

REPUBLIC OF TÜRKİYE
YILDIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

**SYNTHESIS AND CHARACTERIZATION OF POLYMERIC
DRUG DELIVERY SYSTEM IN SUB-MICRO SCALE**

Ayşegül KAHRAMAN

DOCTOR OF PHILOSOPHY THESIS

Department of Chemistry

Program of Chemistry (English)

Supervisor

Prof. Dr. Dolunay ŞAKAR DAŞDAN

August, 2024

REPUBLIC OF TÜRKİYE
YILDIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
SYNTHESIS AND CHARACTERIZATION OF POLYMERIC
DRUG DELIVERY SYSTEM IN SUB-MICRO SCALE

A thesis submitted by Ayşegül KAHRAMAN in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** is approved by the committee on 12.08.2024 in Department of Chemistry, Program of Chemistry (English).

Prof. Dr. Dolunay ŞAKAR DAŞDAN
Yıldız Technical University
Supervisor

Approved By the Examining Committee

Prof. Dr. Dolunay ŞAKAR DAŞDAN, Supervisor
Yıldız Technical University

Doç. Dr. Özlem YAZICI, Member
Yıldız Technical University

Dr. Öğr. Üye. Volkan UĞRAŞKAN, Member
Yıldız Technical University

Doç. Dr. Argun Talat GÖKÇEÖREN, Member
Istanbul Technical University

Doç. Dr. Pınar ŞEN, Member
Uskudar University

I hereby declare that I have obtained the required legal permissions during data collection and exploitation procedures, that I have made the in-text citations and cited the references properly, that I haven't falsified and/or fabricated research data and results of the study and that I have abided by the principles of the scientific research and ethics during my Thesis Study under the title of "Synthesis And Characterization of Polymeric Drug Delivery System In Sub-Micro Scale" supervised by my supervisor, Prof. Dr. Dolunay ŞAKAR DAŞDAN. In the case of a discovery of false statement, I am to acknowledge any legal consequence.

Ayşegül KAHRAMAN

Signature



This student was supported under “100/2000 YÖK PhD scholarship programme specialized in micro- and nanotechnology” by “Yükseköğretim Kurulu (YÖK)”.

Dedicated to my beloved late grandfather...

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to Prof. Dr. Dolunay Şakar Daşdan for her guidance, her enthusiasm for the project, her support, encouragement and patience. The meetings and conversations were vital for the progression of the project. Her knowledge and plentiful experience have encouraged me in all the time of my academic research.

I want to thank Prof. Dr. Melda Altıkatoğlu Yapaöz, for conducting anti-bacterial studies and her precious discussions and her guidance.

I would like to express my gratitude to my husband, my mum and my child. My dear husband, Furkan, accompanied me and waited for long hours outside the lab during pandemics and helped with house chores. My dear mum, always took care of my daughter and gave quiet encouragement and positive belief in my success during my studies. Also, my dear daughter, Gülsüm Feyza, patiently waited for me at home while I worked at the lab, and she always gave me heartwarming hugs. Without their tremendous understanding and encouragement in the past few years, it would be impossible for me to complete my study. I would also thank to my dear sister Tuğba for her motivation and constant belief in me.

I am also deeply thankful to my beloved late grandfather Durkaya Topçubaşı and I dedicated to this thesis to him who always wanted to see me as a Dr. but sadly passed away before graduation. I hope I did you proud.

I would like to thank The Turkish Council of Higher Education “YÖK” for the supports within the scoop of YÖK 100/2000 scholarship programme specialized in micro- and nanotechnology.

Lastly, I want to thank my dear friends Charlene Kane and Dr. Büşra Yusufoglu for their contributions to my study and their emotional support. I want to thank Kadir Çakıral from research team for his contributions to the research.

