REPUBLIC OF TÜRKİYE

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

MICELLES FROM CATIONIC POLYMERS FOR DRUG DELIVERY

Hanan PAŞAOĞLU BELAL

MASTER OF SCIENCE THESIS

Department of Bioengineering

Program of Bioengineering

Supervisor

Assoc. Prof. Dr. Murat TOPUZOĞULLARI

Co-Supervisor

Prof. Dr. Serap DERMAN

June, 2024

REPUBLIC OF TÜRKİYE

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

MICELLES FROM CATIONIC POLYMERS FOR DRUG DELIVERY

A thesis submitted by Hanan PAŞAOĞLU BELAL in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE is approved by the committee on 01.05.2023 in Department of Bioengineering, Program of Bioengineering.

Assoc.Prof. Dr. Murat TOPUZOĞULLARI	Prof. Dr. Serap DERMAN
Yıldız Technical University	Yıldız Technical University
Supervisor	Co- supervisor

Approved By the Examining Committee

Assoc.Prof. Dr. Murat TOPUZOĞULLARI, Supervisor

Yildiz Technical University _____

DOÇ.DR. EMRAH ŞEFİK ABAMOR

Yildiz Technical University _____

DOÇ.DR. MUHAMMET Ü. KAHVECİ

İstanbul Teknik University _____

I affirm that I have acquired the necessary legal permissions for data collection and utilization processes, included appropriate in-text citations, and cited references accurately. I confirm that I have not falsified or fabricated any research data or results within the study. Throughout my Thesis Study titled "Micelles from Cationic Polymers for Drug Delivery," under the guidance of my supervisor, Assoc. Prof. Dr. Murat TOPUZOĞULLARI, I have adhered to the principles of scientific research and ethics. I acknowledge that any false statements may lead to legal consequences.

Hanan PAŞAOĞLU BELAL

Signature

This work was supported by the project from the Scientific Research Projects (BAP) Coordination Unit of Yıldız Technical University (Project No: FCD-2022-4900).

Dedicated to my family and my best friend

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Assoc. Prof. Dr. Murat TOPUZOĞULLARI, for his invaluable guidance, continuous support, and patience throughout my research. His insightful feedback and encouragement have been instrumental in the successful completion of this work.

Besides my supervisor, I would like to thank my co-supervisor Prof. Dr. Serap DERMAN for giving me her time, interest, devoting a great deal of her time, and sharing her great knowledge throughout my study.

My sincere thanks also go to Assoc. Prof. Dr. Emrah Şefik ABAMOR and his doctoral student Büşra AKGÜL for their contribution and assistance in conducting biological activity tests.

Special thanks to my friends Shifa ALHAMVİ and Maria DAABOUL for their motivation, encouragement, and endless support during my thesis work. Also, I would like to thank my lab mates Merve KAYA, Eda Nur YETIŞKIN MORKAN, and Hatice DEMIR and all the Biopolymer and Vaccine Laboratory (KMC306-307) personnel who contributed with sustained assistance.

On a more personal note, I would like to thank my family and friends for their unwavering support and encouragement. Also, I am eternally grateful to my partner, Zaher BELAL, for his love, patience, and understanding during the many hours I spent working on this thesis. Your support was invaluable, and I could not have done it without you.

Finally, I would like to thank the Scientific Research Projects Coordination Unit of Yildiz Technical University for their support within the scope of the project numbered FCD-2022-4900.

Hanan PAŞAOĞLU BELAL

TABLE OF CONTENTS

ACKNOWLEDGEMENTS **VI**

TABLE OF CONTENTS **VII**

LIST OF SYMBOLS **IX**

LIST OF ABBREVIATIONS **X**

LIST OF FIGURES **XII**

LIST OF TABLES **XIII**

ABSTRACT **XIV**

ÖZET **XVI**

1 INTRODUCTION **1**

1.1 Literature Review 1

1.2 Objective of the Thesis 3

1.3 Hypothesis 3

2 THEORETICAL INFORMATION **4**

2.1 Polymeric micelles 4

2.2 Types of polymeric micelles 5

2.3 Polymeric micelles preparation methods 7

2.4 Cationic polymers 8

2.4.1 Types of cationic polymers 8

2.5 Application areas of polymeric micelles 11

2.5.1 Gene delivery 11

2.5.2 Anticancer drug delivery 12

2.5.3 Delivery of antifungal agent 13

2.5.4 Inherently antibacterial and delivery of antibacterial agents 13

3 MATERIAL AND METHOD **17**

3.1 Materials 17

3.2 Equipment 18

3.3 Experimental method 19

3.3.1 Synthesis of Poly(4VP-co-OEGMA) Random Copolymer 20

3.3.2 Quaternization of poly(4VP-co-OEGMA) with 1-bromohexane (BrH) and 1-bromohexadecane (BrHC) 21

3.3.3 Synthesis of quaternized poly(4VP-co-OEGMA) micelles 23

3.3.4 Encapsulation of Juglone into quaternized poly(4VP-co-OEGMA) micelles 23
3.3.5 Characterization 23
3.4 In-vitro Studies 26
3.4.1 Cytotoxicity test 26
4 RESULTS AND DISCUSSION **28**
4.1 Synthesis of the Poly(4VP-co-OEGMA) Copolymer 28
4.2 Synthesis of the Quaternized Poly(4VP-co-OEGMA) Copolymer 31
4.3 Production of the Polycationic Micelle 32
4.4 Biological Investigation of the Micelles 35
5 CONCLUSION **37**
REFERENCES **38**
PUBLICATIONS FROM THE THESIS **60**

LIST OF SYMBOLS

A	Absorbance
cm	Centimetre
Da	Dalton
˚C	Degree Celsius
g	Gram
h	Hour
$[I]_0$	Initial Molarity of Initiator
$[M]_0$	Initial Molarity of Monomer
kDa	Kilodalton
l	Liter
µm	Micrometer
µl	Microliter
mg	Milligram
ml	Milliliter
mV	Millivolts
min	Minutes
M	Molarity
Mw	Molecular Weight
nm	Nanometer
Mn	Number Average Molecular Weight

LIST OF ABBREVIATIONS

ACVA	4-4'Azobiscyanoleric Acid
APs	Antibacterial Polymers
ATR	Attenuated Total Reflectance
BrH	1-Bromohexane
BrHC	1-Bromohexadecane
CMC	Critical Micelle Concentration
CPP	Critical Packing Parameter
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle Medium
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DSSC	Dye Sensitized Solar Sells
ELS	Electrophoretic Light Scattering
EPS	Extracellular Polymeric Substances
FT-IR	Fourier Transform Infrared Spectroscopy
^{1}H-NMR	Proton Nuclear Magnetic Resonance
LC	Drug Loading Capacity
MIC	Minimum Inhibition Concentrations
N_2	Nitrogen
NMR	Nuclear Magnetic Resonance
OEGMA	Poly (ethylene glycol) methyl Ether Methacrylate
PAA	poly(acrylic acid)
PAE	Polyamidoamine-epichlorohydrin

PBS	Phosphate Buffer Solution
PdI	Polydispersity Index
PDMAEMA	poly[2-(dimethylamino) ethyl methacrylate]
PEG	Poly(ethylene glycol)
PEI	Polyethylenimine
PEO	Polyethylene Oxide
PLGA	Poly (Lactic-co-Glycolic Acid)
PLL	Poly(L-lysine)
PM	Polymeric Micelle
PVP	Poly(4-vinylpyridine)
QD	Quaternization Degree
RE	Reaction Yield
TEM	Transmission Electron Microscopy
4VP	4-Vinylpyridine

LIST OF FIGURES

Figure 2.1 The core-shell structure of polymeric micelle. 4
Figure 2.2 Types of polymeric micelles. 5
Figure 2.3 Self-assembling of amphiphilic polymer into spherical shaped PM. 6
Figure 2.4 Self-assembling of amphiphilic polymer into reverse PM. 6
Figure 2.5 Direct dissolution tecnique for micelles preparation. 7
Figure 2.6 Dialysis method for micelle formation. 8
Figure 2.7 The chemical structure of PVP. 11
Figure 2.8 Overview of gene delivery process. 12
Figure 2.9 The mechanism action of cationic polymer against bacteria. 13
Figure 2.10 Diffusing of antibacterial agent through outer wall of gram positive and gram negative bacteria. 15
Figure 2.11 The chemical structure of Juglone. 16
Figure 3.1 Schematic representation of the experimental method. 20
Figure 3.2 Free radical polymerization of poly(4VP-co-OEGMA) copolymer. 21
Figure 3.3 Quaternization reaction of poly(4VP-co-OEGMA) copolymer with 1-bromohexane 22
Figure 3.4 Second quaternization reaction of quaternized poly(4VP-co-OEGMA) copolymer with 1-bromohexadecane. 22
Figure 4.1 The FT-IR spectrum of produced poly(4VP-co-OEGMA) (CP) and poly(4VP-co-OEGMA) quaternized with 1-bromohexane (QCP-H) and 1-bromohexadecane (QCP-HC) 29
Figure 4.2 ^{1}H-NMR spectrum of obtained poly(4VP-co-OEGMA) copolymer. 30
Figure 4.3 The GPC chromatogram of the produced poly(4VP-co-OEGMA) copolymer 31
Figure 4.4 The fluorescence spectra of pyrene (left) and the intensity ratio of I_{338}/I_{333} (right) depending on the QCP-HC concentration 32
Figure 4.5 Hydrodynamic size distribution of free (black line) and Jugloneloaded (red line) micelle. 33
Figure 4.6 TEM images of free (left) and juglone-loaded micelle (right) at 160 kX magnifications. 34
Figure 4.7 Calibration curve of juglone dissolved in acetone acquired by absorbance at 415 nm. 34
Figure 4.8 The cumulative release profile of juglone from the QCP-HC micelles 35
Figure 4.9 The viability of L929 cells against the samples after 24 h of incubation. 36

LIST OF TABLES

Table 2.1 Examples of natural derived cationic polymers ……..…..…… 9
Table 2.2 Examples of synthetic cationic polymers……..…… 10
Table 3.1 Materials used in the thesis study…….……. 17
Table 3.2 Names and brands of devices used in the thesis study ……..….18

ABSTRACT

MICELLES FROM CATIONIC POLYMERS FOR DRUG DELIVERY

Hanan PAŞAOĞLU BELAL

Department of Bioengineering

Program of Bioengineering

Master of Science Thesis

Supervisor: Assoc. Prof. Dr. Murat TOPUZOĞULLARI

Co-supervisor: Prof. Dr. Serap DERMAN

The increased problems related with using therapeutic agents which include limited specificity and off-target effects, poor bioavailability, rapid clearance and short half-life, physical and chemical instability and other limitations, require urgent intervention to find suitable alternatives to these conventional therapeutic approaches. Polymeric micelles have been chosen as a promising alternative to traditional therapeutic agents, due to their unique mechanism in loading therapeutic agents. Cationic amphiphilic copolymers can be utilized to develop polymeric micelles because of their increased interaction with cell membranes resulting in a better internalization of the micelles.

In this study, polymeric micelles carrying Juglone as a model hydrophobic agent were produced using cationic co-polymers of 4-vinylpyridine (4VP) and oligo (ethylene glycol) methyl ether methacrylate (OEGMA) to obtain polymeric micelles as a delivery system. The copolymers were produced with free radical polymerization and modified with quaternization reaction to obtain an amphiphilic polycation suitable for micelle formation. The juglone loaded micelles were prepared using nanoprecipitation technique. While the produced copolymers were characterized by NMR, FT-IR, dynamic light scattering (DLS) spectroscopies and

GPC, the micelles were investigated by FT-IR, DLS spectroscopies and scanning electron microscopy. The average hydrodynamic diameter of the produced cationic micelles loaded with Juglone 135.7 nm and the polydispersity index was 0.141. While juglone was cytotoxic in all studied concentrations, Juglone loaded micelles were less cytotoxic in the related concentrations.

Keywords: 4-vinylpyridine, Juglone, polycation, polymeric micelles.

ÖZET

İLAÇ TAŞIMAYA YÖNELİK KATYONİK POLİMERLERDEN MİSELLER

Hanan PAŞAOĞLU BELAL

Biyomühendislik Anabilim Dalı

Yüksek Lisans Tezi

Danışman: Doç. Prof. Dr. Murat TOPUZOĞULLARI

Eş-Danışman: Dr. Serap DERMAN

Terapötik ajanların kullanımıyla ilgili artan problemler arasında; sınırlı özgüllük ve hedef dışı etkiler, zayıf biyoyararlanım, hızlı temizlenme ve kısa yarı ömür, fiziksel ve kimyasal kararsızlık ve diğer sınırlamalar, bu geleneksel terapötik yaklaşımlara uygun alternatiflerin bulunması için acil müdahale gerekmektedir. Polimerik miseller, terapötik ajanların yüklenmesindeki benzersiz mekanizmaları nedeniyle geleneksel terapötik ajanlara umut verici bir alternatif olarak seçilmiştir Katyonik amfifilik kopolimerik bloklar tarafından oluşturulan polimerik miseller, yüksek stabiliteleri ve artan biyouyumlulukları nedeniyle ilaç dağıtım alanında yüksek verimli görünmektedir.

Bu çalışmada, bir dağıtım sistemi olarak polimerik miseller elde etmek için 4-vinilpiridin (4VP) ve oligo (etilen glikol) metil eter metakrilatın (OEGMA) katyonik kopolimerleri kullanılarak model hidrofobik ajan olarak Juglon taşıyan polimerik miseller üretildi. Katyonik kopolimerler serbest radikal polimerizasyonu ile üretildi ve misel oluşumuna uygun bir amfifilik polikatyon elde etmek için kuaternizasyon reaksiyonu ile modifiye edildi. Juglon yüklü misel nanoçöktürme tekniği kullanılarak hazırlandı. Üretilen kopolimerler NMR, FT-IR, dinamik ışık saçılımı (DLS) spektroskopisi ve GPC ile karakterize edilirken, miseller FT-IR,

DLS spektroskopisi ve transmisyon elektron mikroskobu ile analiz edildi. Juglon 135,7 nm ile yüklenen üretilen katyonik misellerin ortalama hidrodinamik çapı ve polidispersite indeksi 0,141 idi. Juglon çalışılan tüm konsantrasyonlarda sitotoksik iken, Juglon yüklü miseller ilgili konsantrasyonlarda daha az sitotoksik bulunmuştur.

