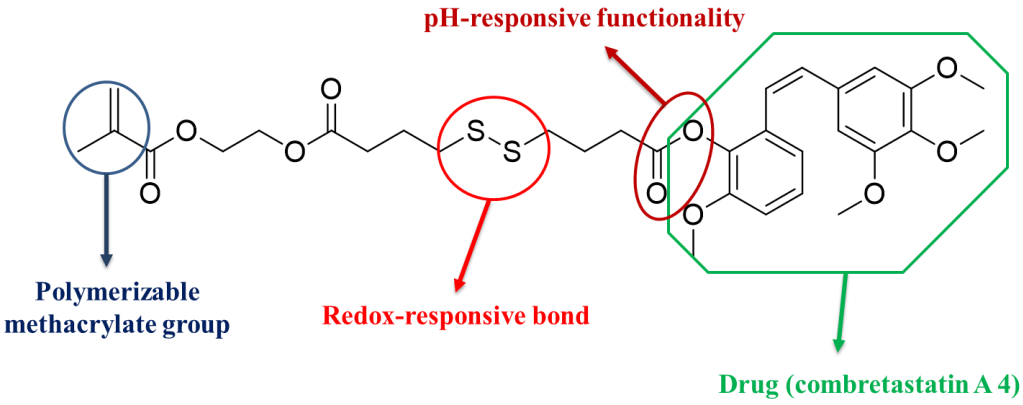

Figure 2.1. Drug bearing monomer.

Tumor site bears 30-40 times more glutathione than other tissuesin the body. Having disulfide bond on the linker makes it sensitive to the glutathione so that it can be cleaved in the tumor tissue. Via a two step mechanism combretastatin A4 will be released (Figure A.4.).

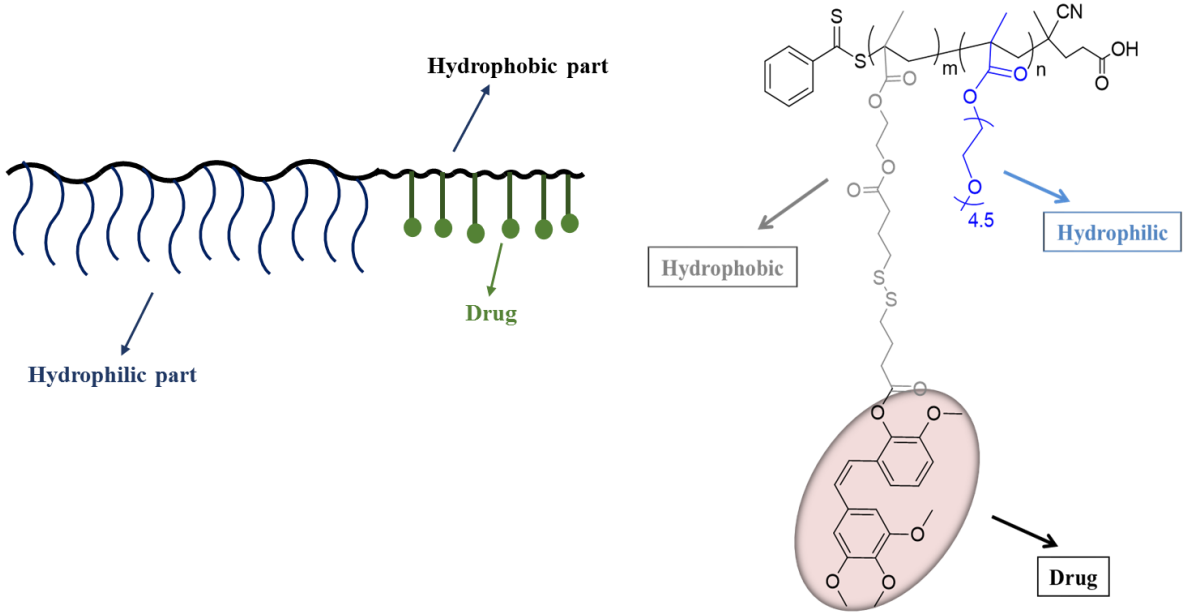

Figure 2.2. General demonstration of diblock copolymer.

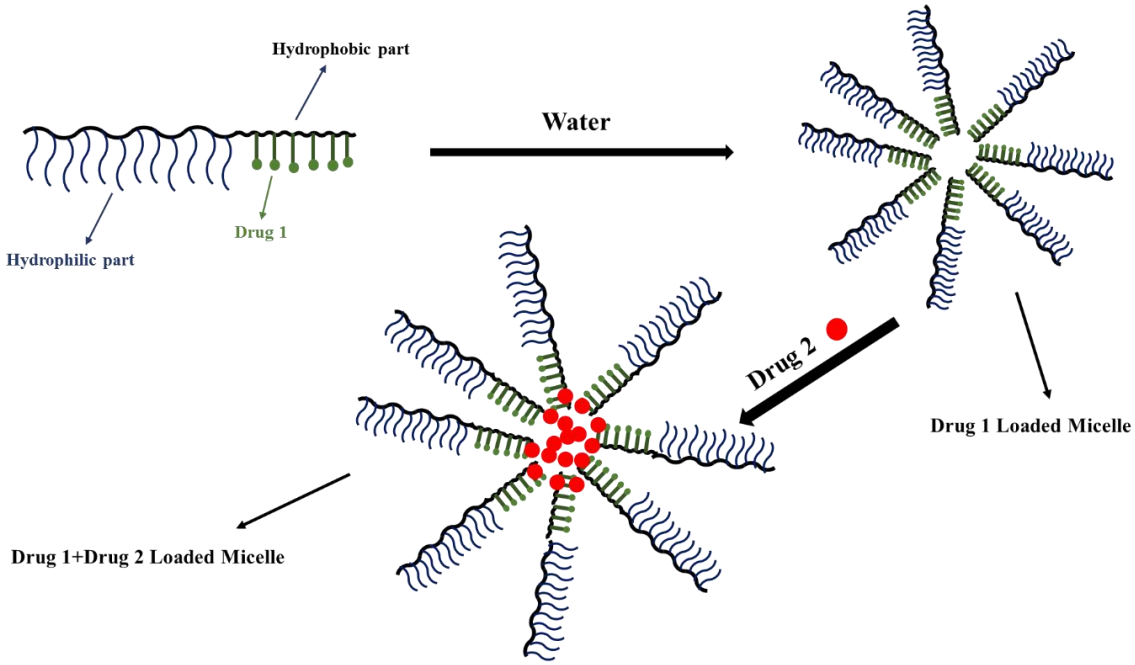

Figure 2.3. General scheme of micelle formation.

3. RESULTS AND DISCUSSION

3.1.Synthesis of Block A

OEGMEMA was used to obtain the Block A for its biocompatible and hyrophilic properties. RAFT polymerization was used to polymerize OEGMEMA with chain transfer agent (CTA) to obtain macro CTA. (Figure 3.1). The reaction was carried out under nitrogen atmosphere since RAFT polymerization is sensitive to oxygen.

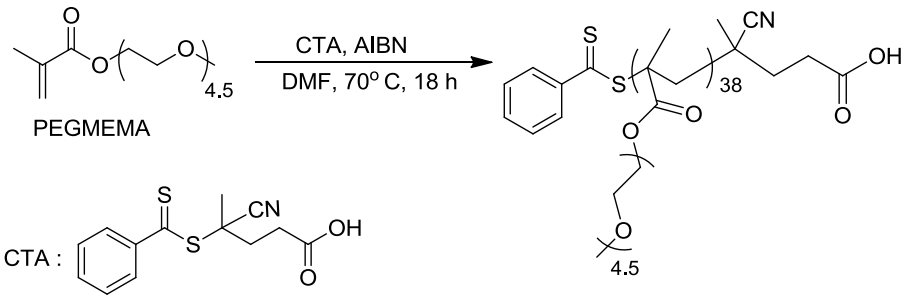

Figure 3.1. Synthesis of the Block A.