Ayşegül KAHRAMAN

TABLE OF CONTENTS

LIST OF ABBREVIATIONS	x
LIST OF FIGURES	xi
LIST OF TABLES	xiii
ABSTRACT	xvi
ÖZET	xviii
1 INTRODUCTION	1
1.1 Summary of Literature	1
1.2 Aim of the Study	4
1.3 Hypothesis	5
2 β-LACTAM ANTIBIOTICS & DRUG DELIVERY SYSTEMS	6
2.1 β -Lactam Antibiotics	6
2.1.1 Penicillins	7
2.1.2 Cephalosporins	10
2.2 Polymeric Drug Delivery Systems	12
2.3 Maleic Anhydride Derived Copolymers	14
2.4 Characterization Techniques	16
2.4.1 FTIR/ATR	16
2.4.2 ^1H NMR	18
2.5 Factors Affecting of β -Lactam Antibiotics	18
2.6 Activity and Zeta-sizer Measurements	19
2.6.1 Activity	20
2.6.2 Particle size	20
2.6.3 PDI	21
2.6.4 Mobility and Zeta Potential	21
2.7 Anti-bacterial Susceptibility Tests	23
3 EXPERIMENTAL PART	24
3.1 Materials	24
3.2 Synthesis of Drug Delivery Systems	24
3.2.1 Synthesis of PMVEMA-AMP DDS	25
3.2.2 Synthesis of PIBMA-AMP DDS	26
3.2.3 Synthesis of PEMA-AMP DDS	26
3.2.4 Synthesis of PMVEMA-CEF DDS	27

3.2.5 Synthesis of PIBMA-CEF DDS	27
3.2.6 Synthesis of PEMA-CEF DDS.....	28
3.3 Structural Characterization.....	28
3.4 Stability And Activity Measurements	28
3.5 Anti-Bacterial Tests.....	31
4 RESULTS AND DISCUSSION	32
4.1 Structural Characterization of PMVEMA, AMP, PMVEMA-AMP Copolymer-Drug Delivery Systems.....	32
4.2 Structural Characterization of PIBMA, AMP, PIBMA-AMP Copolymer- Drug Delivery Systems	34
4.3 Structural Characterization of PEMA, AMP, PEMA-AMP Copolymer-Drug Delivery Systems	36
4.4 Structural Characterization of PMVEMA, CEF, PMVEMA-CEF Copolymer-Drug Delivery Systems.....	38
4.5 Structural Characterization of PIBMA, CEF, PIBMA-CEF Copolymer-Drug Delivery Systems	40
4.6 Structural Characterization of PEMA, CEF, PEMA-CEF Copolymer-Drug Delivery Systems	42
4.7 Calculation Of Drug Binding Percentage To Copolymers, <i>DPW</i> and <i>M_w</i> .	44
4.8 Stability of PMVEMA, PIBMA, PEMA, AMP, AMP Conjugated DDSs at Different pH Values	48
4.9 Stability of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated DDSs at Different pH Values	50
4.10 Temporal In Vitro Stability Tests of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In Simulated Body Fluids.....	53
4.10.1. Zetasizer Measurements of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In 5% Dextrose Solution	53
4.10.2. Zetasizer Measurements of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In PBS Solution.....	56
4.10.3. Zetasizer Measurements of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In 0.9% Isotonic NaCl Solution	59
4.11 Temporal In Vitro Stability Tests of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In Simulated Body Fluids.....	63
4.11.1. Zetasizer Measurements of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In 5% Dextrose Solution	63

4.11.2. Zetasizer Measurements of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In PBS Solution.....	67
4.11.3. Zetasizer Measurements of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In 0.9% Isotonic NaCl Solution	71
4.12 UV/VIS Spectra Of Copolymers At Different pH Values	75
4.13 UV/VIS Spectra Of AMP And AMP-Conjugated DDSs At Different pH Values.....	76
4.14 UV/VIS Spectra of CEF and CEF-Conjugated DDSs at Different pH Values.....	77
4.15 UV/VIS Spectra of Copolymers at Different Body Fluids	78
4.15.1. UV/VIS Spectra of Copolymers in 5% Dextrose Solution	78
4.15.2. UV/VIS Spectra of Copolymers in PBS Solution.....	79
4.15.3. UV/VIS Spectra of Copolymers in 0.9% Isotonic NaCl Solution .	80
4.16 UV/VIS Spectra of AMP and AMP-Conjugated DDSs at Different Body Fluids.....	82
4.16.1. UV/VIS Spectra of AMP and AMP-Conjugated DDSs in 5% Dextrose Solution	82
4.16.2. UV/VIS Spectra of AMP and AMP-Conjugated DDSs in PBS Solution.....	84
4.16.3. UV/VIS Spectra of AMP and AMP-Conjugated DDSs in 0.9% Isotonic NaCl Solution	85
4.17 UV/VIS Spectra of CEF and CEF-Conjugated DDSs at Different Body Fluids.....	86
4.17.1. UV/VIS Spectra of CEF and CEF-Conjugated DDSs in 5% Dextrose Solution	86
4.17.2. UV/VIS Spectra of CEF and CEF-Conjugated DDSs in PBS Solution.....	87
4.17.3. UV/VIS Spectra of CEF and CEF-Conjugated DDSs in 0.9% Isotonic NaCl Solution	89
4.18 Evaluation of Activity of Drug Delivery Systems	90
4.19 Antimicrobial Susceptibility Tests.....	91
5 CONCLUSION	93
REFERENCES	95
PUBLICATIONS FROM THE THESIS	105