Anahtar Kelimeler: 4-vinilpiridin, Juglon, polikatyon, polimerik miseller.

1

INTRODUCTION

1.1 Literature Review

Polymers are found to be promising candidates for development of new strategies to be used in many medical issues including drug delivery, gene delivery and tissue engineering. There are a lot of studies made on polymers either as drug carriers or as antimicrobial agents [1]. Polymeric micelles are one of drug carrier types that can be created by various polymers and have exhibited noticeable efficiency in transporting diverse medical agents. The polymeric micelles are widely studied especially as delivery systems, since they can be a vehicle to deliver different therapeutic agents to treat numerous diseases.

In this field, cationic polymers have shown outstanding performance especially in the field of delivery systems due to their positive charges which make them capable to react with cellular membrane or with negatively charged genetic material making them suitable candidates in genetic delivery and drug delivery. In addition, polycations can be designed to have the features of lower toxicity, biocompatibility, long-term activity, chemical stability, and ease of production and modification [2].

The cationic polymers can be used to prepare particulate systems e.g. micelles. The polymeric micelles can be produced by self-assembly of amphiphilic copolymers, of which can involve cationic groups to form cationic polymeric micelles.

Cationic micelles have many unique properties. In addition to being positively charged, they have additional features include low toxicity, increased drug loading capacity, relatively high stability and solubility, biocompatibility, core-shell arrangement, lower dissociation rate and thanks to their small size and surface modifications they have good targeting properties. All these mentioned distinctive properties make cationic micelles suitable for using as delivery system whether to deliver antimicrobials, different drugs or even genetic material like DNA or RNA.

There are several studies made on cationic micelles as nanocarrier for either gene delivery and drug delivery. One of these studies is that of Wang et al. [1]. In this study, poly (ethylene glycol) (PEG) with different molecular weights (Mn=550,

1100, and 2000) was conjugated to P(MDS-co-CES) with varying degrees of cholesterol grafting. The objective was to enhance the stability of micelle/DNA complexes in the bloodstream for systemic in vivo gene delivery. The DNA binding capacity, gene transfection efficacy, and cytotoxicity of P(MDS-co-CES) and PEGylated P(MDS-co-CES) micelles were evaluated and juxtaposed. As a result, PEGylated P(MDS-co-CES) micelles exhibited significantly lower cytotoxicity; however, they demonstrated reduced gene transfection efficiency. Micelles that self-assemble from PEGylated P(MDS-co-CES) exhibited high stability and this was crucial for effective gene transfection. Despite their reduced DNA binding capacity and relatively larger particle size, these micelles demonstrated enhanced gene transfection ability.

A previous study of our group is a good example of antibacterial polycationic micelles [2]. In the study of Kadayifci et al. [2], amphiphilic copolymer of poly(4-vinylpyridine)-b-(oligoethylene glycol methyl ether methacrylate) was used to prepare core cross-linked micelles to obtain inherently antibacterial micelles. The resulting material was effective against *E. coli*. To increase and broaden the antibacterial efficacy of these micelles, antibacterial small molecules can be encapsulated into these structures.

Juglone (5-hydroxy-1,4-naphthoquinone) is a small organic molecule derived from walnut tree and is found to have high antibacterial activity against various types of bacteria. Few studies have characterized its bacterial activity when conjugated with polymeric nanoparticles, among these study there is Arasoglu et al. study which produced Juglone encapsulated PLGA nanoparticles by the modified single emulsion solvent evaporation method [3]. Then, the antibacterial activity of Juglone loaded PLGA nanoparticles and free juglone was investigated. As a result, Juglone encapsulated nanoparticles revealed higher and longer antibacterial activity than free Juglone. However, Juglone encapsulated polycationic micelles have not been reported previously.

In this study, quaternized poly(4VP-co-OEGMA) copolymers and Juglone were used to produce Juglone loaded polycationic amphiphilic micelles as drug delivery systems.

1.2 Objective of the Thesis

The purpose of this study is production of polymeric micelles from cationic polymers of poly(4VP-co-OEGMA) random copolymer for Juglone delivery which has been chosen as a model hydrophobic active agent.

To achieve this purpose,

1. Synthesis of poly(4VP-co-OEGMA) random copolymer,
2. Quaternization of poly(4VP-co-OEGMA) with 1-bromohexane (BrH) and 1-bromohexadecane (BrHC),
3. Encapsulation of Juglone into cationic quaternized poly(4VP-co-OEGMA) micelles.
4. Investigation of biocompatibility of produced polymeric materials were targeted.

1.3 Hypothesis

Various systems are being developed and utilized to transport therapeutic agents with the goals of targeting damaged or diseased tissues, prolonging their circulation time within the body, and making hydrophobic molecules water-soluble. Juglone, a molecule with toxic effects, also possesses antimicrobial and anticancer properties. Additionally, as a hydrophobic molecule, it has very low solubility in water. Therefore, it is important to integrate it with a carrier system to increase its solubility in water, reduce its toxic effects, and enhance its therapeutic efficacy.

In this thesis, the copolymer poly(4VP-co-OEGMA) was used, where the OEGMA units ensure the polymer's solubility in water. By quaternizing the 4VP units with hexyl and hexadecyl groups, the structure gains both positive charges and long hydrophobic chains, resulting in an amphiphilic copolymer. This design is intended to allow the polymer to aggregate and form micelles in water.

It is hypothesized that micelles produced by encapsulating Juglone in the cationic polymer used in this thesis will rise the water availability of Juglone and decrease its toxicity. Furthermore, because the outer part of these micelles is cationic, they are expected to have higher interaction with the target cells and thus higher cellular uptake. This material, beyond its use with Juglone as a model, is also considered to be potentially applicable for the delivery of other hydrophobic drugs.

2

Theoretical Information

2.1 Polymeric micelles

In recent years, many research have paid special attention to polymeric micelles, due to their unique ability in creating drug delivery systems. Polymeric micelles are commonly created by spontaneous self-assembly of amphiphilic block copolymers, containing a hydrophobic part and a hydrophilic part, as a result of hydrophobic interaction in an aqueous solution [4], [5]. Many distinct advantages of polymeric micelles make them a perfect choice for targeted drug delivery. These distinct properties include low critical micelle concentration (CMC) due to increased molecular weight, low toxicity, increased drug loading capacity, relatively high stability and solubility, biocompatibility, core-shell arrangement, lower dissociation rate and thanks to their small size and surface modifications they have good targeting properties. The structure of polymeric micelle is formed in core-corona shape in which the hydrophobic block composes the core of the micelle giving the capability to encapsulate various hydrophobic drugs, while the hydrophilic block forms the outer shell (corona) of the micelle which protects and stabilizes the hydrophobic core in the aqueous medium and increases the water solubility of polymers [4], [5], [6], [7], [8]. The typical structure of polymeric micelle is shown in Figure 2.1.

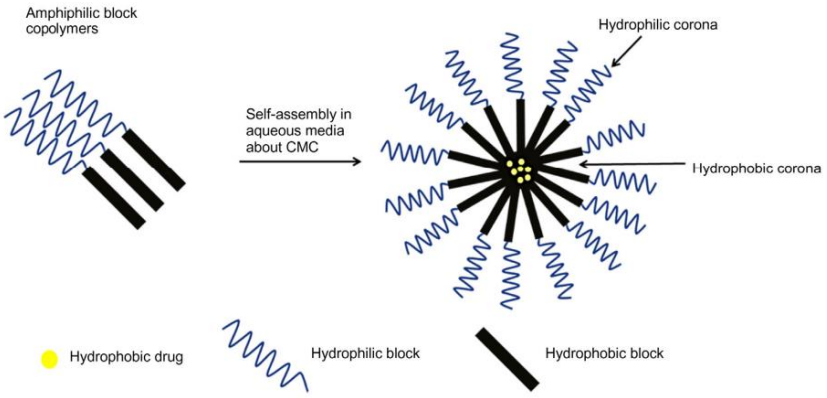


Figure 2. 1 The core-shell structure of polymeric micelle [6].

2.2 Types of polymeric micelles

The morphologies of micelles vary according to the composition of their hydrophobic and hydrophilic segments, as well as the conditions of the solvent. Also, the self-assembling procedure to create polymeric micelles (PMs) can be affected by the type of polymer utilized in the process. Therefore, di-block, tri-block, multi-block copolymers, graft polymers, stimuli-sensitive polymers, and other types can develop several types of PM structures. Thus, PM can found in various shapes such as spheres, tubules, inverse micelles, bottle-brush shapes, and more [9], [10].

In addition to that, there are some features that affect the variation of PMs such as pH, ionic strength, solvent type, polymer concentration. Polymeric micelles can be categorized into two classes based on their assembly properties. Self-assembled micelles and covalently assembled micelles (such as unimolecular star micelle and unimolecular dendritic micelle) are shown in Figure 2.2 [11].

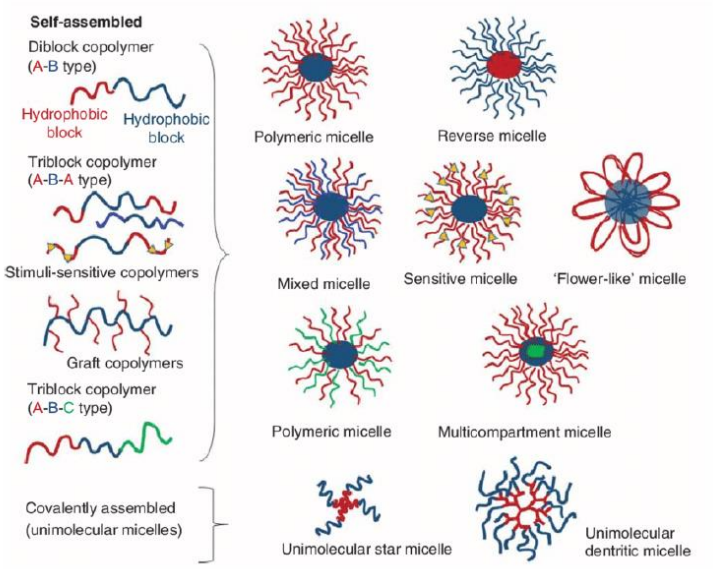


Figure 2. 2 Types of polymeric micelles [11].

The lengths of hydrophilic and hydrophobic blocks directly influence the morphology of micelles. When the hydrophobic part is longer than the hydrophilic part, PMs tend to take on rod-like or lamellar shapes. Conversely, a longer hydrophilic part typically results in spherical PMs [11].

Spherical shaped PMs can be obtained by using amphiphilic polymer with hydrophilic segment longer than hydrophobic segment. In which the hydrophobic

part assembles inward forming the core of micelle, while the hydrophilic section is directed outward forming the shell of micelle as shown in Figure 2.3 [10], [12].

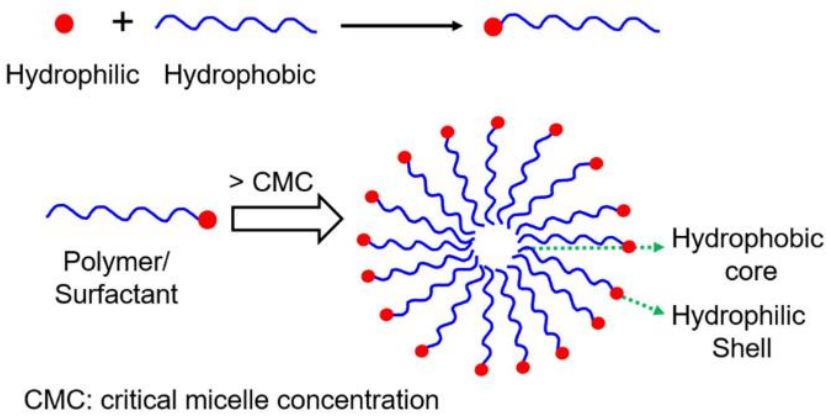


Figure 2. 3 Self-assembling of amphiphilic polymer into spherical shaped PM [9].

In contrast, for reverse micelles, the hydrophilic block of the amphiphilic polymer forms the inner core, while the hydrophobic block constitutes the outer shell of the polymeric micelle as shown in Figure 2.4 [9].

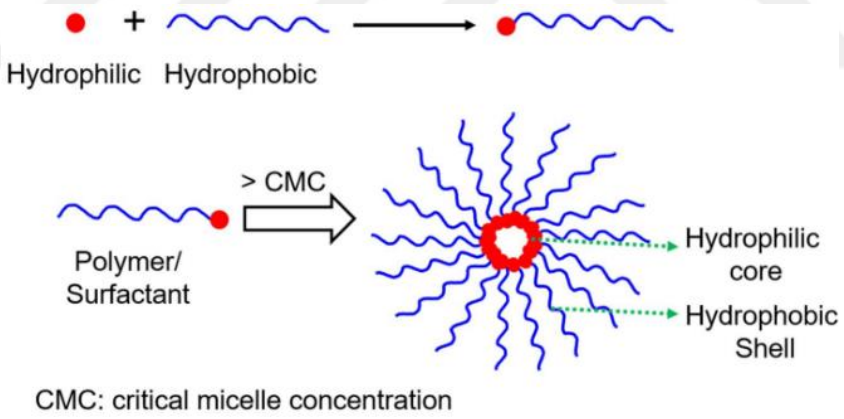


Figure 2. 4 Self-assembling of amphiphilic polymer into reverse PM [9].

Reverse micelles can be obtained using different types of macromolecules including branched or hyperbranched polymer, star-shaped polymer and water-soluble dentriticts [13].

The morphology of the micelles can be predicted by using critical packing parameter, which is a theoretical parameter calculated by the Formula 2.1.

$$\text{CPP} = \frac{hydrophobic\ volume}{hydrophobic\ length * interfacial\ area} \quad (2.1)$$

For example, when CPP <0.35 the aggregation form will be spherical micelles [14].