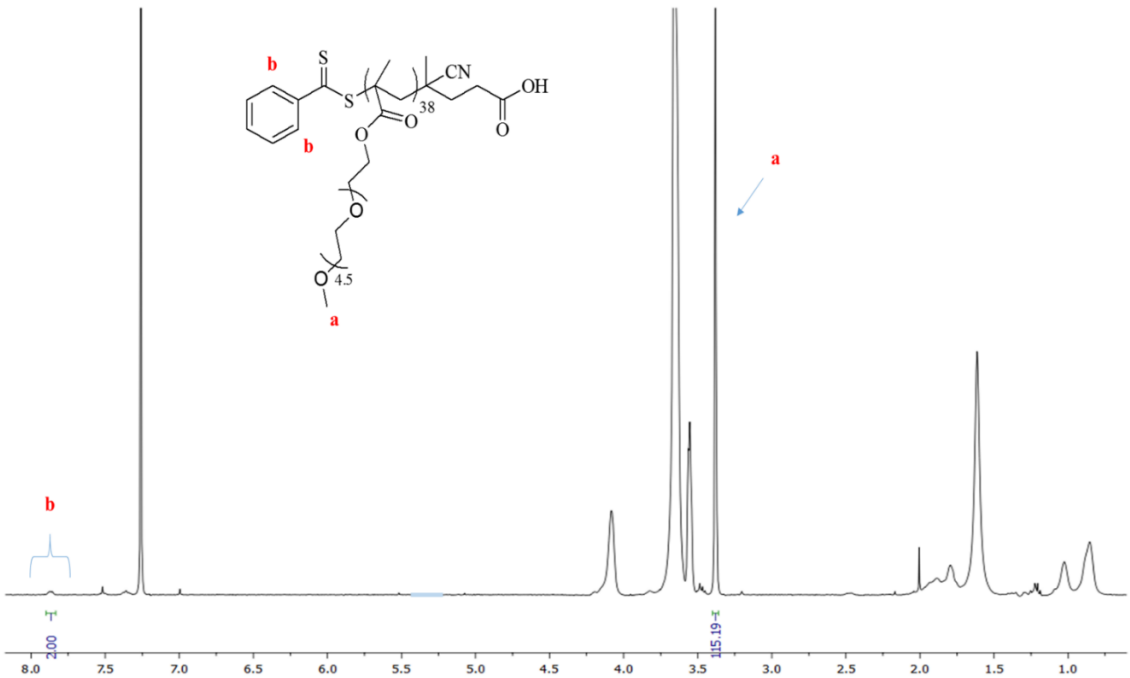

Figure 3.2. ^{1}H NMR spectrum of the Block A.

Peak seen at 7.87 ppm represent 2 protons of aromatic ring of the CTA. Methoxy peak of OEGMEMA is seen at 3.38 ppm which correspond to 3 protons. By integrating the peaks of CTA to 2, integration of POEGMEMA was found as 115. Since it corresponds to 3 protons, by dividing it by 3, we obtain the repeating unit of POEGMEMA as 38. After multiplying the repeating unit with the molecular weight of OEGMEMA (300 g/mol), the NMR molecular weight of POEGMEMA was found as 11.5 K (Table 3.1, item 4).

Table 3.1. Polymerization conditions of Block A.

No	**OEGMEMA (Eq.)**	**CTA (Eq.)**	**Mn_{SEC} (KDa)**	**Mn_{NMR} (KDa)**	**Mn/Mw**
1	250	9	6.3	10.7	1.07
2	125	9	5.2	7.7	1.17
3	250	9	9.8	12.1	1.19
4	300	9	8.5	11.5	1.24

3.2.Synthesis of Block B

In order to synthesize the drug containing monomer, disulfide bond containing carboxylic acid linker was synthesized. DCC coupling reaction was used for synthesis of the linker. The reaction took place at room temperature for 24 h.

The diacid linker was further used to form the methacrylate bearing acid. Combretastatin A4 (CA4), a vascular disrupting agent, was attached to this linker via esterification reaction. The reaction took place at room temperature for 24 h under N_2 atmosphere.

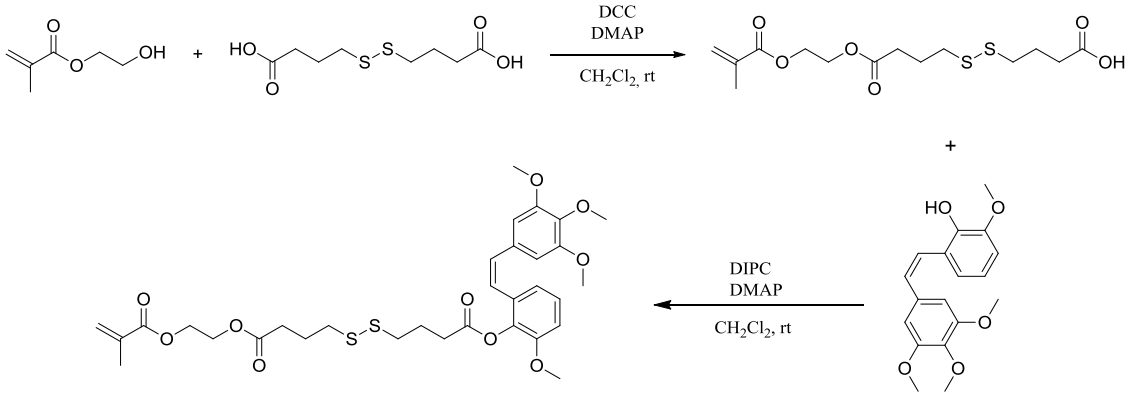

Figure 3.3. Synthesis of the linker and Block B.

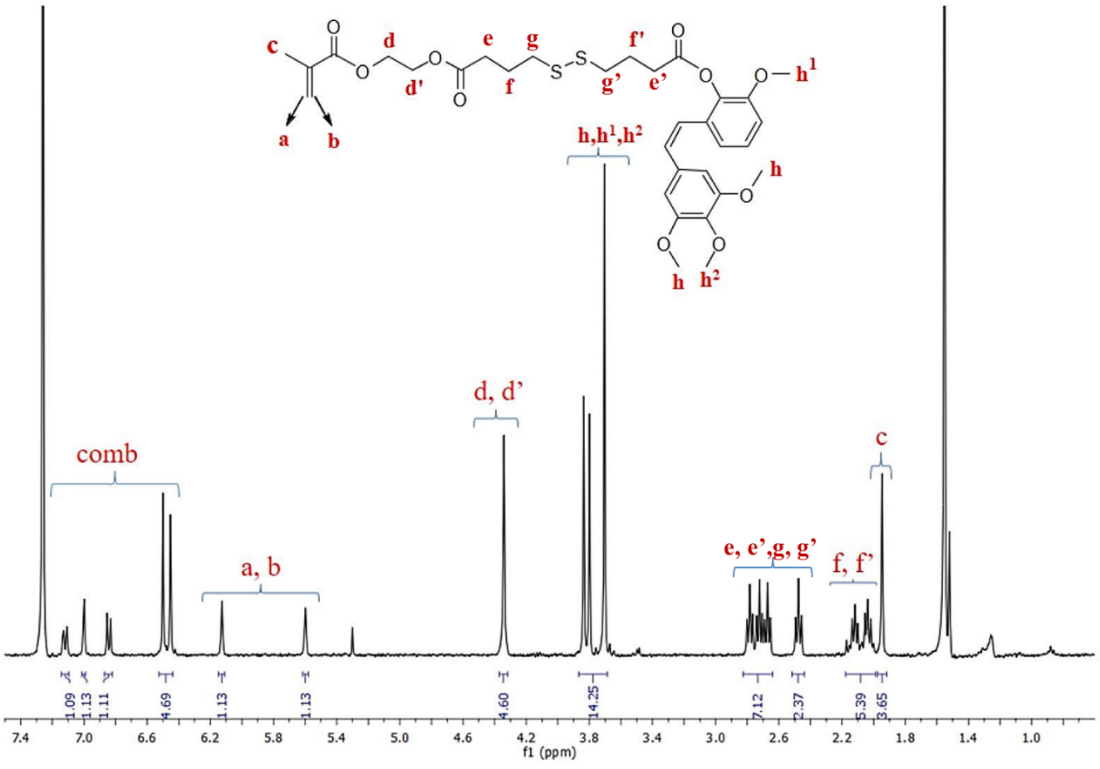

Figure 3.4. ^{1}H NMR spectrum of the Block B.

As can be seen in the Figure 3.2., ^{1}H NMR analysis of the drug bearing monomer proved that CA4 molecule was successfully attached to the linker. Peaks at

5.5 pm and 5.92 pm represent the double bond protons of methacrylate group. Peaks between 6.43 and 7.11 correspond to the 7 hydrogens of CA4 and prove the conjugation of the drug molecule to the linker. Methacrylate group makes the monomer polymerizable so that diblock copolymers can be obtained at the end.

After obtaining drug containing monomer, again RAFT polymerization was used for copolymerization and POEGMEMA was used as macro CTA. The reaction took place for 6 days under oxygen free conditions to prevent the deactivation of free radicals. Subsequently, ^{1}H NMR analysis was done after termination of the polymerization. Polymerization was conducted multiple times with a range of conditions and obtained results can be seen at Table 3.2. For micelle preparation a block copolymer that has similar block size is used (Table 3.2, item 5).