LIST OF SYMBOLS

$\overline{M}_w$	Average Molecular Weight of Polymer
k	Boltzmann constant
δ	Chemical Shift
DP_w	Degree of Polymerization
ϵ_e	Dielectric constant of solvent
D	Diffusion coefficient
μ_e	Electrophoretic mobility
d_H	Hydrodynamic diameter
μL	Micro Liter
M_1	Molecular Weight of Repeating Unit of Polymer
η	Solvent Viscosity
T	Temperature
ν	Wave Number
λ	Wavelength
ζ	Zeta Potential

LIST OF ABBREVIATIONS

AMP	Ampicillin Trihydrate
ATR	Attenuated Total Reflectance
CEF	Cefalexin (Cephalexin) Monohydrate
CFU	Colony Forming Unit
DDS	Drug Delivery System
DMF	N, N-Dimethylformamide
DMSO	Dimethyl Sulfoxide
FTIR	Fourier Transform Infrared
NMR	Nuclear Magnetic Resonance
PBS	Phosphate Buffer Saline
PDDS	Polymeric Drug Delivery Systems
PEMA	Poly (Ethylene alt-maleic anhydride)
PIBMA	Poly (Isobutyl alt-maleic anhydride)
PMVEMA	Poly (Methyl vinyl ether alt-maleic anhydride)
UV	Ultraviolet
VIS	Visible

LIST OF FIGURES

Figure 1.1 a. Bacterial density vs. Time, b. Antibiotic concentration vs. Time.....	2
Figure 2.1 Chemical Structure of β -lactam ring	6
Figure 2.2 Chemical structure of penicillin G	7
Figure 2.3 Chemical Structure of Penams	8
Figure 2.4 Chemical Structure of Ampicillin Trihydrate.....	9
Figure 2.5 Degradation reaction of Ampicillin.....	9
Figure 2.6 Chemical Structure of Cephems	11
Figure 2.7 Chemical Structure of Cefalexin	11
Figure 2.8 Representation of amide reaction for maleic anhydride.....	15
Figure 2.9 Disk Diffusion test- Inhibition zones	23
Figure 3.1 Final physical appearances of PMVEMA-AMP DDS (a), PIBMA-AMP DDS (b), PEMA-AMP DDS (c), PMVEMA-CEF DDS (d), PIBMA- CEF DDS (e), PEMA-CEF DDS (f)	26
Figure 3.2 Synthesis of PMVEMA-AMP drug delivery system	26
Figure 3.3 Synthesis of PIBMA-AMP drug delivery system	26
Figure 3.4 Synthesis of PEMA-AMP drug delivery system.....	26
Figure 3.5 Synthesis of PMVEMA-CEF drug delivery system.....	27
Figure 3.6 Synthesis of PIBMA-CEF drug delivery system.....	27
Figure 3.7 Synthesis of PEMA-CEF drug delivery system	28
Figure 4.1 FTIR/ATR spectra of PMVEMA, AMP, PMVEMA-AMP DDS.....	33
Figure 4.2 ^1H NMR spectrum of PMVEMA-AMP DDS	34
Figure 4.3 FTIR/ATR spectra of PIBMA, AMP, PIBMA-AMP DDS.....	35
Figure 4.4 ^1H NMR spectrum of PIBMA-AMP DDS	36
Figure 4.5 FTIR/ATR spectra of PEMA, AMP, PEMA-AMP DDS.....	37
Figure 4.6 ^1H NMR spectrum of PEMA-AMP DDS.....	38
Figure 4.7 FTIR/ATR spectra of PMVEMA-CEF DDS, CEF, PMVEMA.....	40
Figure 4.8 ^1H NMR spectrum of PMVEMA-CEF DDS.....	41
Figure 4.9 FTIR/ATR spectra of PIBMA, CEF, PIBMA-CEF DDS	42
Figure 4.10 ^1H NMR spectrum of PIBMA-CEF DDS	42
Figure 4.11 FTIR/ATR spectra of PEMA, CEF and PEMA-CEF DDS.....	43
Figure 4.12 ^1H NMR spectrum of PEMA-CEF DDS	44
Figure 4.13 Zeta Potential vs. Time (5% Dextrose Solution).....	67