2.3 Polymeric micelles preparation methods

There are different methods to produce micelles, which include;

1. Direct dissolution method,
2. Solvent evaporation method,
3. Dialysis method,
4. Emulsion method,
5. Nanoprecipitation method

The suitable method for micelle preparation can be chosen depending on the physicochemical properties of the block copolymers. In addition to that, particularly, the choose of the proper technique usually depends on the solubility of the copolymer forming the micelle block in an aqueous medium [6], [15]. For example, the appropriate method to form micelles from copolymers, that have relatively high-water solubility, is direct dissolution method, where both the copolymers and drug are directly dispersed in aqueous media which can be distilled deionized water or buffer. Drug encapsulation within micelles can be induced through various methods such as heating the solution, stirring, or ultrasound treatment as shown in Figure 2.5 [6], [15].

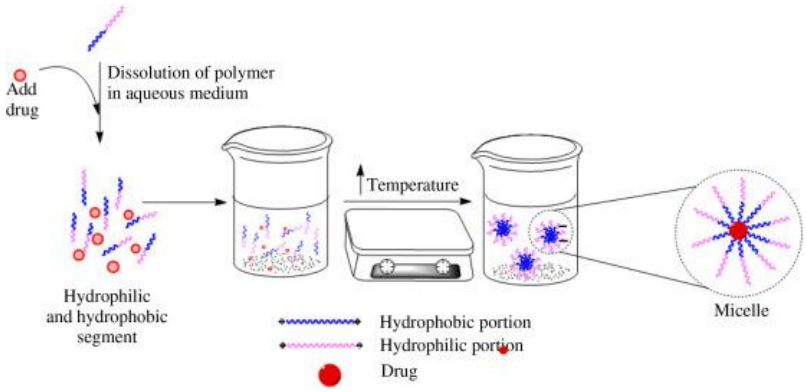


Figure 2. 5 Direct dissolution technique for micelles preparation [6].

In the case of copolymers with low water solubility, the dialysis method is frequently applied to produce polymeric micelles. In this method an appropriate organic solvent is used to dissolve both the copolymer and active agents, then the copolymer-drug mixture is dialyzed against water. Thereby, entering water to the

dialysis bag stimulates self-assembly of the block copolymer resulting in micelles formation as shown in Figure 2.6 [6], [16].

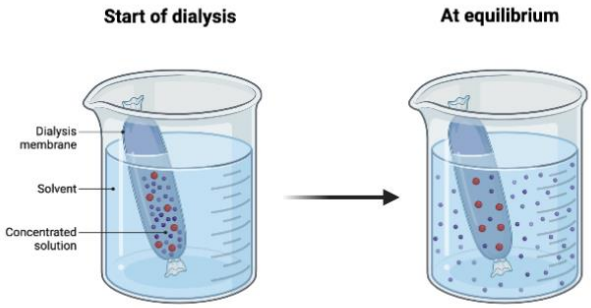


Figure 2. 6 Dialysis method for micelle formation [17].

Also, the emulsion method is frequently applied to form micelles. Here, copolymer and drug are solved in water-immiscible solvents like acetone, then the solution is dripped slowly into distilled water. The mixture is left for 24 hours to allow the solvent to evaporate and to complete self-assembly process of copolymer block resulting in micelles formation [18].

2.4 Cationic polymers

Cationic polymers are defined as macromolecules containing positive charges, which can be found on the side chains of polymer or on its backbone, or both. Their unique properties make them as promising candidates for using in many therapeutic applications involving drug delivery, gene delivery, tissue engineering and in the development of antibacterial systems. The positive charges present in polycations enable them to interact effectively with genetic material or cellular membranes, that makes them suitable agents for such applications. In addition, these types of polymer can be antibacterial agents by themselves thanks to positive charges present on their backbone [19], [20], [21].

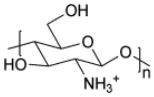
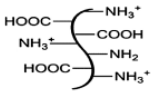
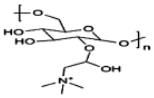
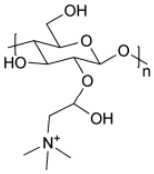
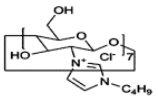
2.4.1 Types of cationic polymers

According to their origin cationic polymers are divided into two groups: natural and synthetic.

Nowadays cationic polymers derived naturally are used as attractive candidates in various medical applications because of their typically non-toxic nature, derivation from renewable sources, biocompatibility, biodegradability, and minimal

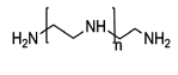
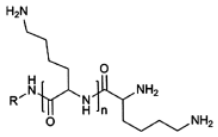
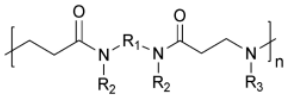
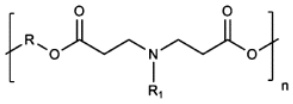
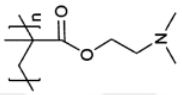
immunogenicity. In addition to the ability of improvement physicochemical features of natural cationic polymer simply by alteration in the reactive sides found on their backbone [21]. Examples of naturally originated cationic polymers and their applications are shown in Table 2.1.

Table 2.1 Examples of natural derived cationic polymers [22].

Cationic polymer	Nature	Structure	Application
Cationic chitosan	Polysaccharide *N*-acetyl glucosamine and D-glucosamine		Drug delivery Tissue engineering Gene delivery
Cationic gelatin	Protein 18 Non-uniformly distributed amino acids		Drug delivery Tissue engineering Gene delivery
Cationic dextran	Polysaccharide Glucose units linked by α-1,6-linkages		Drug delivery Tissue engineering Gene delivery
Cationic cellulose	Polysaccharide Linear glucose units linked by β-1,4-D-linkage		Drug delivery Tissue engineering Gene delivery
Cationic cyclodextrin	Polysaccharide Cyclic glucose units linked by α-1,4-linkage		Drug delivery Tissue engineering Gene delivery

Although natural cationic polymers are widely used in many therapeutic applications, the primary challenges associated with batch-to-batch variation in them still a big disadvantage. So that, to overcome this challenge synthetic cationic polymers emerged to be good alternatives to use in various applications. Synthetically derived cationic polymers provide high ability to be chemically modified to broaden their medical applications. For example, specific polycationic structures can be obtained by conjugating various bioactive molecules and functional groups to their chain. Also the degradation properties and therapeutic efficiency of synthetic cationic polymer can be adjusted [21], [22]. Examples of synthetic cationic polymers and their applications are mentioned in Table 2.2

Table 2.2 Examples of synthetic cationic polymers [22].

Cationic polymer	Nature	Structure	Application
PEI	LPEIs contain all secondary amines BPEIs contain primary, secondary and tertiary amines		Drug delivery Tissue engineering Gene delivery
PLL	Homopolymer of the amino acid L-lysine.		Drug delivery Tissue engineering Gene delivery
PAA	Synthetic cationic polymer containing tertiary amino groups.		Drug delivery Tissue engineering Gene delivery
PAE	Amine containing polyesters		Drug delivery Tissue engineering Gene delivery
PDMAEMA	Synthetic cationic polymer containing tertiary amino groups.		Drug delivery Tissue engineering Gene delivery

Recently, cationic polymers with quaternary ammonium groups are found to have highly developed therapeutic properties, since they can react with cellular membrane and genetic material. To obtain polymer with quaternary ammonium groups, alkylating agents is chemically introduced to the backbone of polymer bearing amine groups [23]. Quaternization is found to be a suitable procedure to produce polycation with quaternary ammonium groups. Polymer containing amine groups can be functionalized with different functional groups by quaternization reaction. The most important parameters to be taken in consider when designing such polycation are the length and number of hydrophobic groups and the strength and number of cationic groups, since it affects the efficiency of therapeutic performance of these polymer [24], [25]. Poly(4-vinylpyridine) (P4VP) are widely studied polymers to produce cationic bearing quaternary ammonium groups by quaternization reaction.

- Quaternized poly(4-vinylpyridine) (PVP)

Poly(4-vinylpyridine) is a linear polymer containing pyridine rings (Figure 2.8). It is a hydrophobic polymer in a polar solvent but converts to a polycation at low pH (pH<5) and becomes water soluble, so that it is a pH sensitive polymer due to the presence of the tertiary nitrogen in its pyridine ring [26], [27]. Thanks to pyridine groups, poly(4-vinylpyridine) can be used in several application areas [28].

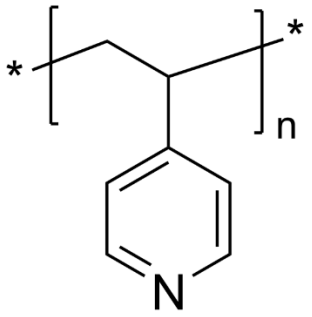


Figure 2. 7 The chemical structure of PVP [29].

Quaternized PVP has been recently studied widely for development of drug delivery systems. As a result of quaternization reaction, permanent positively charged groups can be introduced to the backbone of PVP leading to electron delocalization phenomena producing positively charged quaternary ammonium PVP. It has been found that a strong polycation with high therapeutic properties can be produced by quaternizing PVP with especially n-alkylbromides. But due to its hydrophobicity and cytotoxicity, it has been found that introducing of a hydrophilic polymer such as oligoethyleneglycol methacrylate (OEGMA) can result in producing quaternized amphiphilic copolymer with enhanced properties like water soluble, non-toxic and biocompatible [24], [25], [28].

2.5 Application areas of polymeric micelles

Due to their numerous advantages, polymeric micelles have been used in different medical areas including;

2.5.1 Gene delivery

Nowadays, cationic micelles self-assembled of biodegradable amphiphilic copolymer are widely used as nanocarriers in the field of gene delivery because of their unique features beginning with their positive charges that enable them to react with cellular membrane and also to bind with negatively charged DNA leading to form stable and size-controlled polyplex. Moreover, considering the safety concerns that viral vectors continue to encounter during the advancement through clinical trials, polycationic micelles have emerged as a promising alternative due to their reduced safety concerns and improved ability to react more efficiently and stably with cells compared to other chemically engineered nonviral vectors [1], [30].

In gene delivery process, the pDNA-polycation complex undergoes many intra and extracellular steps including; pDNA condensation, cellular uptake, endosomal release, nuclear transport, pDNA–polycation complex unpacking, and gene expression. All mentioned steps are shown in Figure 2.8.

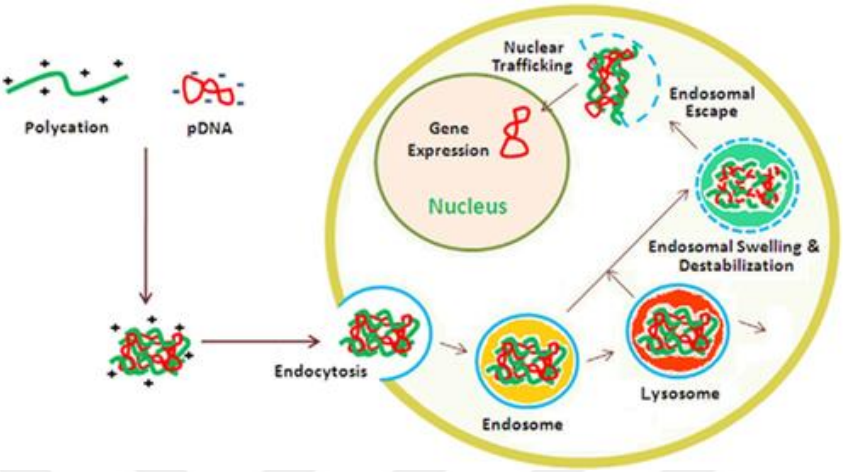


Figure 2. 8 Overview of gene delivery process [31].

As shown in Figure 2.8, the polycationic gene delivery process requires several key steps. Firstly, polycationic carrier, typically consisting of positively charged molecules, forms a complex with negatively charged nucleic acids, such as DNA or RNA, resulting in the formation of stable polyplexes. The primary function of these polyplexes is to shield nucleic acids from degradation and facilitate their transportation to target cells. When reaching the target cells, the polyplexes react with the cell membrane through various mechanisms, such as endocytosis or direct membrane fusion, allowing internalization into the cytoplasm. After being inside the cell, the nucleic acids are released from the polyplexes and can enter the nucleus (in the case of DNA) or undergo translation (in the case of RNA), finally leading to the desired genetic expression or therapeutic effect. Throughout this process, the properties of the polycation carriers, involving their size, charge, and surface modifications, play crucial role in determining the efficiency and specificity of gene delivery [31].

2.5.2 Anticancer drug delivery

In the field of cancer treatment, one of the major problems was that chemotherapy targeting all cells that divide quickly both the abnormal and normal ones. It has been demonstrated in many studies that the use of polymeric micelles as a drug

delivery system to deliver chemotherapeutic agents only to cancerous tissues has a highly positive effect in increasing drug efficacy. Since, they can target actively the tissue by attaching targeting specific ligands on their surface. In addition to their prolonged circulation time in blood what lead to superior accumulation of drug in cancerous tissue [32].

2.5.3 Delivery of antifungal agent

For fungal infection, the low stability and systemic toxicity of antifungal agents form significant problems that lead to decrease the efficiency of these agents in treatment procedure. So that, polymer micelles are found to be ideal as delivery system for the treatment of systemic fungal infection, due to their high stability and their ability to solubilize the drug leading to reduce its toxicity [33].

2.5.4 Inherently antibacterial and delivery of antibacterial agents

It has been demonstrated that polymeric micelles can exhibit antibacterial potency when conjugating with cationic groups that increases their positive charges what make them antibacterial by themselves thus, they can combat bacteria by disruption their negatively charged membrane. The mechanism action of cationic polymers starts with the electrostatic interaction between the cationic moieties and phosphate groups found on the anionic membrane of targeted bacteria. At the same time, the hydrophobic alkyl chains of cationic polymer penetrate and bind to the hydrophobic parts of lipids found in the bacterial membrane leading to membrane disruption resulting in outflow of intracellular components, degradation of proteins and nucleic acids, cell wall lysis and cell death (as shown in Figure 2.9) [19], [20], [24]. Thus, the antibacterial performance of cationic polymer can be essentially achieved by two components the cationic group and the hydrophobic group. [34].