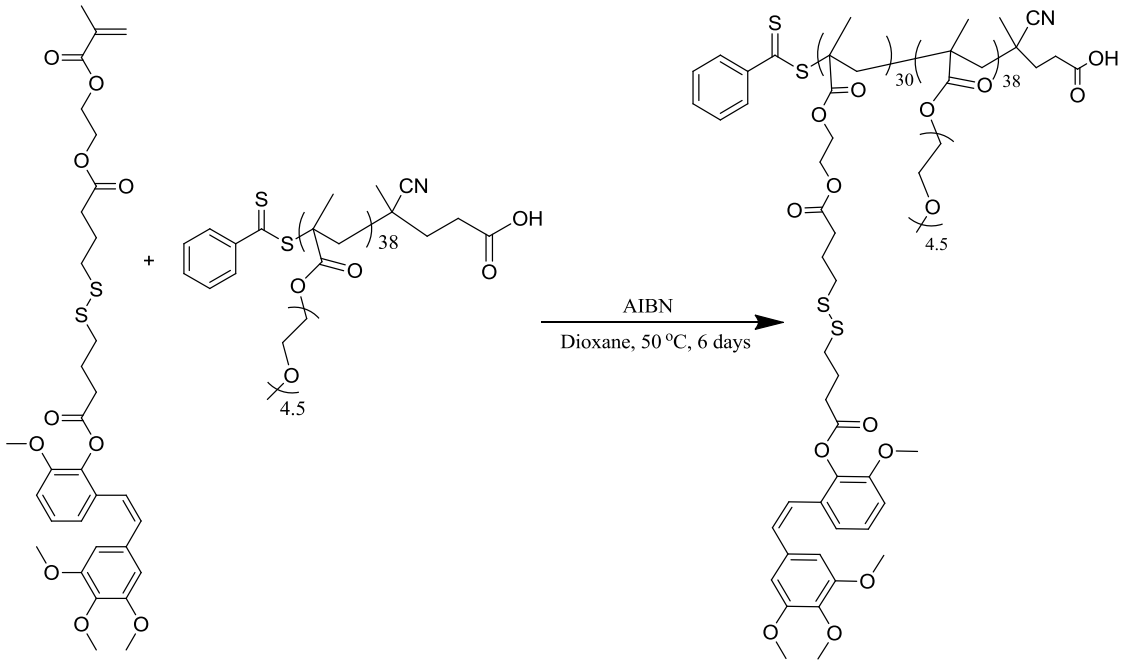

Figure 3.5. Synthesis pathway of the diblock copolymer (Block AB).

Table 3.2. Polymerization conditions of diblock copolymers.

No	Block A No	Block A Eq.	Mn_{SEC} (K Da)	Drug Monomer (Eq.)	Block A:Block B (from NMR)
1	1	5	6.3	50	35:10
2	1	5	6.3	50	35:5
3	2	9	5.2	108	25:10
4	3	9	6.8	188	40:2
5	4	11	8.5	400	38:30

Appearance the peaks at between 6.43 and 6.48 ppm belong to the CA4, and disappearance of the double bond peaks of methacrylate which belongs to the drug monomer indicate that polymerization was successful (Figure 3.6). According to the known repeating units of the Block A which is 38, the repeating units of the Block B was calculated. Peaks at between 6.43 and 6.48 ppm gives the value of 2 protons that are coming from CA4. After integration, it was found as 60 which corresponds to 30 repeating units for the Block B.

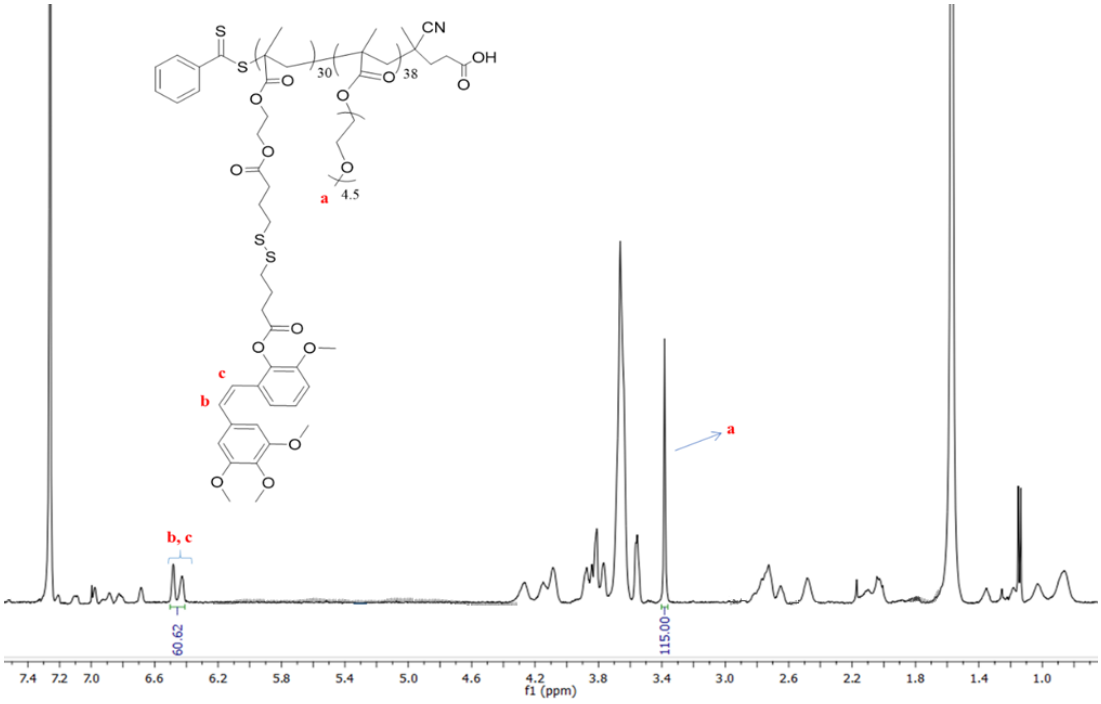

Figure 3.6. ^{1}H NMR spectrum of the diblock copolymer Table 3.2 item 5.

3.3.Micelle Formation

Micelles have many advantages for drug delivery such as high stability and drug loading capacity, biodebrability, improved releaase profile, accumulation in tumor site via EPR effect, and easy preparation. In this study, hydrophilic part (Block A) and hydrophobic part (Block B) bearing diblock copolymers were synthesized. Since micelle formation requires amphiphilic copolymers, our diblock copolymers were put in aqueous media to give micellar structure.

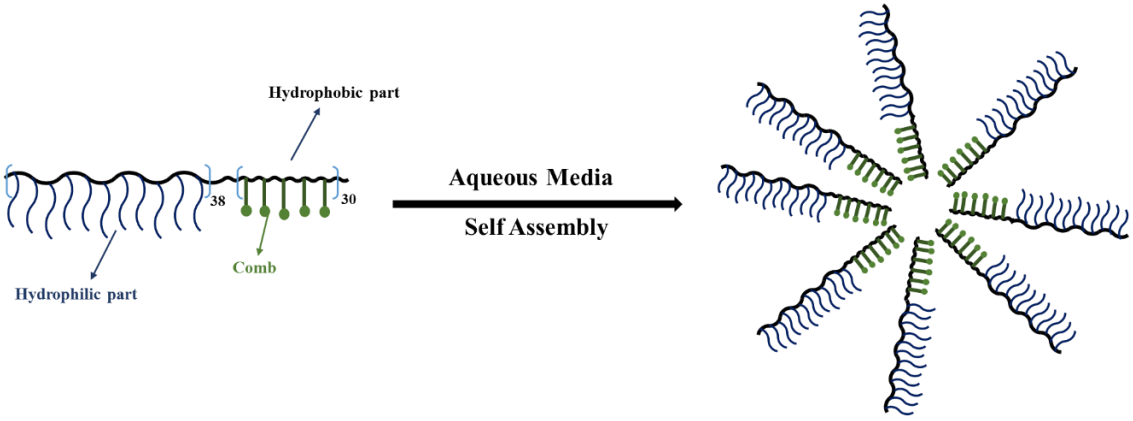

Figure 3.7. Micelle formation from diblock copolymers.

3.3.1.Critical Micelle Concentration (CMC) Measurement

Critical micelle concentration is the minimum concentration required for the successful micelle formation to occur. To calculate the CMC value of micelles, ranging between $2x10^{-5}$ to $2x10^{-8}$ M of diblock copolymer solutions were prepared. Nile Red was used for the fluorescence measurements. CMC value was calculated with respect to fluorescence measurement results.

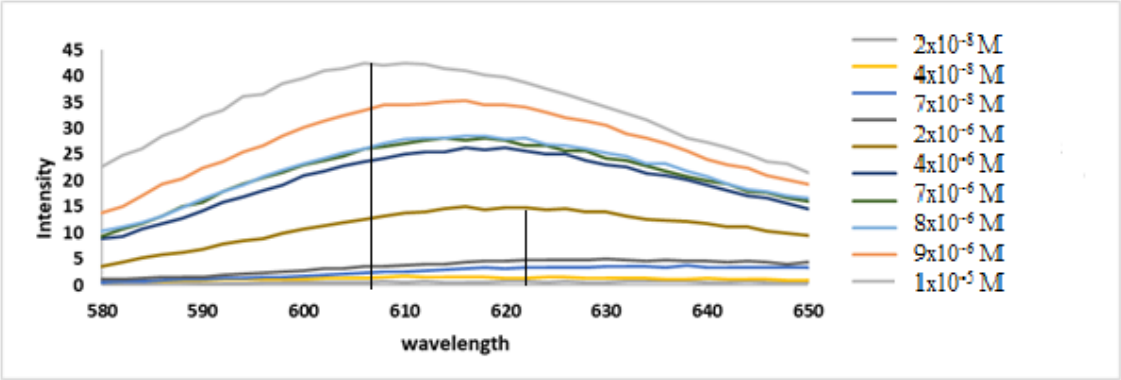

Figure 3.8. Fluorescence graph of Nile Red added micelles.