Figure 4.14 Zeta Potential vs. Time (PBS Solution)	72
Figure 4.15. Zeta Potential vs. Time (0.9% NaCl Solution).....	75
Figure 4.16 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at different pHs	77
Figure 4.17 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at different pHs.....	78
Figure 4.18 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at different pH values	79
Figure 4.19 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at 5% Dextrose solution	80
Figure 4.20 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at PBS solution.....	81
Figure 4.21 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at 0.9% NaCl solution.....	82
Figure 4.22 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at 5% dextrose solution ...	84
Figure 4.23 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at PBS solution.....	85
Figure 4.24 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at 0.9% NaCl solution	86
Figure 4.25 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at 5% Dextrose solution	88
Figure 4.26 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at PBS solution.....	88
Figure 4.27 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at 0.9% NaCl solution	90
Figure 4.28 Diameters of inhibition zones of samples against gram-positive bacteria (<i>S. aureus</i>) and gram-negative bacteria (<i>E. coli</i>).....	92

LIST OF TABLES

Table 3.1	Solution concentrations (mg/mL) of samples for zetasizer and UV/VIS measurements.....	31
Table 4.1	Percentage of drug binding to copolymers, $\overline{Mw}$ (g/mol), DP_w , M_1 (g/mol) values belong to copolymers and DDSs	48
Table 4.2	The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles at the different pH values.....	49
Table 4.3	The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles at the different pH values	50
Table 4.4	The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles at the different pH values	50
Table 4.5	The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS at the different pH values.....	51
Table 4.6	The PDI values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles at the different pH values.....	52
Table 4.7	The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles at the different pH values.	52
Table 4.8	The mobility values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles at the different pH values	53
Table 4.9	The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS at the different pH values.....	54
Table 4.10	The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 5% Dextrose solution	55
Table 4.11	The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 5% Dextrose solution	55

Table 4.12	The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 5% Dextrose solution	55
Table 4.13	The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS in 5% Dextrose solution.....	57
Table 4.14	The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in PBS solution.....	58
Table 4.15	The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in PBS solution	58
Table 4.16	The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in PBS solution.....	59
Table 4.17	The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS in PBS solution	59
Table 4.18	The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 0.9% NaCl solution.....	61
Table 4.19	The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 0.9% NaCl solution	62
Table 4.20	The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 0.9% NaCl solution	63
Table 4.21	The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS in 0.9% NaCl solution.....	64
Table 4.22	The PDI values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 5% Dextrose solution	65
Table 4.23	The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 5% Dextrose solution	65
Table 4.24	The mobility values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 5% Dextrose solution	66
Table 4.25	The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS in 5% Dextrose solution.....	67

Table 4.26	The PDI of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in PBS solution.....	68
Table 4.27	The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in PBS solution.....	69
Table 4.28	The mobility of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in PBS solution.....	70
Table 4.29	The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS in PBS solution	71
Table 4.30	The PDI values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 0.9% NaCl solution	72
Table 4.31	The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 0.9% NaCl solution	73
Table 4.32	The mobility values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 0.9% NaCl solution	74
Table 4.33	The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS in 0.9% NaCl solution.....	75
Table 4.34	pH values and time at which maximum and minimum absorbance values of AMP, CEF, PMVEMA, PIBMA, PEMA, PMVEMA-AMP DDS, PIBMA-AMP DDS, PEMA-AMP DDS, PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS.....	91