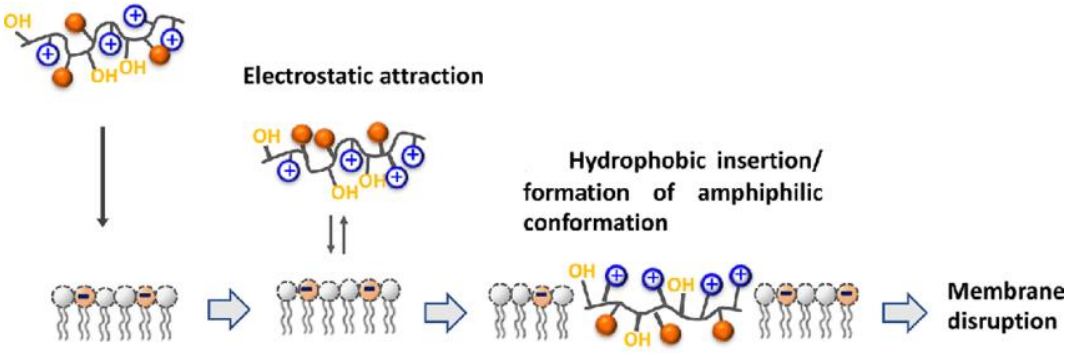


Figure 2. 9 The mechanism action of cationic polymer against bacteria [35].

Thereby, antibacterial polymeric micelles form a promising alternative due to their high positive charge density, increased surface area, versatility, biocompatibility and nonimmunogenicity. Besides being antibacterial by themselves, polymeric micelles can act as an antibacterial agent delivery system, what increase the effectiveness of treatment against bacteria [2].

The polycationic micelles can also be used to deliver antibacterial agents which are defined as chemical drugs that used to treat bacterial infections. The antibacterial agents can be classified into two groups, first group is bactericidal which work on killing the target bacterium and fungus, the second one is bacteriostatic which work on inhibition of their growth and multiplication without hurting cells of the host [36]. The antibacterial agents prevent bacteria or fungi from growth by many mechanisms:

- Inhibiting cell wall synthesis
- Increasing the permeability of cell membrane
- Targeting bacterial nucleic acid (DNA) and its associated processes like protein synthesis and ribosomal function [37], [38].

Inhibition of bacterial cell wall synthesis

As mentioned before, some types of bacteria are classified with respect to their differences in cell wall structure, among these types there are gram negative and gram-positive bacteria. The antibacterial agents that work on inhibit bacterial cell wall synthesis attack critically peptidoglycan layer present in the cell wall of these bacteria. The main role of peptidoglycan layer is keeping bacteria survive in hypotonic environments, those targeting this layer leads to destroy the rigidity of the bacterial cell wall resulting in cell lysis and death (as shown in Figure 2.10) [38].

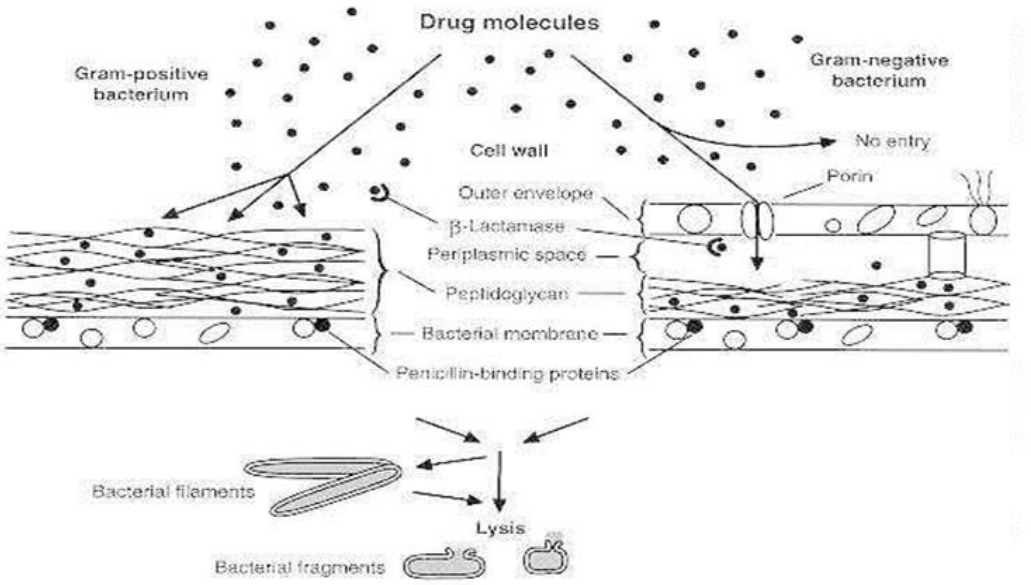


Figure 2. 10 Diffusing of antibacterial agent through outer wall of gram positive and gram negative bacteria [38].

Antibacterial agents can be either of natural origin or manufactured. The natural antibacterial agents are derived from plants, microorganisms and animals [39]. Historically, plants were one of the most important source of novel drug components and had an important role in the enhancement of human health [40]. Juglone is one of the natural origin drugs derived from a walnut plant with high antimicrobial and antitumor activities [41].

Juglone

Juglone (5-hydroxy-1,4-naphthoquinone) is a small organic molecule derived from walnut tree. It is present almost in all of parts of walnut tree and characterized by its high antibacterial, antifungal, antiviral and antitumor activities. It is an aromatic organic compound with a distinct phenyl group (as shown in Figure 2.10) since it belongs to the Naphthoquinones family that are wide- spread phenolic compounds in nature [42], [43], [44]. Juglone is widely utilized in the curation of many diseases include cancer, fungal infections, bacterial infections, and viral infections [41]. Besides, Juglone, with a dark reddish-brown color, has wide uses in cosmetic field as colorant to dye hair and fabrics. Its bioactivities are acquired by its high ability to covalently bind to proteins [45].

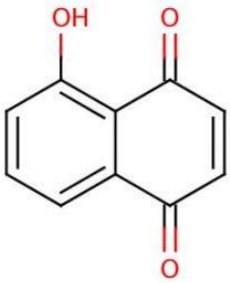


Figure 2. 11 The chemical structure of Juglone [46].

Many studies have been done on Juglone to discover its effects on microorganisms, and have revealed that Juglone has high potential effect to inhibit a wide spectrum of bacteria involving gram positive and gram negative bacteria like Helicobacter pylori. Also Juglone is approved to have high antifungal activity compared with another known antifungal agent such as selenium sulfide [44]. The action mechanism of Juglone is from its high toxicity that affects microorganisms in several way including transcription inhibition, cancelation of cell membrane potential, cell cycle disruption, cell death, inhibition of miRNA synthesis, DNA modification and/or generation of hydrogen peroxide [3], [47]. One of most important characteristic of Juglone is its biodegradability since it can degrade to non-toxic products, thus it can be utilized safely in human body [48]. In this study, Juglone has been chosen as a model hydrophobic active agent to be encapsulated in a cationic polymeric micelle.

3

Material and Method

3.1 Materials

All chemicals used in this thesis are mentioned in Table 3.1.

Table 3. 1 List of chemicals used in the study

Chemical Name	Company	Purpose of usage
4-Vinylpyridine	Sigma-Aldrich	Polymer synthesis
Poly (ethylene glycol) methyl ether methacrylate, Mw 500 Da	Sigma-Aldrich	Polymer synthesis
4-4'-Azobis(4-cyanovaleric acid)	Sigma-Aldrich	Initiator
1-Bromohexane	Sigma-Aldrich	Quaternization agent
1-Bromohexadecane	Sigma-Aldrich	Quaternization agent
Dimethylformamide	Sigma-Aldrich	Solvent
Diethyl ether	Sigma-Aldrich	Solvent
Ethanol	Sigma-Aldrich	Solvent
Acetone	ISOLAB	Solvent
Mouse fibroblast cell line (L929)	YTU- Cell Culture and Tissue Engineering Laboratory	Cytotoxicity Test - Cell culture
Dulbecco's Modified Eagle Medium (DMEM/F12)	Capricorn	Cytotoxicity Test - Cell culture
FBS (Fetal Bovine Serum)	Capricorn	Cytotoxicity Test - Cell culture

Table 3.1 list of chemicals used in the study (continued)

Penicillin-streptomycin	Capricorn	Cytotoxicity Test - Cell culture
Dimethyl sulfoxide	Sigma Aldrich	Cytotoxicity Test - Cell culture
Trypsin-EDTA (0.25%)	Capricorn	Cytotoxicity Test - Cell culture
MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide]	Biomatik	Cytotoxicity Test - Cell culture
E. Coli cell line (NCTC 9001)	YTU- Cell Culture and Tissue Engineering Laboratory	Antibacterial activity assay
S. aureus cell line (NCTC 10788)	YTU- Cell Culture and Tissue Engineering Laboratory	Antibacterial activity assay
Sodium chloride	Merck	Antibacterial activity assay
Yeast Extract	Merck	Antibacterial activity assay
Bacto agar	Merck	Antibacterial activity assay
Peptone	BD	Antibacterial activity assay

3.2 Equipment

All instruments used in this thesis are mentioned in Table 3.2.

Table 3. 2 Names and brands of devices used in the study

Device Name	Brand/Model
NMR Spectrometer	Bruker Avance 500
FT-IR Spectrometer	Nicolet™ iS™ 10
UV-Vis Spectrometer	Shimadzu UV-1700

Table 3.2 Names and brands of devices used in the study (continued)

GPC	Viscotek TDA302
Dynamic and Electrophoretic Light Scattering Spectroscopy	Malvern Zetasizer NanoZS
Vortex	Heidolph Reaxtop
Analytical Balance	Precisa
Refrigerated Centrifuge	Sigma
Vacuum Oven	Binder
Ultra-Pure Water Device	Millipore MilliQ Gradient
Magnetic Stirrer	Heidolph
Orbital Shaking Water Bath	N-Biotek-303
Lyophilizator	Telstar Cryodos
Laminer flow cabin	Faster
incubator	Binder
Invert microscope	Olympus
Centrifuge	Himac
Microplate reader	Multiskan™ FC
Laminer flow cabin	Thermo Scientific Hera safe
Incubator	Innova/CO-170

3.3 Experimental method

The purpose of this study is production of polymeric micelles from cationic polymers of poly(4VP-co-OEGMA) random copolymer for Juglone delivery which has been chosen as a model hydrophobic active agents and examine their biocompatibility and cytotoxicity.

In order to achieve the aim of this study several experimental steps were followed, and these steps include:

1. Synthesis of poly(4VP-co-OEGMA) random copolymer
2. Quaternization of poly(4VP-co-OEGMA) with 1-bromohexane (BrH) and 1-bromohexadecane (BrHC)
3. Encapsulation of Juglone into quaternized poly(4VP-co-OEGMA) polymers to form micelles
4. Characterization of all the produced materials
5. Investigation of biocompatibility of synthesized products.

By Figure 3.1 the experimental methods carried out can be shown schematically.

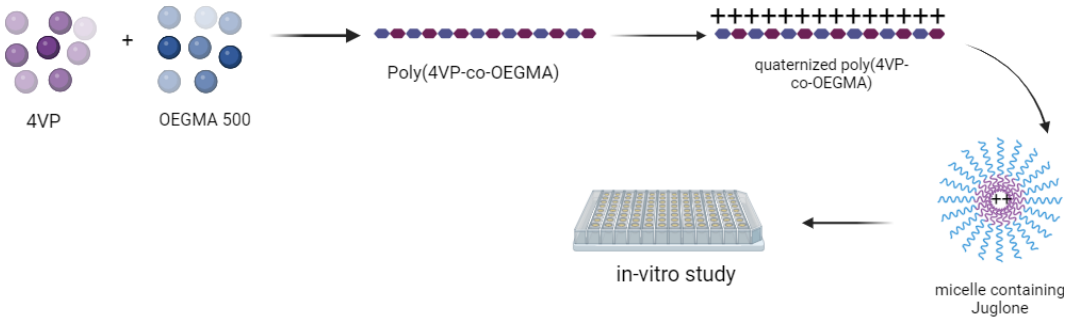


Figure 3. 1 Schematic representation of the experimental method.

3.3.1 Synthesis of Poly(4VP-co-OEGMA) Random Copolymer

In the study, Juglone encapsulated polymeric micelles with antibacterial properties were produced during several steps. Firstly, poly(4VP-co-OEGMA) random copolymer was synthesized by free radical polymerization of 4VP and OEGMA (M_w = 500 Da) monomers. 4-4'azobiscyanoleric acid (ACVA) was used as the initiator, and dimethylformamide (DMF) was used as a solvent as shown in Figure 3.2.

A solution containing 0.4 M of 4VP and 0.1 M of OEGMA monomers dissolved in 20 ml of DMF was prepared. The solution underwent N_2 gas purging for 15 minutes to eliminate dissolved oxygen, after which it was heated to 70°C. The polymerization reaction commenced upon addition of ACVA solution to the

monomer solution. The initial molar ratio of the monomer to the initiator ($[M]_0/[I]_0$) was 200:1. The free radical polymerization reaction was conducted for 24 hours.

After 24 hours, precipitation process was carried out by adding excess cold diethyl ether to obtain copolymer and washing many times in order to eliminate the unreacted monomers and the remaining DMF. Finally, vacuum oven was used overnight at 50°C to dry the obtained copolymer. The chemical analysis of gained poly(4VP-co-OEGMA) copolymer was performed by using FTIR and NMR spectroscopies and the molecular weight was determined using GPC.