Nile Red was used to determine the CMC value of micelle because it gives a visible color and can be easily calculated with flourescence measurement.

When the micelle formation takes place, Nile Red keeps its color, however, if micelle formation is failed, its color change from pink to colorless. The reason for this is that, Nile Red becomes colorless when it is dissolved into an organic solvent. In the case of a successful micelle formation, Nile Red is entrapped into the core of micelle because of its hydrophobicity. Therefore, it keeps its color stable. From the Figure 3.9., color change can be easily seen.The critical micelle concentration of the micelle formed from polymer (Table 3.2, item 5) was found as 1.76 x 10 $^{-6}$ M.

Figure 3.9. Nile Red added micelles.

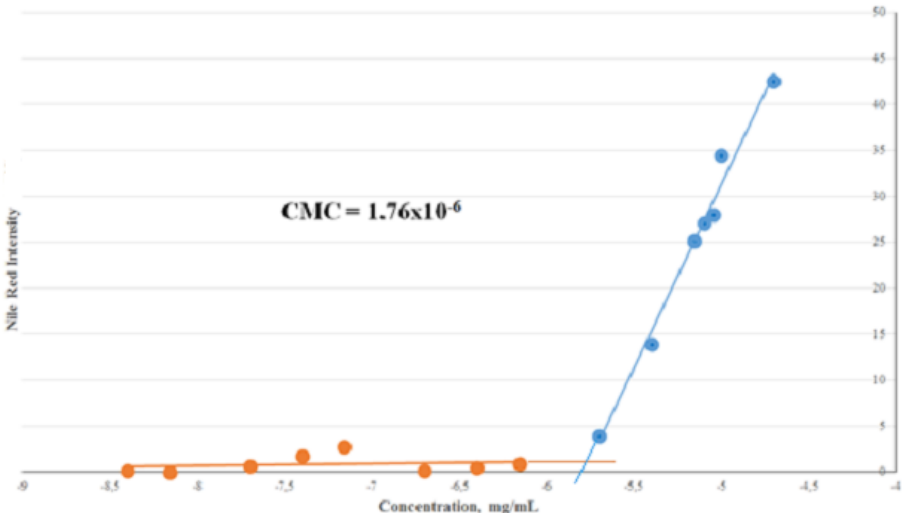

Figure 3.10. Nile Red emission spectra of micelles.

3.2.2. DLS Measurements of Micelles

After calculating the CMC value of the polymers, dynamic light scattering (DLS) measurements were performed to determine the diameter and size distrubitions.

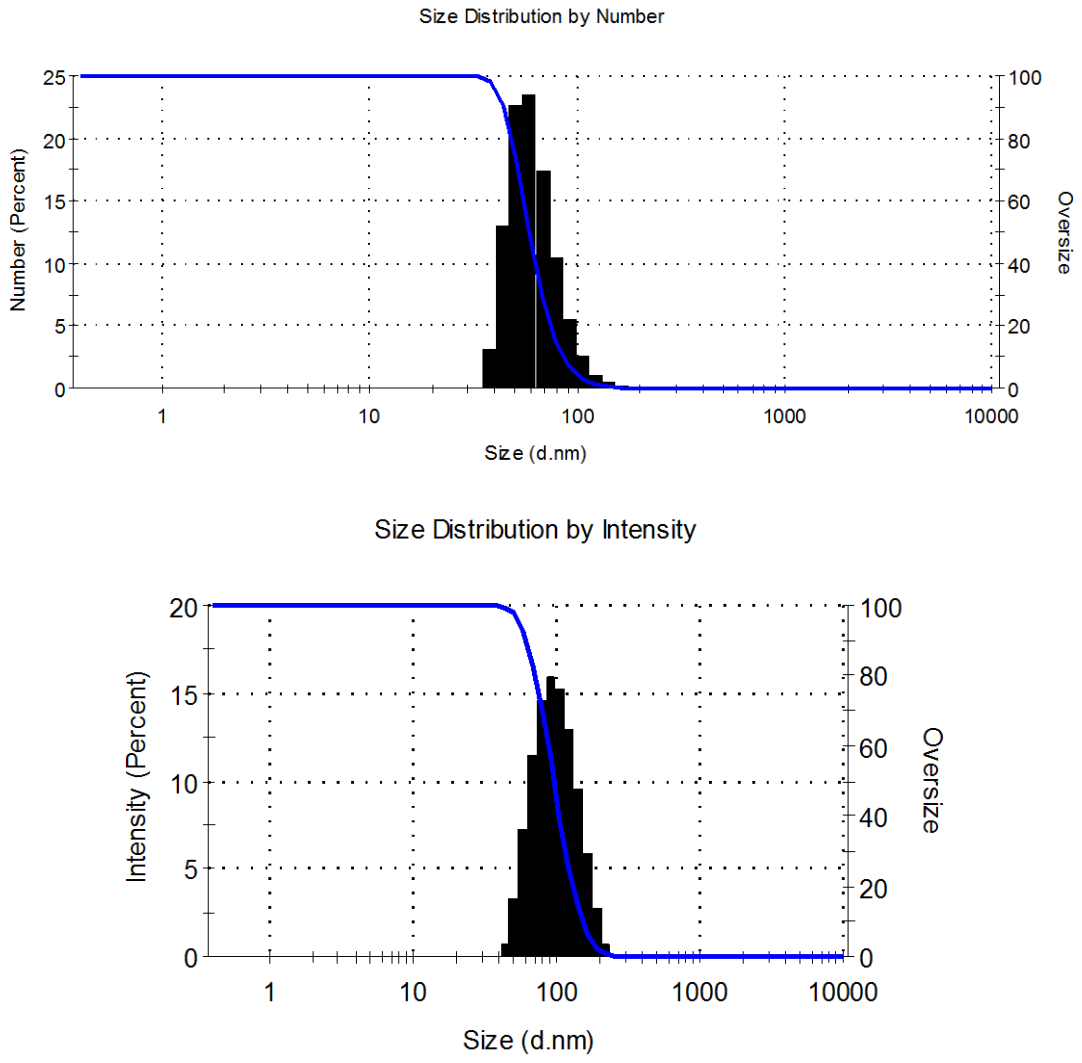

Figure 3.11. DLS results of micelle.

Table 3.3. DLS results of micelle.

		Intensity PSD	Volume PSD	Number PSD
Micelle DLS Result	Z-Average (d.nm): 91.73 PdI : 0.148	Peak 1 : 184.0 100%	Peak 1 : 196.9 100%	Peak 1 : 122.3 100%

3.4.Second Drug Loading

Docetaxel is an anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, and non-small cell lung cancer.

For second drug loading, docetaxel (DTX) was chosen. 1.2 mg of DTX, 3 mg of copolymer were dissolved in 1 mL acetone and 3 mL distilled water was added dropwise. The solution was evaporated to remove acetone and filtered twice. After DTX entrapment, DLS results were taken (Figure 3.13).

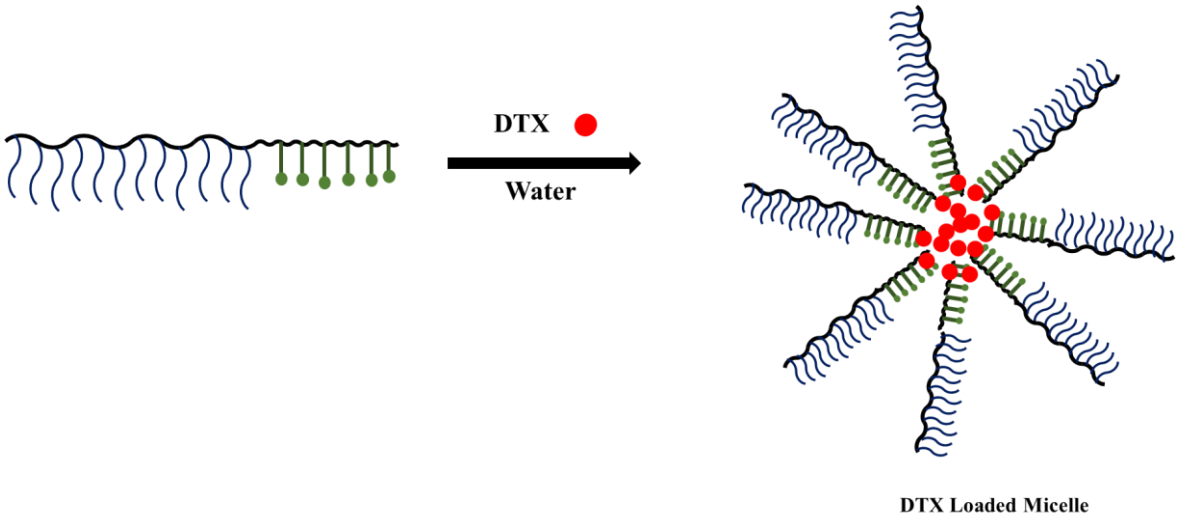

Figure 3.12. Loading of second drug into the micelle.