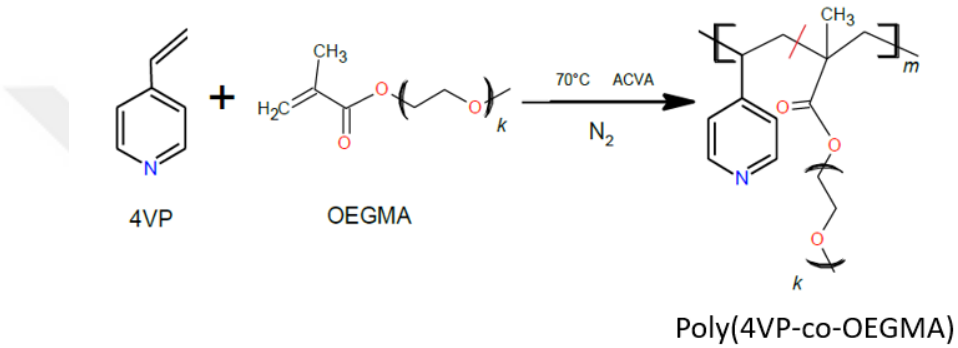


Figure 3. 2 Free radical polymerization of poly(4VP-co-OEGMA) copolymer.

3.3.2 Quaternization of poly(4VP-co-OEGMA) with 1-bromohexane (BrH) and 1-bromohexadecane (BrHC)

In order to acquire cationic copolymer, the produced poly(4VP-co-OEGMA) copolymer was quaternized with 1-bromohexane (BrH) (Figure 3.3). So that, the copolymer was solved in 2.5 ml ethanol and 45 µl of BrH was added to obtain 40% of quaternization degree. The reaction was carried out at 70°C for 3 days. After that, excess cold diethyl ether was used again to precipitate the gained polycation and eliminate the remaining BrH and ethanol, and then it was dried in a vacuum oven at 50°C for 24 h. The quaternized copolymer was chemically analyzed by FTIR spectroscopy.

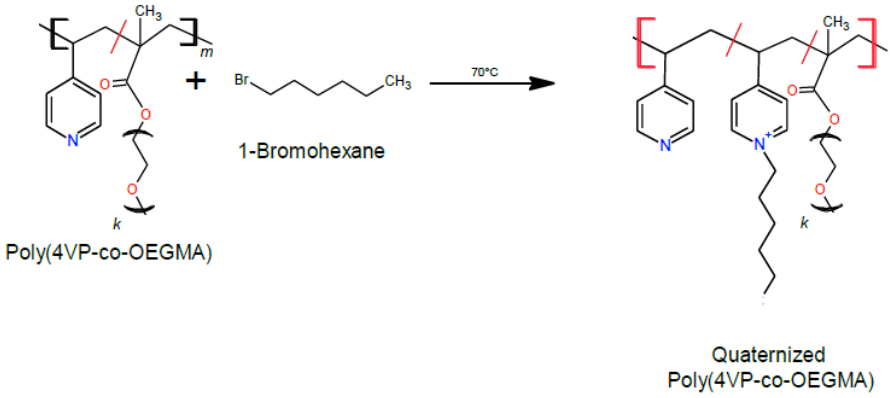


Figure 3. 3 Quaternization reaction of poly(4VP-co-OEGMA) copolymer with 1-bromohexane.

To increase hydrophobic character of the acquired polycation, it was necessary to rise the quaternization degree to 70% by adding a more hydrophobic agent which is 1-bromohexadecane (BrHC). In this process the obtained polycation was dissolved in 2.5 ml ethanol and 118 µl of BrHC was added (Figure 3.4). Then, the reaction was left to carry out for one week. In the next step, precipitation process was done by excess cold diethyl ether and finally the produced polycation was dried in vacuum oven at 50°C for one night. The quaternized copolymer was chemically analyzed by FTIR spectroscopy.

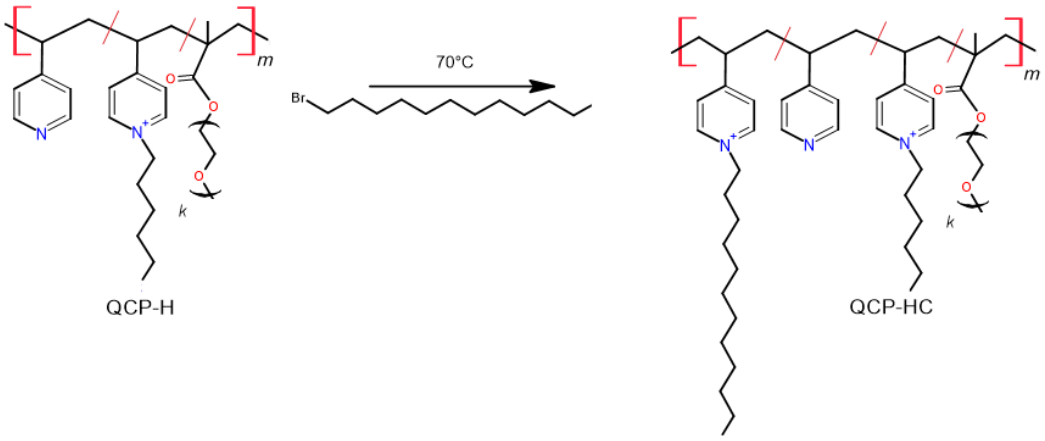


Figure 3. 4 Second quaternization reaction of quaternized poly(4VP-co-OEGMA) copolymer with 1-bromohexadecane.

3.3.3 Synthesis of quaternized poly(4VP-co-OEGMA) micelles

In order to form quaternized poly(4VP-co-OEGMA) micelles, 8 mg of copolymer was solved in 1 ml acetone. Then the solution was poured drop by drop to 5 ml ultra-pure water under vigorous stirring. The solution was left for one night to ensure evaporation of acetone. After that, the hydrodynamic size and zeta potential value of the synthesized cationic micelles were adjusted by DLS and ELS, respectively. In the end, the solution was dried by lyophilization to obtain micelles as powder for further analysis.

3.3.4 Encapsulation of Juglone into quaternized poly(4VP-co-OEGMA) micelles

In the purpose of formation Juglone encapsulated quaternized poly(4VP-co-OEGMA) micelles, 8 mg of copolymer and 1 mg of Juglone were solved in 1 ml acetone. Then the solution was poured drop by drop to 5 ml ultra-pure water under vigorous stirring. The solution was left for one night to ensure evaporation of acetone. After that, in order to exclude the non-binding drug, the solution was filtered using 0.22 mm filter. The hydrodynamic size and zeta potential value of the synthesized cationic micelles were defined by DLS and ELS, respectively.

3.3.5 Characterization

- Fourier Transform Infrared Spectroscopy (FT-IR)

The chemical composition of our samples was detected in every step using Shimadzu IRPrestige-21 FTIR with attenuated total reflectance (ATR) attachment at wavenumber range between 4000 and 650 cm^{-1}. In addition, the quaternization degree of the polymers was determined using Equation 3.1 [25] . All samples were dried before applying them on FTIR analysis.

$$Q\% = \frac{A_{1640}}{A_{1640} + (1.68 + A_{1600})} \times 100 \qquad (3.1)$$

Where,

Q% is the quaternization degree, and

A_{1640} and A_{1600} are the absorbance values of the material at the wavenumbers of 1640 and 1600 cm^{-1}, respectively.

- Gel Permeation Chromatography (GPC)

The average molecular weight and molecular weight distribution of the produced poly(4VP-co-OEGMA) were detected by utilizing GPC system. The analysis was performed using Viscotek TDA302 GPC instrument and the used column was Eprogen CatSEC300. The flow rate during analysis was 0.3 ml/min. The mobile phase was prepared using a solution composed of 0.1 M acetic acid (CH_3COOH) and 0.50 M sodium chloride (NaCl). The concentration of prepared polymer was 2 mg/ml. Both the prepared polymer and the mobile phase solution were filtered with 0.45 µm filters and degassed in an ultrasonic water bath before the analysis. The used calibration standard of GPC system in this analysis was Polyethylene oxide (PEO) (M_W = 23.8 kDa, M_w/M_n = 1.013).

- Nuclear Magnetic Resonance (NMR) Spectroscopy

The molecular structure of the poly(4VP-co-OEGMA) copolymer and the monomer quantities in its chain were determined using NMR spectroscopy. H-NMR spectrum of the copolymer was acquired by using Bruker Ascend 500 MHz nuclear magnetic resonance spectroscopy. The polymer sample was prepared by dissolving in deuterated dimethyl sulfoxide (DMSO).

- Dynamic and electrophoretic light scattering spectroscopy

The zeta potential, hydrodynamic diameter and hydrodynamic size distribution of both polycationic micelles and Juglone encapsulated polycationic micelles were assigned by DLS and ELS techniques. The samples were measured as being dissolved in ultra-pure water using Malvern Zetasizer Nano-ZS device equipped with 4.0 mV He-Ne laser at 633 nm at scattering angle with 173˚.

- Reaction yield (RY%) measurement of produced micelle

The weight of the lyophilized micelles was measured and recorded with a precision balance, and the reaction yield (RY%) was calculated according to Equation 3.2.

$$\text{RY\%} = \frac{Amount\ of\ micelles\ produced\ (mg)}{Amount\ of\ copolymer\ (mg) + juglone\ (mg)\ added\ at\ the\ beginning} \times 100 \qquad (3.2)$$

- UV-Vis spectroscopy

The encapsulation efficiency (EE%) of Juglone loaded into poly(4VP-co-OEGMA) micelles was calculated by using Equation 3.3. In this purpose, UV-Vis spectroscopy technique was performed in which, 1 ml of micelles was solved in 1 ml of acetone. In this way the structure of micelles was dissociated allowing Juglone molecules to be released. The quantity of released Juglone was calculated by utilizing the equation obtained from calibration curve created by dissolving Juglone in acetone at different concentrations. Then, the absorbance of samples was determined by Shimadzu UV-1700 UV-VIS spectrophotometer. UV-Vis spectra were acquired between 200-800 nm and the Juglone displayed absorbance at 415 nm. Thus, by measuring the absorbance of released juglone at 415 nm the amount of juglone loaded micelles was calculated.

$$\text{EE\%} = \frac{the\ amount\ of\ encapsulated\ Juglone\ in\ micelles}{the\ initial\ Juglone\ amount} \times 100 \qquad (3.3)$$

- Drug Loading Capacity (LC%)

The loading capacity of Juglone was calculated by using Equation 3.4.

$$\text{LC\%} = \frac{the\ amount\ of\ encapsulated\ Juglone\ in\ micelles}{Total\ micelle\ amount\ (mg)} \times 100 \qquad (3.4)$$

- In-vitro Release Study

The controlled release study of Juglone encapsulated micelles was performed by dialysis method. For this purpose, 2 mg of dried loaded micelle was dissolved in 1 ml Phosphate buffer solution (PBS) then, the solution was taken into dialysis tubing cellulose membrane (avg. flat width 25 mm). For the outer phase a 10 ml solution composed of PBS and 10% DMSO was prepared. The dialysis was applied in an orbital shaking water bath at 37 ºC. The complete dialysate was withdrawn at predetermined time intervals and fresh release medium was added. Then, the absorbance of each withdrawn solution was measured using UV-Vis spectroscopy to calculate the concentration of released Juglone by time.

- Determination of Critical Micelle Concentration (CMC)

The CMC, which is the lowest concentration at which the quaternized poly(4VP-co-OEGMA) copolymer can form micelles, was defined by utilizing pyrene as a probe since it is a hydrophobic and fluorescent molecule. For this purpose, firstly pyrene solution was prepared by dissolving 0.121 mg of pyrene in 50 ml acetone to keep the concentration of pyrene at 0.000012 M. In the next step, different concentrations (ranging between 0.000025-0.4 mg/ml) of quaternized poly (4VP-co-OEGMA) copolymer dissolved in distilled water were prepared and 50 µl of pyrene solution was added to each sample. All samples were stirred for one hour to allow evaporation of acetone. To prevent pyrene from being affected by light, the glass bottles in which the reaction was carried out were wrapped with aluminum foil. After 1 hour, the samples were analyzed with a fluorescence spectrophotometer with an excitation wavelength range of 300-360 nm and an emission wavelength of 390 nm. The ratio of fluorescence intensities of pyrene at 338 and 333 nm (I_{338}/I_{333}) was determined. These ratios were plotted against polymer concentrations to find the critical micelle concentration (CMC).

- Transmission Electron Microscopy

The morphological analysis of both free micelles and drug-loaded micelles was conducted utilizing TEM. Initially, the lyophilized micelles were dispersed in water, and a droplet of the sample was deposited onto a 400-mesh copper grid coated with a carbon film. Excess water was eliminated by blotting with filter paper, and the sample was left to air-dry at room temperature prior to examination. TEM images were captured at various magnifications and annotated with corresponding scale bars.

3.4 In-vitro Studies

3.4.1 Cytotoxicity test

- Cell culture

L929 fibroblast cells were used for cytotoxicity tests. Cells were cultured in DMEM/F12 medium containing 10% FBS and 1% penicillin streptomycin [49]. Cells that reached 80-90% density in the culture dishes were collected from the surface using 0.25% trypsin-EDTA and sedimented in a centrifuge. After this

process, the cell pellet was suspended in 1 ml of medium and the cells were counted on the thoma slide using trypan blue. The required number of cells to be used in the experiments were suspended in the growth medium and planted in 96-well plates. After the 24-hour incubation period, the plates were replaced with fresh medium from the control group, while the experimental groups were replaced with the determined concentrations of the samples. Three replicates were performed for each experimental group. Cell viability analysis was performed after the plates were treated with samples for 24 and 72 hours.