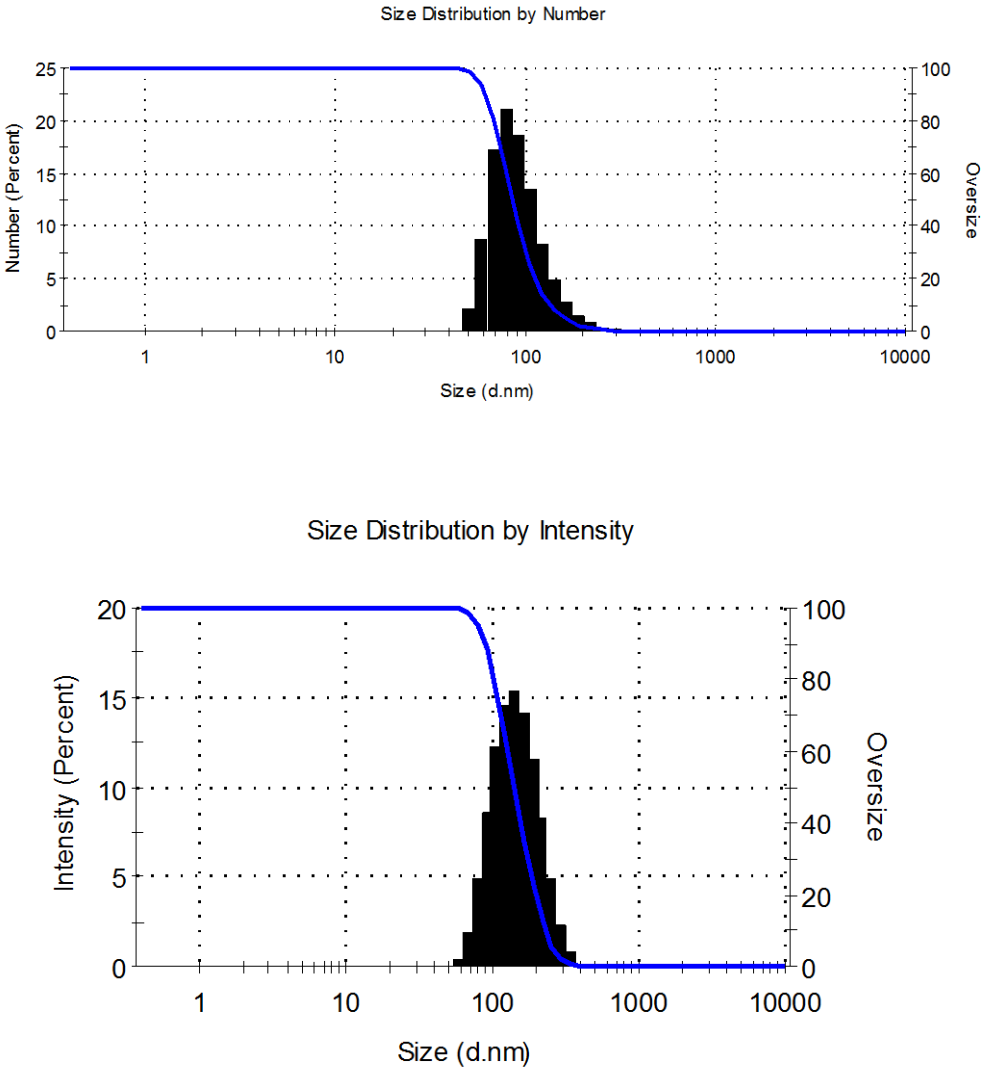

Figure 3.13. DLS results of DTX loaded micelles.

3.4.1.Drug Loading Efficacy

After loading DTX into the micelles, drug loading efficiancy was calculated. 40%, 50%, 60%, 80%, and 100% raito of DTX bearing micelles were prepared. Drug loaded micelles were lyophilized to determine the dry weight. Afterwards, they were dissolved in acetonitrile and LC-MS-MS analysis was performed to obtain the drug loading capacity of micelles. Results are tabluated at Table 3.4.

Table 3.4. Drug loading capacities of micelles.

No	DTX Loading	Loaded DTX
1	40%	10.5% ±0.5
2	50%	31% ±1
3	60%	29% ±7
4	70%	23% ±2
5	80%	31.5% ±13.5
6	100%	18% ±2

As seen from the Table 3.5 50% drug loading showed the best reproducibility. This experiment was repeated 3 times.

Table 3.5. Repeating of 50% DTX loading into the micelle.

Experiment	DTX Loading	Loaded DTX
1	**50%**	**30%**
2	**50%**	**32%**
3	**50%**	**31%**

4. EXPERIMENTAL

4.1.General Methods and Materials

4,4′-Dithiodibutyric acid, *N, N'*-dicyclohexylcarbodiimide (DCC), 2-Hydroxymethyl methacrylate (HEMA), Poly(ethylene glycol) methyl ether methacrylate (OEGMEMA), Nile Red, 4-cyano-4[(dodecylsulfanylthiocarbonyl)-sulfanyl]pentanoic acid (CTA), AIBN, Combretastatin A4 (CA4), and Docetaxel (DTX) were purchased from Sigma Aldrich. N,N'-Diisopropylcarbodiimide (DIPC) was purchased from TCI. All solvents were purchased from Merck. Ultra pure water was obtained by Milli-Q Water Purification System (Milli-Q system, Millipore, Billerica, MA, USA).

^{1}H NMR analysis were carried out by Varian 400 Mhz spectrometer. LC-MS analysis was done using LCMS-2020-Mass Spectrometer System (Shimadzu, Japan). Varian Cary Eclipse Fluorescence Spectrometer (Varian, Agilent, USA) was used for CMC calculations. Micelles were analyzed with Malvern/Zetasizer (Malvern, Worcestershire, UK).

4.2. Synthesis and Characterization of Diblock Copolymers

4.2.1.Synthesis of Block A

Olygo(ethylene glycol) methyl ether methacrylate (OEGMEMA, 1 g, 3.3 mmol) and CTA (28 mg, 0.1 mmol) were dissolved in dioxane. As initiator, AIBN (1.8 mg, 0.011 mmol) was added to the mixture and stirred under N_2 atmosphere to remove the oxygen for 1 h. After purging, the reaction mixture was heated at 70 ^{o}C for 18 h. The reaction was proceeded via RAFT polymerization and terminated via air exposure and cooling down in an ice bath. The solvent was evaporated and crude was dissolved in DCM. Subsequently, the mixture was prufied by precipitating in cold diethyl ether two times. After drying under *vacuo*, 600 mg product with an average of approximately 38 OEGMA repeating units was obtained (60% yield). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.87 (d 2H, *J* = 2.05, ArH), 7.52 (s, 1H), 7.36 (d, *J* = 6.47, 2H), 4.08 (s, -OCH_2), 3.38 (s, 3H, -OCH_3), 1.02 (s, CH_3), 0.85 (s, CH_3).

4.2.2.Synthesis of Linker

4,4′-Dithiodibutyric acid (1831 mg, 7.68 mmol), DCC (1585 mg, 7.68 mmol), and 4-Dimethylaminopyridine (DMAP, 281 mg, 2.31 mmol) were dissolved in dichloromethane (DCM, 54 mL). 2-Hydroxyethyl methacrylate (HEMA, 500 mg, 3.84 mmol) was added to the mixture. The reaction was carried out under N_2 atmosphere at room temperature for 24 h. The reaction was stopped by air exposure and the reaction mixture was put at -20 ^{o}C to let DCU precipitate.

DCM was evaporated after the mixture was filtered with sintered glass, and the crude was dissolved in 1,5 mL of DCM and purified by column chromatography on SiO_2 (EtOAc/Hexane, 20:80). The product was dried under *vacuo* yielding 380 mg of compound with 76% yield. ^{1}H NMR ($CDCl_3$, δ, ppm) 6.13 (s, 1H), 5.60 (s, 1H), 4.35 (s, 4H), 4.12 (q, *J* = 7.11, 4H), 2.73 (q, *J* = 6.53, 4H), 2.49 (q, *J* = 7.75, 4H), 2.04 (s, 3H) 1.95 (s, 3H).

4.2.3.Synthesis of Monomer

To a solution of linker (300 mg, 0.85 mmol), N,N'-Diisopropylcarbodiimide (DIPC) (157.5 μL, 1.04 mmol), and 4-Dimethylaminopyridine (DMAP, 103 mg, 0.86 mmol) in DCM (6 mL) was added Combretastatin A 4 (CA4, 270.4 mg, 0.85 mmol). The reaction was stirred under N_2 atmosphere at room temperature for 24 h. After exposure of air, solvent was evaporated and the crude was dissolved in 1 mL DCM for purification by column chromatography on SiO_2 (EtOAc/Hexane, 25:75). 456 mg of drug-monomer was obtained with 80% yield after dried under *vacuo*. ^{1}H NMR ($CDCl_3$, δ, ppm) 7.12 (d, *J* = 8.6, 2H), 7.0 (s, 1H), 6.84 (d, *J* = 8.5, 2H), 6.5 (s, 1H), 6.45 (s, 1H), 6.13 (s, 1H, CH_2), 5.6 (s, 1H, CH_2), 4,34 (s, 4H), 3.83 (s, 3H, -OCH_3), 3.81 (s, 3H, -OCH_3), 3.70 (s, 3H, -OCH_3), 2.14-2.10 (m, 2H), 2.06-2.02 (m, 2H), 1.95 (s, CH_3).

4.2.4.Synthesis of Diblock Copolymer

AIBN (3.5 mg, 0.02 mmol) and as a macro CTA, Block A (64 mg, 0.0056 mmol) were dissolved in anhydrous dioxane (0.3 mL) and the mixture was purged under N_2 atmosphere for 45 min. Thereafter, the mixture was heated at 70 ^oC for 1 h. While the temperature was dropped at 50 ^oC for 1 h, the monomer (128 mg, 0.2 mmol) was purged in 0.3 mL anhydrous dioxane under N_2. Following purging, Block B mixture was added to the former mixture and stirred for 6 days. Polymerization was terminated via air exposure and cooling down in an ice bath, then purified by precipitation in cold diethyl ether and dried under *vacuo* to give 96 mg diblock copolymer with an average of approximately 30 repeating monomer units (50% yield). ^1H NMR ($CDCl_3$, δ, ppm) 7.09 (s, 1H), 6.98 (s, 1H), 6.89 (s, 1H), 6.82 (s, 1H), 6.69 (s, 1H), 6.48 (s, 1H, CH), 6.42 (s, 1H, CH), 4.28 (s, 4H), 3.66 (s, 12H), 3.38 (s, 3H, $-OCH_3$), 1.57 (s, 6H).

4.2.5.Micelle Preparation and Characterization

1 mg diblock copolymer was dissolved in 0.2 mL acetone and 3 mL double distilled water was added slowly to the mixture. The mixture was put on rotary evaporator to abolish the solvent. After evaporation of the organic solvent, DLS result was taken.

For preparation of micelles from diblock copolymers, copolymer stock solutions ($1x10^{-4}$ M) in acetone were prepared. Ranging between $2x10^{-5}$ to $4x10^{-9}$ M of polymer solutions were prepared in glass vials and using 100 μL syringe, 50 μL of 0.03 mg/mL stock solution of Nile Red in acetone was added to each vial. Finally, 3 mL of distilled water was added dropwise to the vials respectively. The solutions were left under dark overnight to let the organic solvent remove and fluorescence measurements were taken.

4.2.6.DLS Measurements

DLS analysis was performed after second drug loading and results were found as figure 4.1. and 4.2..

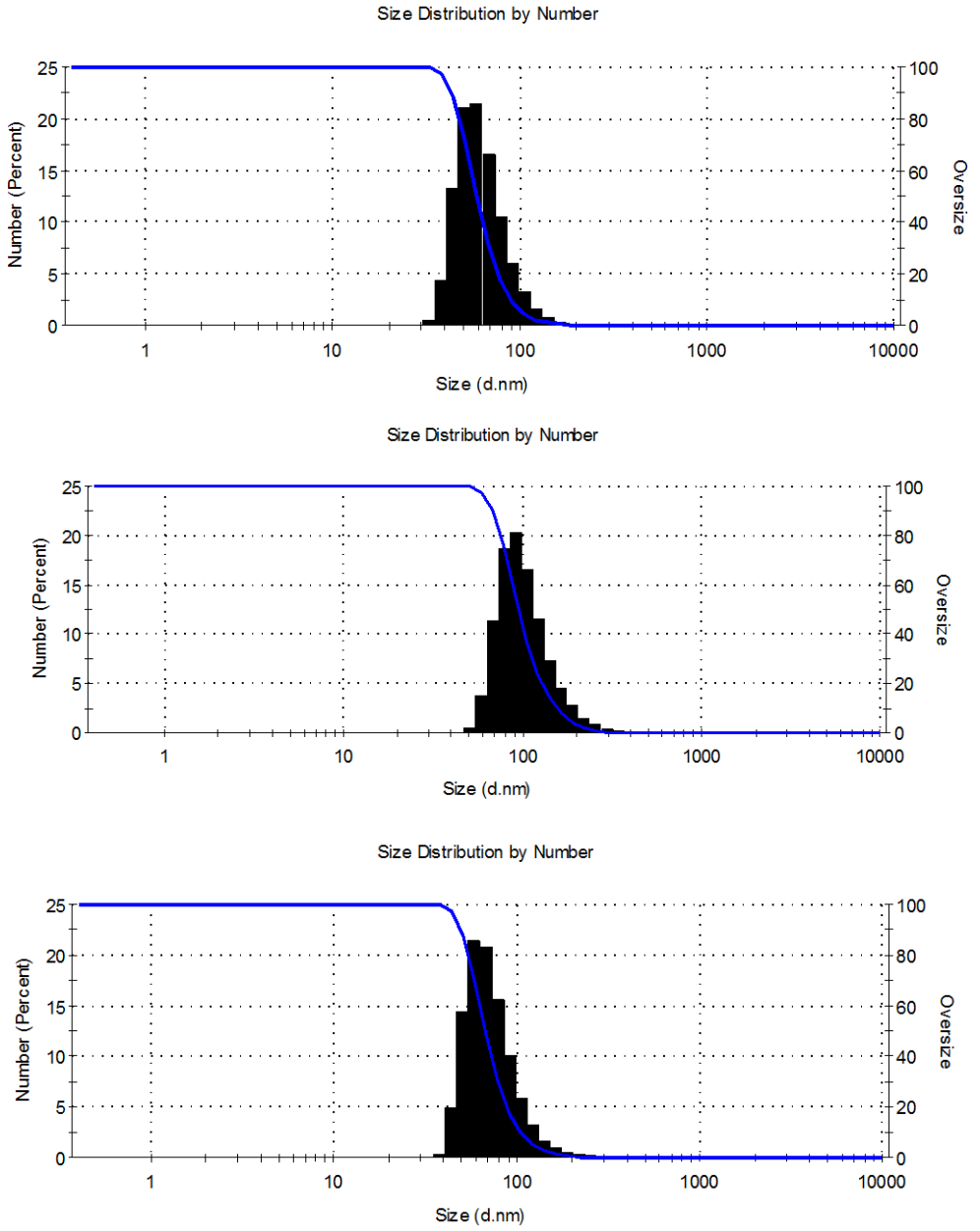

Figure 4.1. Size distributions of 40%, 50%, 60%, 70%,80%, 100% DTX loaded micelles by number, respectively.

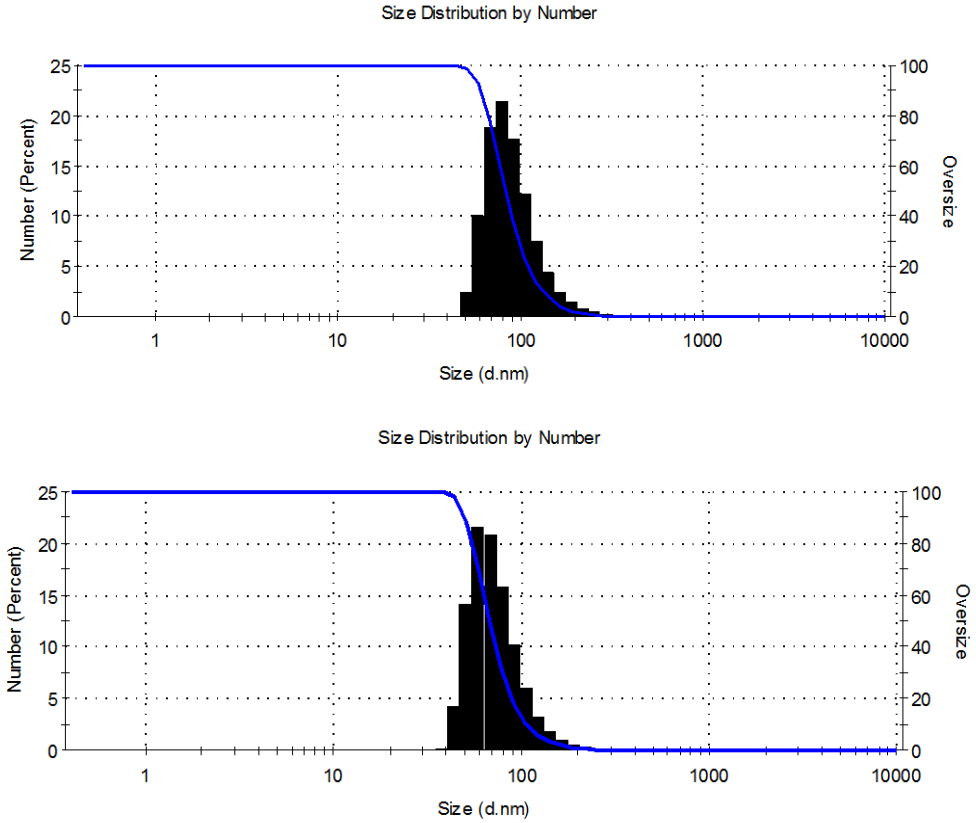

Figure 4.1. Size distributions of 40%, 50%, 60%, 70%,80%, 100% DTX loaded micelles by number, respectively. (cont.)