- MTT method

The MTT technique was applied to define the cytotoxicity of the samples on fibroblast cells [50]. 10 µl of MTT solution was added to each well, and after 4 hours of incubation, 100 µl of DMSO was added to crystals converted from tetrazolium to formazan by living cells. The plates were incubated in the dark for 30 minutes. Then, the optical density in the wells was measured at 570 nm in a microplate reader. ±SD was calculated with the absorbance data obtained (n = 3). At the same time, cell viability (%) was calculated using these data by Equation 3.5.

$$cell\ viability\ (\%) = \frac{sample\ absorbance}{control\ absorbance} \times 100 \qquad (3.5)$$

4

RESULTS AND DISCUSSION

In this study, Juglone loaded quaternized poly(4VP-co-OEGMA) micelles were synthesized. To achieve this;

- Poly(4VP-co-OEGMA) random copolymer was prepared by a free-radical polymerization reaction.
- The produced poly(4VP-co-OEGMA) was quaternized by 1-bromohexane and 1-bromohexadecane.
- Quaternized poly(4VP-co-OEGMA) micelles were produced and characterized.
- Juglone encapsulated quaternized poly(4VP-co-OEGMA) micelles were synthesized and characterized.
- The biocompatibility tests of all the materials produced were performed on mammalian cells.

4.1 Synthesis of the Poly(4VP-co-OEGMA) Copolymer

The produced poly(4VP-co-OEGMA) copolymer was investigated by FT-IR, NMR spectroscopies and GPC.

The chemical structure of the produced copolymer was detected using FT-IR spectroscopy. The FT-IR spectrum of the poly(4VP-co-OEGMA) is given by Figure 4.1. In the figure, CP is the poly(4VP-co-OEGMA). According to acquired spectrum, the bands at 1600 cm^{-1} and 1415 cm^{-1} (show in green lines) indicate the pyridine rings in 4VP. Since the characteristic peak at about 1600 cm^{-1} was shown due to the stretching vibration of C=N and C=C of the aromatic rings in the 4VP monomer. While the OEGMA monomer was detected because of the peak at 1725 cm^{-1} (shows in purple line) which ensures the presence of C=O group of the OEGMA monomer.

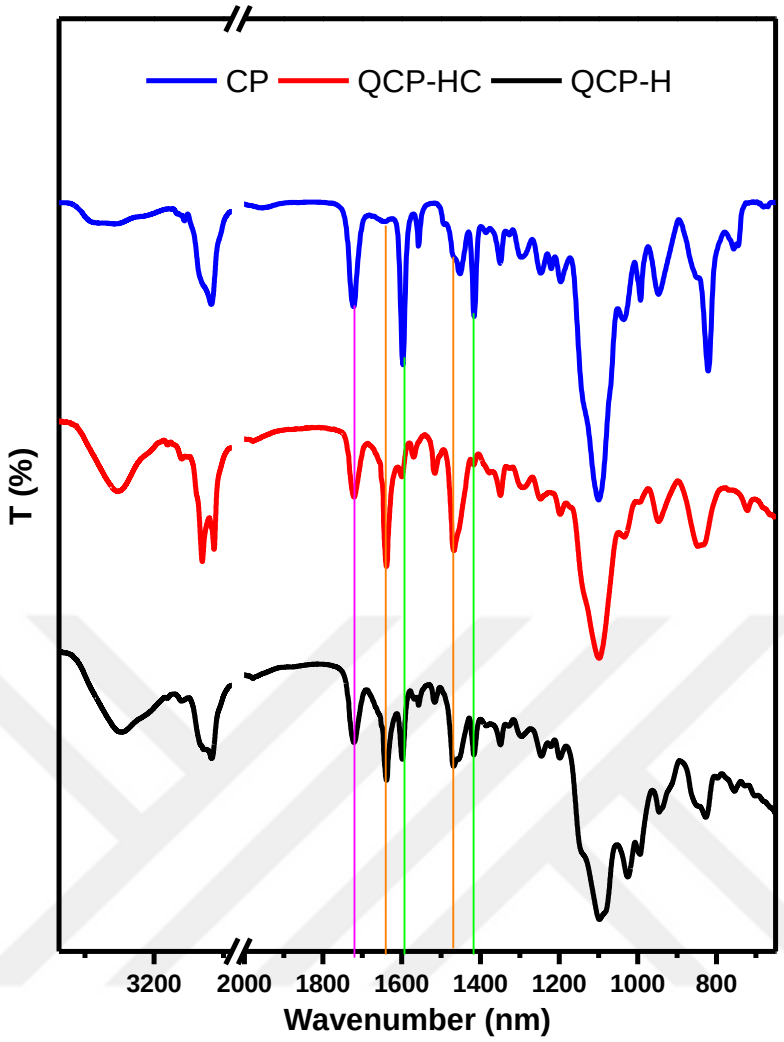


Figure 4. 1 The FT-IR spectrum of produced poly(4VP-co-OEGMA) (CP) and poly(4VP-co-OEGMA) quaternized with 1-bromohexane (QCP-H) and 1-bromohexadecane (QCP-HC).

For further chemical analysis, NMR spectroscopy technique was performed on produced copolymer. From ^{1}H-NMR spectrum of the polycation (Figure 4.2) a peak at 3.50 can be clearly observed which refers to the presence of methyl protons in the methyl ether groups found in the side chain ends of OEGMA monomer. the aromatic protons in the pyridine rings displayed two peaks at 8.25 ppm and 6.6 ppm [24], also, the protons of the vinyl chain of the 4VP monomer was observed obviously from the peak at 1.5 ppm. The solvent peaks are also given at 3.3 ppm and 2.5 ppm which refer to water and DMSO, respectively.

By using peak areas of pyridine rings (at 8.25 and 6.6 ppm) and OEGMA chains (at 3.5 ppm) the monomer quantities of copolymer were measured to be 80% 4VP and

20% OEGMA by mole. It can be observed that the chemical composition of the synthesized copolymer that determined before (80% 4VP and 20% OEGMA) is identical to the mole percentage of 4VP and OEGMA monomers used in the feed of polymerization. In addition, from the spectrum there is no peak appeared for any monomer what means that all unreacted monomers were removed successfully.

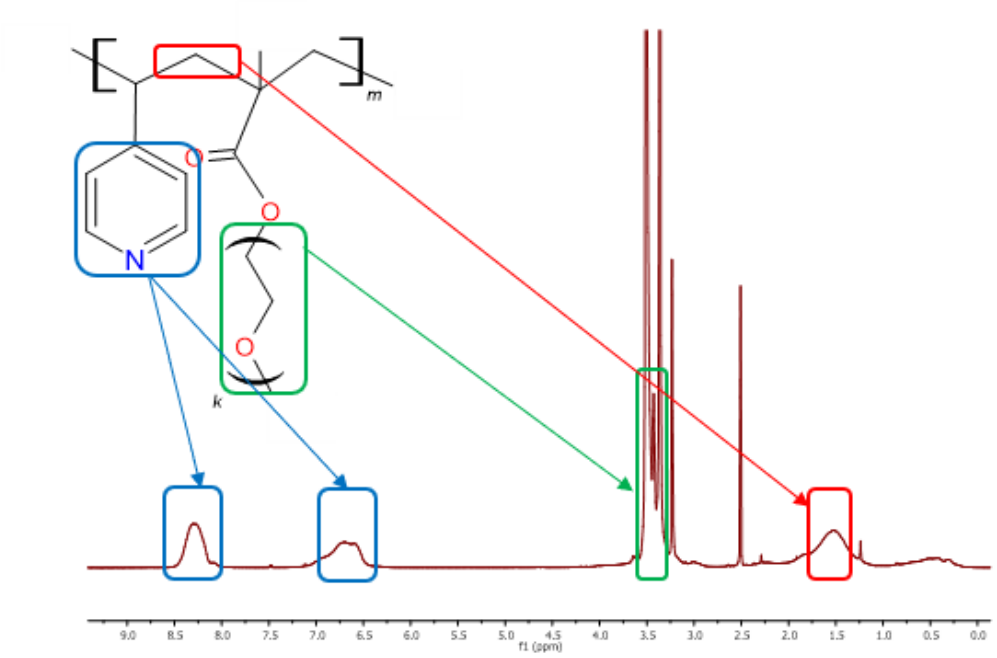


Figure 4. 2 ^{1}H-NMR spectrum of obtained poly(4VP-co-OEGMA) copolymer.

The molecular weight and molecular weight distribution of produced copolymer were defined utilizing GPC analysis. As shown in the chromatogram gained from RALS detector (Figure 4.3) the retention volume of produced copolymer was determined as 2.6 ml. Also, there is no peak observed except the peak of synthesized copolymer, that means all unreacted monomers were removed successfully. According to the GPC analysis, the molecular weight of obtained copolymer was 140 kDa, and dispersity $Đ$ (M_W/M_n) was 1.43.

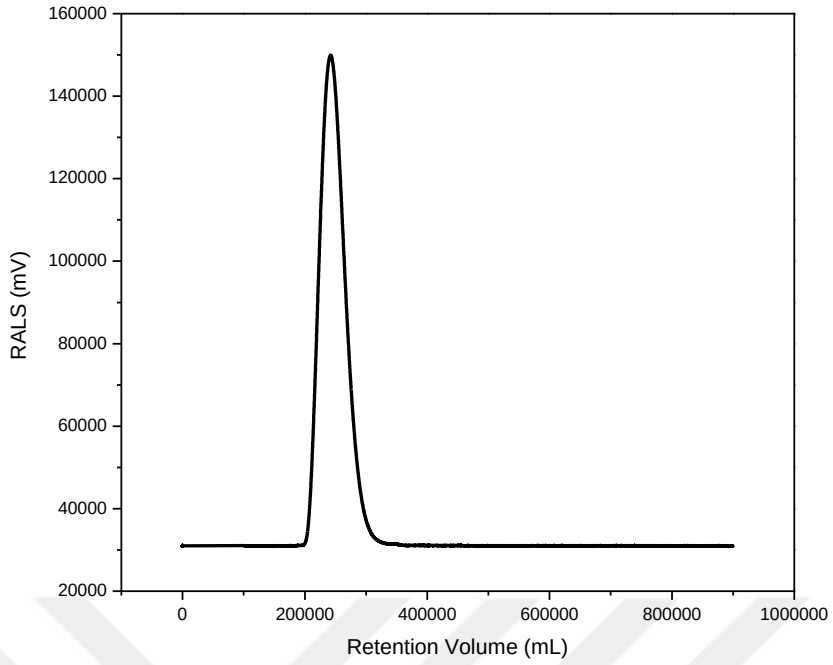


Figure 4. 3 The GPC chromatogram of the produced poly(4VP-co-OEGMA) copolymer

From all results obtained by NMR, FT-IR spectroscopies and GPC chromatography it can be concluded that poly(4VP-co-OEGMA) copolymer was synthesized successfully.

4.2 Synthesis of the Quaternized Poly(4VP-co-OEGMA) Copolymer

After synthesis of the copolymer, the pyridine rings of the copolymer were quaternized alkylbromides in two steps to acquire positively charged copolymer containing quaternary ammonium groups. In the first step of quternization, BrH was used as an alkylating agent to obtain the quaternized copolymer of QCP-H. In Figure 4.1, FTIR spectrum of QCP-H is given. In the FTIR spectrum, new bands at 1640 and 1468 cm^{-1} (show in red lines) were revealed. These new bands were seen as a result of quaternization of pyridine rings in 4VP units. The quaternization degree of the obtained cationic copolymer was detected by using absorbance values in FT-IR spectrum using Equation 3.1. The quaternization degree of QCP-H was calculated after the first quaternization to be 40.8 %.

Then, QCP-H was quaternized with BrHC as the second step of quaternization and the degree of quaternization was 70.8% which reveals 30% quaternization in the second step of quaternization reactions.

4.3 Production of the Polycationic Micelle

Due to the amphiphilic nature of the quaternized copolymer, it is predicted that the QCP-HC can self-assemble to create micelles in aqueous solutions. To prove the micelle formation of QCP-HC copolymer, CMC of the copolymer was defined by fluorescence spectrometry. The fluorescence spectra of pyrene in the range of 300-360 nm, which vary depending on the copolymer concentrations, are seen in Figure 4.4. As shown in the spectra, when the polymer concentration increases, the fluorescence intensity of pyrene also increases. This refers to the fact that the fluorescence features of pyrene change when it passes from the aqueous environment to the hydrophobic core of the micelle [51]. The CMC is determined with the help of shifts in fluorescence intensities. Figure 4.4 shows the change in the I_{338}/I_{333} fluorescence intensity ratio depending on the QCP-HC concentration. The CMC value was found to be 0.056 mg/mL for the copolymer of QCP-HC, which reveals the micelle formation of the copolymer.

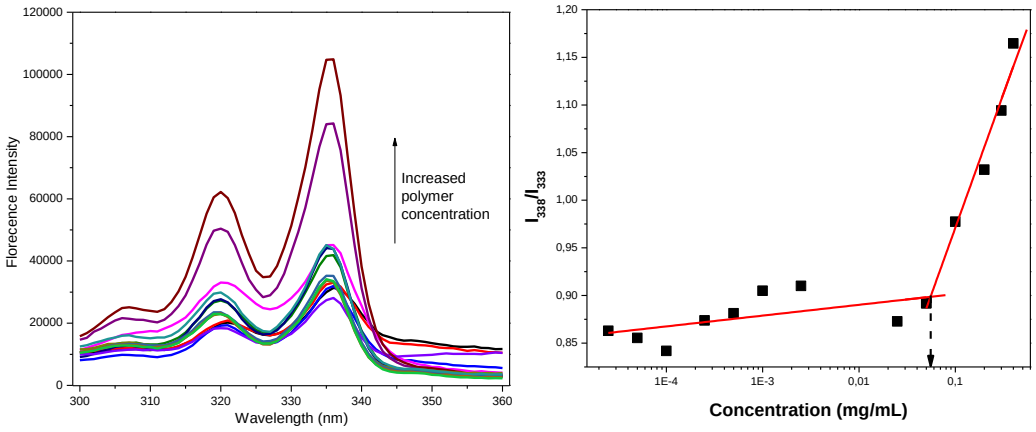


Figure 4. 4 The fluorescence spectra of pyrene (left) and the intensity ratio of I_{338}/I_{333} (right) depending on the QCP-HC concentration.

After revealing the micelle formation of the copolymer, the empty and Juglone-loaded micelles were prepared using QPC-HC. The produced empty and Juglone-loaded micelles were first characterized with DLS and ELS spectroscopy to determine their hydrodynamic diameter, hydrodynamic size distribution, and zeta

potential. The zeta potential of free and Juglone-loaded micelle was found to be +32 mV and +4.05 mV, respectively.