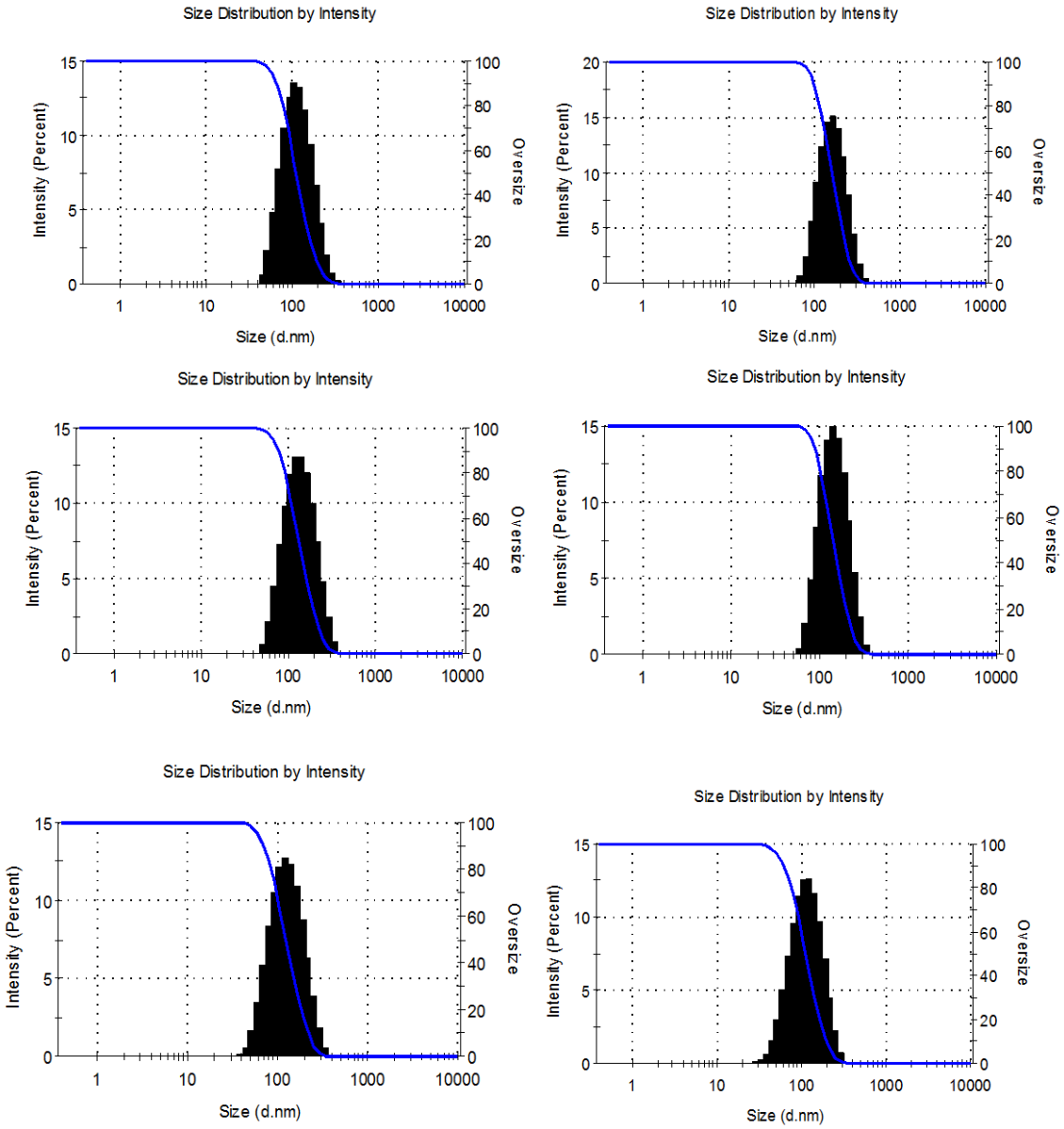

Figure 4.2. Size distributions of 40%, 50%, 60%, 70%,80%, 100% DTX loaded micelles by intensity, respectively.

5. CONCLUSION

In this study, polymeric micelle structure was obtained for drug delivery. For this purpose, diblock copolymers were synthesized. Drug containing monomer was successfully synthesized and well-characterized. Hydrophilic block of the copolymer was prepared by polymerization of OEGMEMA via RAFT polymerization and this Block A was also used as macro initiator for synthesis of the copolymer. After synhesizing the diblock copolymers, micelle formation was carried out and critical micelle concentration of the micelles was calculated. Afterwards, a second drug was loaded and loading efficiency was analyzed.

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APPENDIX A : ADDITIONAL DATA

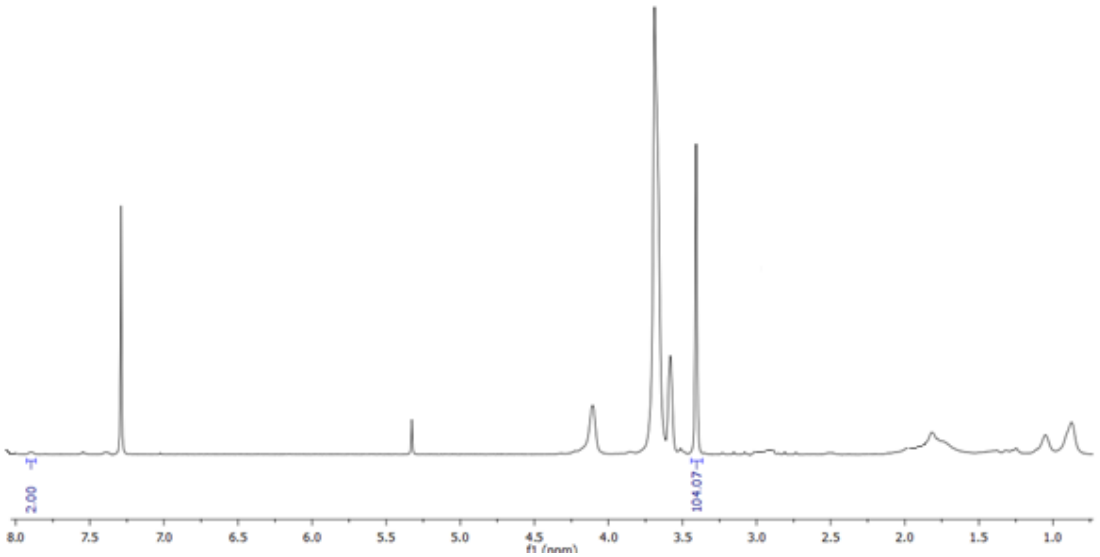

Figure A.1. ^{1}H NMR spectrum of the block polymer (Block A) of Experiment 1.

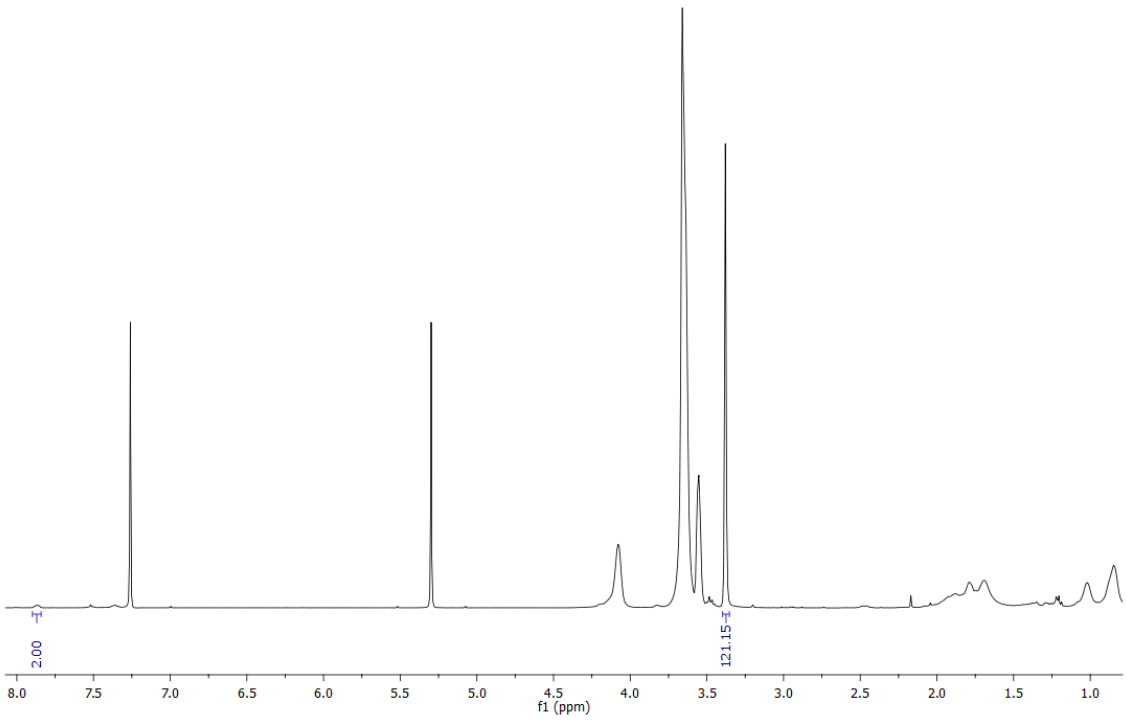

Figure A.2. ^{1}H NMR spectrum of the block polymer (Block A) of Experiment 4.

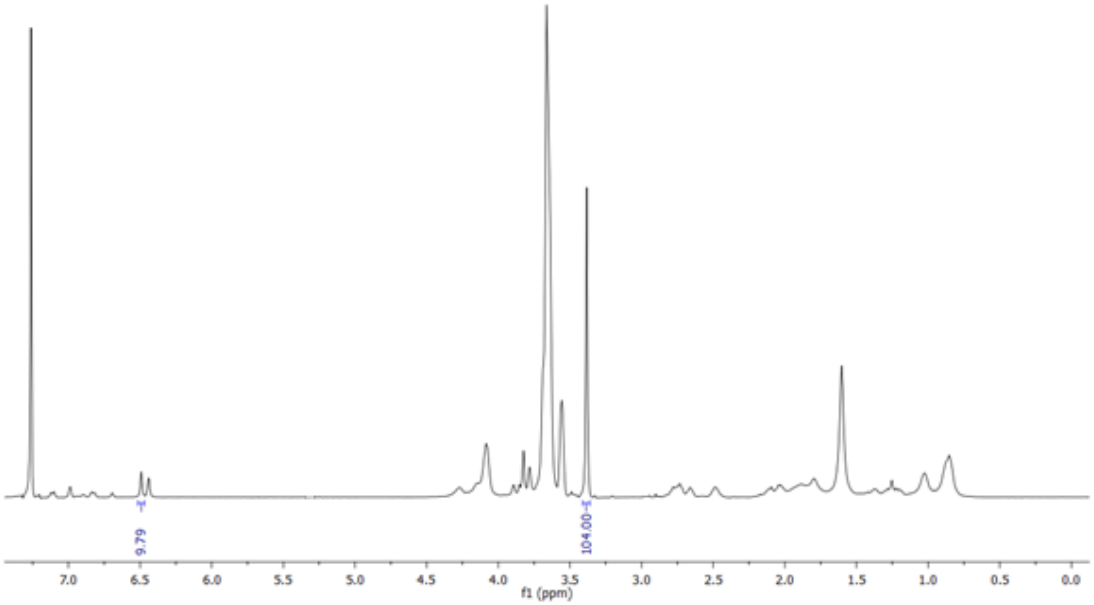

Figure A.3. ^{1}H NMR spectrum of the diblock copolymer (Block AB) of Experiment 1.

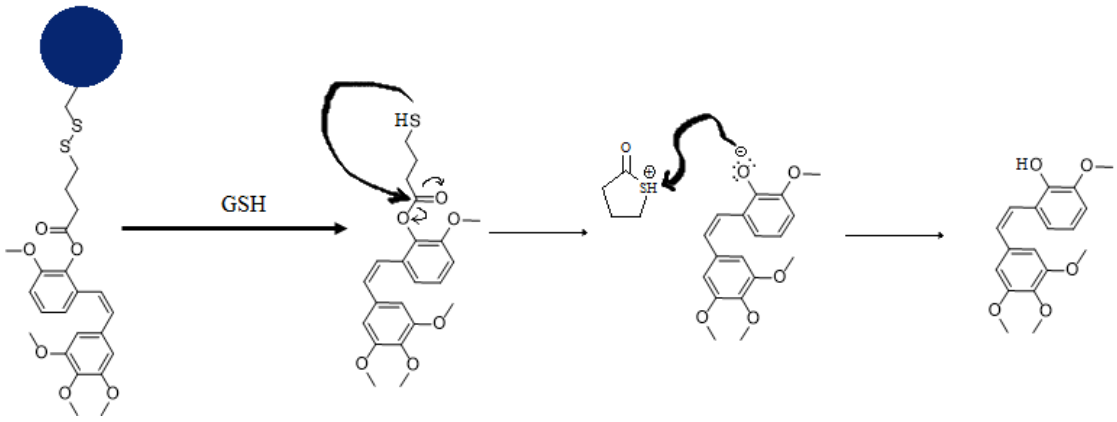

Figure A.4. Release mechanism of combretastatin A4 in the presence of GSH.

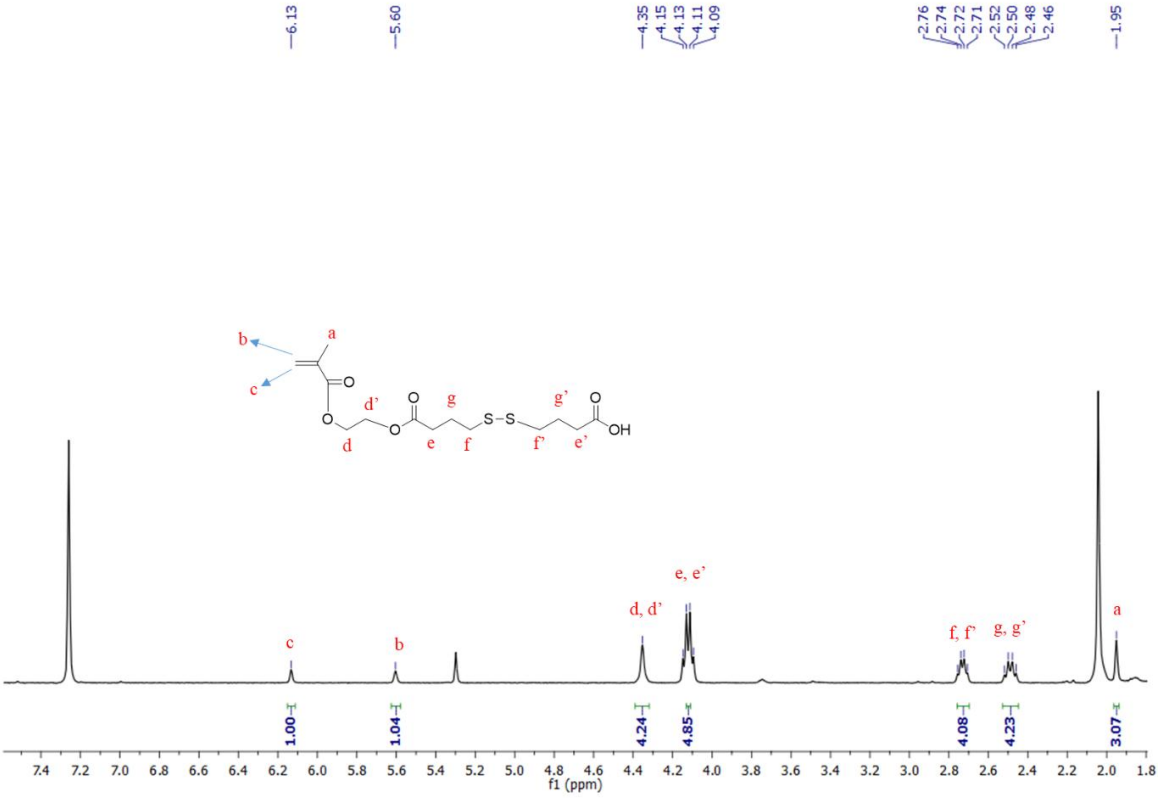

Figure A.5. ^{1}H NMR spectrum of the linker.