Figure 4.5 displays the size distribution of polycationic free and Juglone-loaded micelles by intensity, in which the hydrodynamic diameters of the micelles were 131 and 135 nm, respectively. The polydispersity indices of the free and juglone-loaded micelles (PdI) were 0.389 and 0.141, respectively.

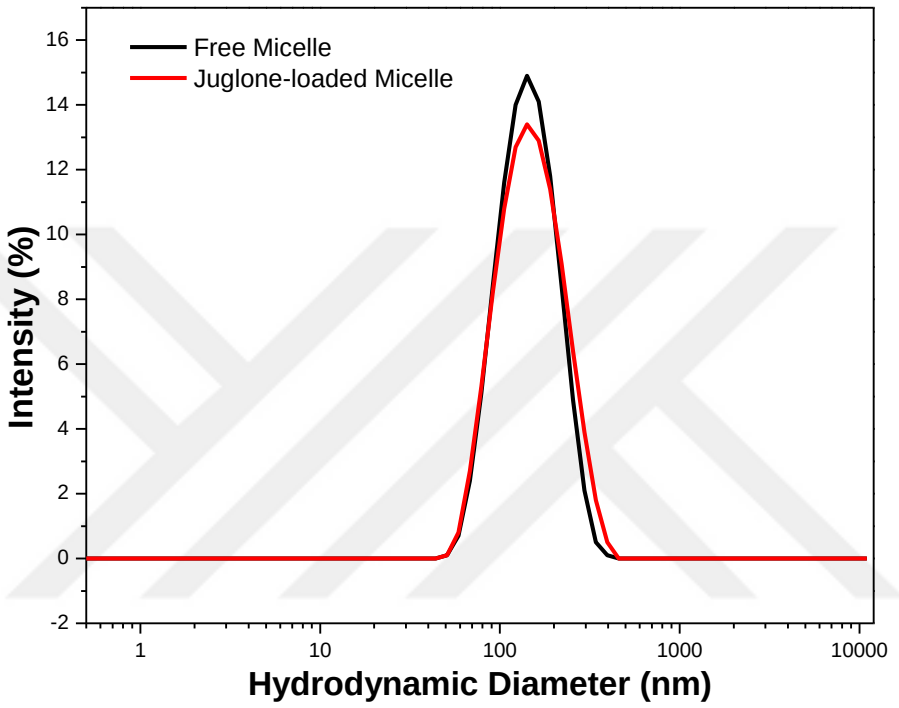


Figure 4. 5 Hydrodynamic size distribution of free (black line) and Juglone-loaded (red line) micelle.

The morphology of the produced micelles is also important, which can be revealed by electron microscopy techniques. In our study, we used TEM to investigate the size and morphology of the micelles. Figure 4.6 shows the TEM images of free and juglone-loaded micelles. Both micelles have rod-like (cylindrical) shapes in the TEM images. Also, the length of these rod-like micelles is between 150-200 nm in both samples. The shape of the micelles can be spherical or rod-like or even vesicular structures can be obtained from amphiphilic copolymers. Here, the critical packaging parameter (CPP) of the copolymer is very important in the shape of its micelle. The CPP value is related to the hydrophobic/hydrophilic balance of the copolymer.

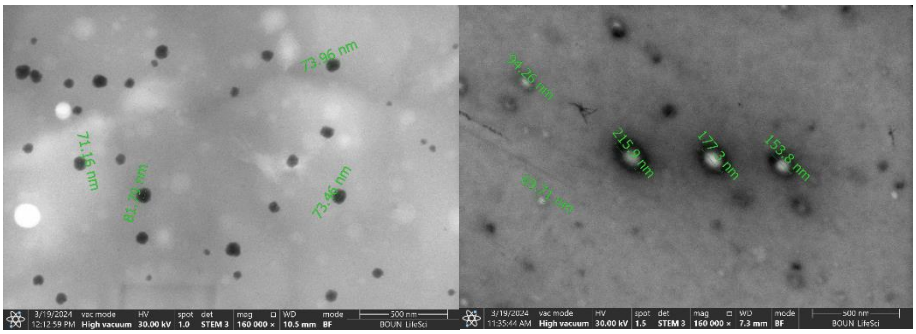


Figure 4. 6 TEM images of free (left) and juglone-loaded micelle (right) at 160 kX magnifications.

After physicochemical characterization of the micelles, the drug content and drug release profile of the micelle were determined. The reaction yield of the lyophilized juglone-loaded micelle was calculated using Equation 3.2 as %56.85.

Encapsulation efficiency of the loaded micelle was calculated by dissolution of the micelles in acetone leading to release of juglone. For this purpose, the initial task was to obtain the calibration curve of juglone by acquiring its absorbance at 415 nm using UV spectroscopy. The obtained calibration curve of juglone is given in Figure 4.7. Then, the encapsulation efficiency of the micelle was found to be 70.4%. Consequently, the drug loading capacity (LC%) was calculated as 13.76% using Equation 3.4.

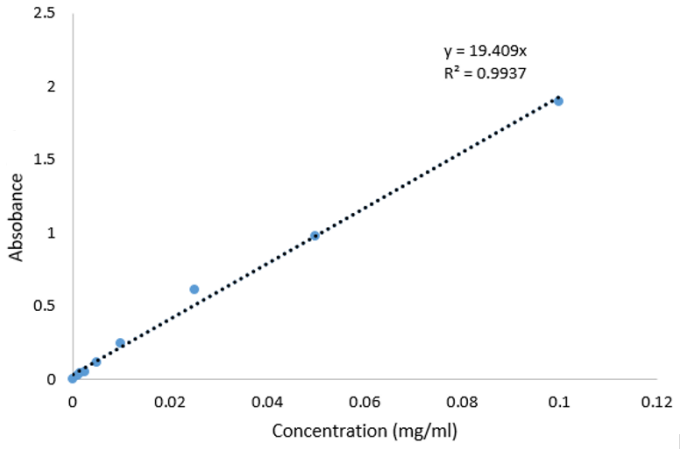


Figure 4. 7 Calibration curve of juglone dissolved in acetone acquired by absorbance at 415 nm.

The cumulative release of juglone from the loaded micelle is given in Figure 4.8. The released juglone into the release medium (PBS) was analyzed with UV spectrometry at specific intervals to acquire absorbance at 415 nm which gives the concentration of the molecule. As seen in Figure 4.8, the release reached 15.1% in the first 6 hours and reached 16.81% at the 7th day, which reveals the release was almost completed after 6 hours. The very low release from the micelle can be relate to the high hydrophobicity of the copolymer which causes interactions with the hydrophobic juglone preventing the release. Also, non-degradability of the copolymer can be another reason to prevent the release of the remaining juglone molecules.

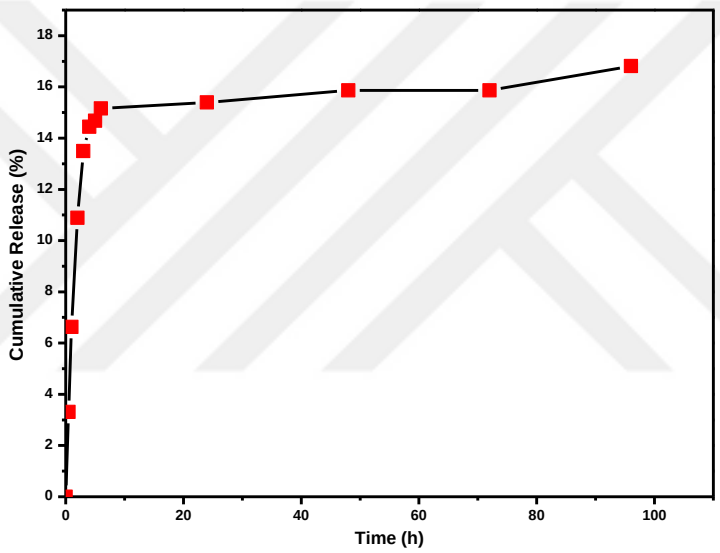


Figure 4. 8 The cumulative release profile of juglone from the QCP-HC micelles.

4.4 Biological Investigation of the Micelles

Figure 4.9 gives the viability values of L929 cells against the samples produced in the thesis. Juglone is a toxin molecule that harms the surrounding organisms around walnut (ATIF). In our study, juglone killed almost all cells at concentrations above 8 µg/mL, which proved the toxin activity of the molecule. The copolymer quaternized with only BrH is biocompatible in all concentrations. The OEGMA groups in the copolymer chain is water-soluble and biocompatible groups that can rise the biocompatibility of the copolymer. In contrast, the copolymers quaternized with BrHC are toxic above 16 µg/mL, which can be the result of very hydrophobic hexadecyl groups attached to the copolymer. These groups can increase the

interactions with the cell membrane and disrupt the cell to cause toxicity. When the copolymer formed micelle, it also exhibited toxicity, but in all concentrations. When the juglone is loaded into the micelle, it increases the cytotoxicity of the micelle slightly.

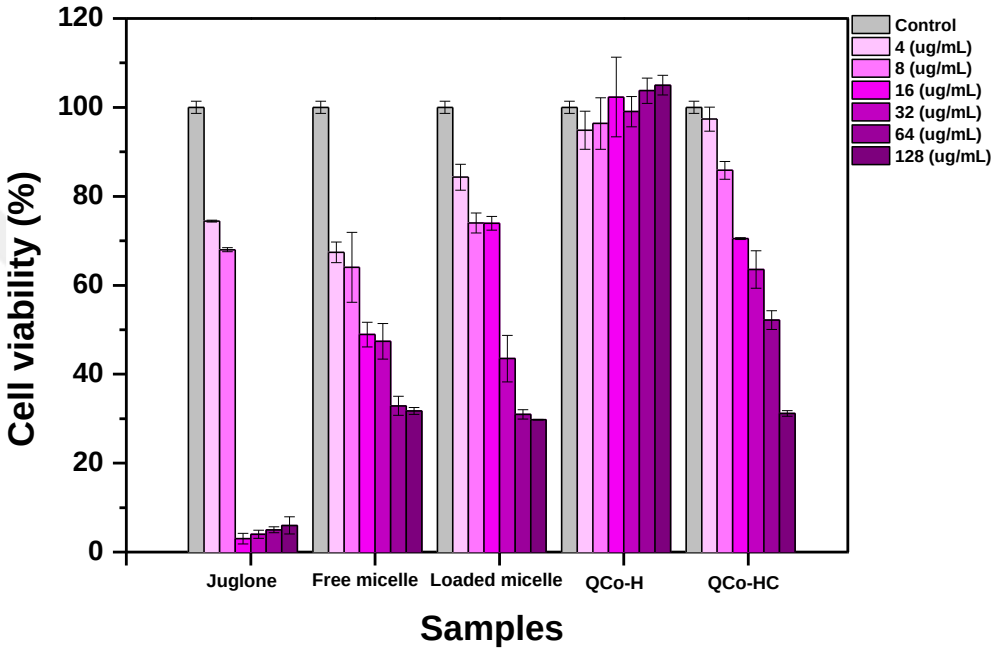


Figure 4. 9 The viability of L929 cells against the samples after 24 h of incubation.

5

CONCLUSION

In the thesis, amphiphilic polycations were used to form micelles to carry Juglone as a model hydrophobic active agent. For this purpose, the copolymer was produced, micelles were prepared from this copolymer and its biological activities were investigated.

In conclusion,

- The copolymers with 140 kDa molecular weight with monomer ratio of n_{4VP}/n_{OEGMA}= 8:2 was obtained.
- The copolymer was successfully quaternized with BrH and BrHC to obtain permanent positive charges on the copolymer and hydrophobic groups, which has an amphiphilic structure.
- The produced quaternized copolymer formed micelles in the aqueous solution above 0.056 mg/mL.
- The free and juglone-loaded micelles formed from QCP-CH copolymer exhibited hydrodynamic diameters smaller than 200 nm and uniformly size distribution. The zeta potential of the micelles was positive.
- The micelles exhibited 70.4% encapsulation efficiency and the drug loading capacity was calculated as 13.76%. The micelles released almost 16% of the juglone in 6 hours.
- The juglone and the micelles exhibited toxicity against the L929 cells, the copolymer quaternized with only BrH was biocompatible.

In the study, juglone-loaded micelles exhibited a higher biocompatibility against L929 cells compared to the free juglone. However, in the future studies, functional groups that can rise biocompatibility of the micelle can be added to the polymer.

REFERENCES

[1] Y. Wang, C.-Y. Ke, C. Weijie Beh, S.-Q. Liu, S.-H. Goh, and Y.-Y. Yang, "The self-assembly of biodegradable cationic polymer micelles as vectors for gene transfection," Biomaterials, vol. 28, no. 35, pp. 5358–5368, Dec. 2007, doi: 10.1016/j.biomaterials.2007.08.013.

[2] M. S. Kadayifci et al., "Core-crosslinking as a pathway to develop inherently antibacterial polymeric micelles," J. Appl. Polym. Sci., vol. 137, no. 8, p. 48393, 2020, doi: 10.1002/app.48393.

[3] T. Arasoglu et al., "Synthesis, characterization and antibacterial activity of juglone encapsulated PLGA nanoparticles," J. Appl. Microbiol., vol. 123, no. 6, pp. 1407–1419, Dec. 2017, doi: 10.1111/jam.13601.

[4] R. Trivedi and U. B. Kompella, "Nanomicellar formulations for sustained drug delivery: strategies and underlying principles," Nanomed., vol. 5, no. 3, pp. 485–505, Apr. 2010, doi: 10.2217/nnm.10.10.

[5] C. Popovici, M. Popa, V. Sunel, L. I. Atanase, and D. L. Ichim, "Drug Delivery Systems Based on Pluronic Micelles with Antimicrobial Activity," Polymers, vol. 14, no. 15, p. 3007, Jul. 2022, doi: 10.3390/polym14153007.

[6] A. Kapse, N. Anup, V. Patel, G. K. Saraogi, D. K. Mishra, and R. K. Tekade, "Chapter 6 - Polymeric micelles: a ray of hope among new drug delivery systems," in Drug Delivery Systems, R. K. Tekade, Ed., in Advances in Pharmaceutical Product Development and Research. , Academic Press, 2020, pp. 235–289. doi: 10.1016/B978-0-12-814487-9.00006-5.

[7] Y.-Y. Diao et al., "Doxorubicin-loaded PEG-PCL copolymer micelles enhance cytotoxicity and intracellular accumulation of doxorubicin in adriamycin-resistant tumor cells," Int. J. Nanomedicine, vol. 6, pp. 1955–1962, 2011, doi: 10.2147/IJN.S23099.

[8] K. Miyata, R. J. Christie, and K. Kataoka, "Polymeric micelles for nano-scale drug delivery," React. Funct. Polym., vol. 71, no. 3, pp. 227–234, Mar. 2011, doi: 10.1016/j.reactfunctpolym.2010.10.009.

[9] S. Perumal, R. Atchudan, and W. Lee, "A Review of Polymeric Micelles and Their Applications," Polymers, vol. 14, no. 12, Art. no. 12, Jan. 2022, doi: 10.3390/polym14122510.

[10] S. Kotta, H. M. Aldawsari, S. M. Badr-Eldin, A. B. Nair, and K. Yt, "Progress in Polymeric Micelles for Drug Delivery Applications," Pharmaceutics, vol. 14, no. 8, Art. no. 8, Aug. 2022, doi: 10.3390/pharmaceutics14081636.

[11] S. M. N. Simões, A. R. Figueiras, F. Veiga, A. Concheiro, and C. Alvarez-Lorenzo, "Polymeric micelles for oral drug administration enabling locoregional and systemic treatments," Expert Opin. Drug Deliv., vol. 12, no. 2, pp. 297–318, Feb. 2015, doi: 10.1517/17425247.2015.960841.

[12] M. Almeida, M. Magalhães, F. Veiga, and A. Figueiras, "Poloxamers, poloxamines and polymeric micelles: Definition, structure and therapeutic

applications in cancer," J. Polym. Res., vol. 25, no. 1, p. 31, Dec. 2017, doi: 10.1007/s10965-017-1426-x.

[13] E. P. Melo, M. R. Aires-Barros, and J. M. Cabral, "Reverse micelles and protein biotechnology," Biotechnol. Annu. Rev., vol. 7, pp. 87–129, 2001, doi: 10.1016/s1387-2656(01)07034-x.

[14] "Critical Packing Parameter | Practical Surfactants Science | Prof Steven Abbott." Accessed: May 03, 2024. [Online]. Available: https://www.stevenabbott.co.uk/practical-surfactants/cpp.php

[15] K. Cholkar, A. Patel, A. D. Vadlapudi, and A. K. Mitra, "Novel Nanomicellar Formulation Approaches for Anterior and Posterior Segment Ocular Drug Delivery," Recent Pat. Nanomedicine, vol. 2, no. 2, pp. 82–95, 2012, doi: 10.2174/1877912311202020082.

[16] Y. H. Feng, X. P. Zhang, J. Y. Li, and X. D. Guo, "How is a micelle formed from amphiphilic polymers in a dialysis process: Insight from mesoscopic studies," Chem. Phys. Lett., vol. 754, p. 137711, Sep. 2020, doi: 10.1016/j.cplett.2020.137711.

[17] "Dialysis | BioRender Science Templates." Accessed: Jan. 15, 2024. [Online]. Available: https://www.biorender.com/template/dialysis

[18] Vaibhav Mahalea, Abhijit Ahirraob, Santoshi Deshmukhc, Pankaj Deshmukhd , Dr.Abhijeeet kulkarni*, "MIXED MICELLE FORMATION METHODS AND IMPORTNACE", [Online]. Available: https://www.jetir.org/papers/JETIR2107760.pdf

[19] J. Guo et al., "Antibacterial activity of cationic polymers: side-chain or main-chain type?," Polym. Chem., vol. 9, no. 37, pp. 4611–4616, Sep. 2018, doi: 10.1039/C8PY00665B.

[20] A. M. Carmona-Ribeiro and L. D. de Melo Carrasco, "Cationic antimicrobial polymers and their assemblies," Int. J. Mol. Sci., vol. 14, no. 5, pp. 9906–9946, May 2013, doi: 10.3390/ijms14059906.

[21] M. Farshbaf, S. Davaran, A. Zarebkohan, N. Annabi, A. Akbarzadeh, and R. Salehi, "Significant role of cationic polymers in drug delivery systems," Artif. Cells Nanomedicine Biotechnol., vol. 46, no. 8, pp. 1872–1891, Nov. 2018, doi: 10.1080/21691401.2017.1395344.

[22] S. K. Samal et al., "Cationic polymers and their therapeutic potential," Chem. Soc. Rev., vol. 41, no. 21, pp. 7147–7194, Oct. 2012, doi: 10.1039/C2CS35094G.

[23] A. G. Grigoras, "Natural and synthetic polymeric antimicrobials with quaternary ammonium moieties: a review," Environ. Chem. Lett., vol. 19, no. 4, pp. 3009–3022, Aug. 2021, doi: 10.1007/s10311-021-01215-w.

[24] "Antibacterial properties of cationic copolymers as a function of pendant alkyl chain length and degree of quaternization - Gokkaya - 2021 - Polymer International - Wiley Online Library." Accessed: Dec. 31, 2023. [Online]. Available: https://onlinelibrary.wiley.com/doi/abs/10.1002/pi.6170

[25] M. Topuzogullari, "Microwave assisted quaternization of poly(4-vinylpyridine) with 1-bromohexane," J. Polym. Res., vol. 25, p. 188, Jul. 2018, doi: 10.1007/s10965-018-1581-8.

[26] L. Cen, K. G. Neoh, and E. T. Kang, “Antibacterial activity of cloth functionalized with N-alkylated poly(4-vinylpyridine),” J. Biomed. Mater. Res. A, vol. 71A, no. 1, pp. 70–80, 2004, doi: 10.1002/jbm.a.30125.

[27] Z. Iatridi and C. Tsitsilianis, “Water-Soluble Stimuli Responsive Star-Shaped Segmented Macromolecules,” Polymers, vol. 3, no. 4, Art. no. 4, Dec. 2011, doi: 10.3390/polym3041911.

[28] K. Mavronasou et al., “Poly(vinyl pyridine) and Its Quaternized Derivatives: Understanding Their Solvation and Solid State Properties,” Polymers, vol. 14, no. 4, p. 804, Feb. 2022, doi: 10.3390/polym14040804.

[29] “Poly(4-vinyl pyridine).” Accessed: Jan. 15, 2024. [Online]. Available: https://www.polymersource.ca/index.php?route=product/category&path=2_2183_14_91_509&order=DESC&subtract=1&categorystart=.1-.2.7.5&serachproduct=

[30] H. Wang, S. Ding, Z. Zhang, L. Wang, and Y. You, “Cationic micelle: A promising nanocarrier for gene delivery with high transfection efficiency,” J. Gene Med., vol. 21, no. 7, p. e3101, 2019, doi: 10.1002/jgm.3101.

[31] K. Modra, S. Dai, H. Zhang, B. Shi, and J. Bi, “Polycation-mediated gene delivery: Challenges and considerations for the process of plasmid DNA transfection,” Eng. Life Sci., vol. 15, no. 5, pp. 489–498, 2015, doi: 10.1002/elsc.201400043.

[32] A. Figueiras et al., “New Advances in Biomedical Application of Polymeric Micelles,” Pharmaceutics, vol. 14, no. 8, Art. no. 8, Aug. 2022, doi: 10.3390/pharmaceutics14081700.

[33] G. S. Kwon, “Polymeric Micelles for Delivery of Poorly Water-Soluble Compounds,” Crit. Rev. Ther. Drug Carr. Syst., vol. 20, no. 5, 2003, doi: 10.1615/CritRevTherDrugCarrierSyst.v20.i5.20.

[34] Y. Yang, Z. Cai, Z. Huang, X. Tang, and X. Zhang, “Antimicrobial cationic polymers: from structural design to functional control,” Polym. J., vol. 50, no. 1, Art. no. 1, Jan. 2018, doi: 10.1038/pj.2017.72.

[35] “Figure 5. Proposed antimicrobial mechanism of cationic amphiphilic...,” ResearchGate. Accessed: Jan. 14, 2024. [Online]. Available: https://www.researchgate.net/figure/Proposed-antimicrobial-mechanism-of-cationic-amphiphilic-copolymers-i-Cationic-polymer_fig4_328297657

[36] R. Anderson, P. W. Groundwater, A. Todd, and A. Worsley, Antibacterial Agents: Chemistry, Mode of Action, Mechanisms of Resistance and Clinical Applications. John Wiley & Sons, 2012.

[37] “Overview of Antibacterial Drugs - Infectious Diseases,” MSD Manual Professional Edition. Accessed: Dec. 05, 2023. [Online]. Available: https://www.msdmanuals.com/professional/infectious-diseases/bacteria-and-antibacterial-drugs/overview-of-antibacterial-drugs

[38] H. C. Neu and T. D. Gootz, “Antimicrobial Chemotherapy,” in Medical Microbiology, 4th ed., S. Baron, Ed., Galveston (TX): University of Texas Medical Branch at Galveston, 1996. Accessed: Dec. 05, 2023. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK7986/

[39] “13.1A: Origins of Antimicrobial Drugs,” Biology LibreTexts. Accessed: Dec. 07, 2023. [Online]. Available: https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Boundless)/13%3A_Antimicrobial_Drugs/13.01%3A_Overview_of_Antimicrobial_Therapy/13.1A%3A_Origins_of_Antimicrobial_Drugs

[40] C. Majdi, V. Duvauchelle, P. Meffre, and Z. Benfodda, “An overview on the antibacterial properties of juglone, naphthazarin, plumbagin and lawsone derivatives and their metal complexes,” Biomed. Pharmacother., vol. 162, p. 114690, Jun. 2023, doi: 10.1016/j.biopha.2023.114690.

[41] J. Wang et al., “Antibacterial Activity of Juglone against Staphylococcus aureus: From Apparent to Proteomic,” Int. J. Mol. Sci., vol. 17, no. 6, p. 965, Jun. 2016, doi: 10.3390/ijms17060965.

[42] Z. Chudhary, R. A. Khera, M. A. Hanif, M. A. Ayub, and L. Hamrouni, “Chapter 49 - Walnut,” in Medicinal Plants of South Asia, M. A. Hanif, H. Nawaz, M. M. Khan, and H. J. Byrne, Eds., Elsevier, 2020, pp. 671–684. doi: 10.1016/B978-0-08-102659-5.00049-5.

[43] P. Babula, V. Adam, L. Havel, and R. Kizek, “[Naphthoquinones and their pharmacological properties],” Ceska Slov. Farm. Cas. Ceske Farm. Spolecnosti Slov. Farm. Spolecnosti, vol. 56, no. 3, pp. 114–120, Jun. 2007.

[44] M. Strugstad and S. Despotovski, “A Summary of Extraction, Synthesis, Properties, and Potential Uses of Juglone: A Literature Review,” J. Ecosyst. Manag., vol. 13, no. 3, Art. no. 3, 2012, doi: 10.22230/jem.2012v13n3a119.

[45] F. Alihosseini and G. Sun, “17 - Antibacterial colorants for textiles,” in Functional Textiles for Improved Performance, Protection and Health, N. Pan and G. Sun, Eds., in Woodhead Publishing Series in Textiles. , Woodhead Publishing, 2011, pp. 376–403. doi: 10.1533/9780857092878.376.

[46] “Juglone | CAS 481-39-0 | SCBT - Santa Cruz Biotechnology.” Accessed: Dec. 07, 2023. [Online]. Available: https://www.scbt.com/fr/p/juglone-481-39-0

[47] M. T. Paulsen and M. Ljungman, “The natural toxin juglone causes degradation of p53 and induces rapid H2AX phosphorylation and cell death in human fibroblasts,” Toxicol. Appl. Pharmacol., vol. 209, no. 1, pp. 1–9, Nov. 2005, doi: 10.1016/j.taap.2005.03.005.

[48] A. Hoş, U. Olgun, and K. Tunç, “Investigation of Antibacterial Activity of Juglone – Poly(ε-caprolactone) - Alumina Composite Films,” Sak. Univ. J. Sci., vol. 25, no. 1, Art. no. 1, Feb. 2021, doi: 10.16984/saufenbilder.650379.

[49] R. Tarrahi, A. Khataee, A. Karimi, M. Golizadeh, and F. Ebadi Fard Azar, “Development of a cellulose-based scaffold for sustained delivery of curcumin,” Int. J. Biol. Macromol., vol. 183, pp. 132–144, Jul. 2021, doi: 10.1016/j.ijbiomac.2021.04.123.

[50] A. Yu et al., “Mucoadhesive dexamethasone-glycol chitosan nanoparticles for ophthalmic drug delivery,” Int. J. Pharm., vol. 575, p. 118943, Feb. 2020, doi: 10.1016/j.ijpharm.2019.118943.

[51] Ö. Topel, B. A. Çakır, L. Budama, and N. Hoda, “Determination of critical micelle concentration of polybutadiene-block-poly(ethyleneoxide) diblock copolymer by fluorescence spectroscopy and dynamic light scattering,” J. Mol. Liq., vol. 177, pp. 40–43, Jan. 2013, doi: 10.1016/j.molliq.2012.10.013.

PUBLICATIONS FROM THE THESIS

Conference Papers

Paşaoğlu H., Topuzoğulları M., Derman S., "Inherently Antibacterial polymeric micelles as drug delivery system", The 6th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2023), Ankara Turkey, October 11-13, 2023.

Projects

“Kendiliğinden Antibakteriyel Polimerik Malzemelerin Geliştirilmesi”, The Scientific Research Projects Coordination Unit of Yildiz Technical University, Project No: FCD-2022-4900, Principal Investigator: Assoc. Prof. Dr. Murat Topuzoğulları, 2022-2024