ABSTRACT

Synthesis And Characterization of Polymeric Drug Delivery Systems in Sub-Micro Scale

Aysegul KAHRAMAN

Department of Chemistry

Doctor of Philosophy Thesis

Supervisor: Prof. Dr. Dolunay SAKAR DASDAN

The effectiveness of drugs with poor pharmacological properties can be improved through drug delivery microstructures that control and regulate various release, absorption, distribution and elimination processes. In this study, to obtain polymeric drug delivery system in sub-micro scale, ampicillin trihydrate and cephalexin monohydrate antibiotics having low pharmacological properties were reacted with poly(methyl vinyl ether-alt-maleic anhydride), poly(ethylene-alt-maleic anhydride) and poly(isobutylene-alt-maleic anhydride) copolymers and 6 different copolymer-drug delivery systems were synthesized by chemical conjugation method based on the ring opening reaction of anhydride ring of copolymer to obtain amide bond with drug. The synthesized copolymer-drug delivery systems were characterized by ^1H NMR and FTIR/ATR for structural identification. Also, analyses were done by UV/VIS spectroscopy and Zeta-sizer instrument in detail with consecutive antibacterial tests. Optimum stability and activity conditions of synthesized copolymer-drug delivery systems in different pH values and simulated body fluids as a function of time to obtain new drug delivery system candidates for 2 different antibiotics were determined. The most suitable drug delivery system candidate has been chosen for each antibiotic with this study.

Keywords: Maleic Anhydride Derived Copolymers, Beta-lactam Antibiotics, Copolymer-Drug delivery systems.



YILDIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

Mikron altı Ölçekte Polimerik İlaç Taşıma Sistemlerinin Sentezi ve Karakterizasyonu

Ayşegül KAHRAMAN

Kimya Anabilim Dalı
Doktora Tezi

Danışman: Prof. Dr. Dolunay ŞAKAR DAŞDAN

Düşük farmakolojik özelliklere sahip ilaçların etkinliği, salınım, emilim, dağılım ve eliminasyon süreçlerini kontrol eden ve düzenleyen ilaç taşıma mikroyapıları aracılığıyla geliştirilebilir. Bu çalışmada mikron altı ölçekte ilaç taşıma sistemi sentezlemek için; düşük farmakolojik etki gösteren amfisilin trihidrat ve cefaleksim monohidrat antibiyotikleri, poli(metil vinil-eter-alt maleik anhidrit), poli(etilen-alt-maleik anhidrit) ve poli(izo butilen-alt-maleik anhidrit) 3 farklı maleik anhidrit kopolimeri ile halka açılması reaksiyonu ile kopolimer-ilaç arasında amid bağı oluşumu ile altı farklı kopolimer-ilaç taşıyıcı sistem sentezlenmiştir. Sentezlenen kopolimer-ilaç taşıyıcı sistemler ^1H NMR ve FTIR/ATR ile yapısal tanımlama için karakterize edildi. Ayrıca; analizler UV/VIS spektroskopisi ve Zeta-sizer cihazı ile yapıldı ve onu takip eden antibakteriyel testler ile tamamlanmıştır. 2 farklı antibiyotik için yeni ilaç taşıyıcı sistem adayları elde etmek amacıyla sentezlenen kopolimer-ilaç taşıyıcı sistemlerinin farklı pH değerlerinde ve simüle edilmiş vücut sıvılarında zamana bağlı olarak optimum stabilite ve aktivite koşulları belirlenmiştir. Bu çalışma ile her bir antibiyotik için en uygun olan ilaç taşıma sistemi seçilmiştir.

Anahtar Kelimeler: Maleik Anhidrit Türevi Kopolimerler, Beta-laktam Antibiyotikleri, Kopolimer-İlaç Taşıma Sistemleri.



1

INTRODUCTION

1.1 Summary of Literature

The terms "antibiotics," "anti-infectious agents," and "antimicrobial drugs" all refer to a class of pharmaceuticals used to treat infections. The main factors that determine category of those pharmaceuticals are biological origin, the way of action, antibacterial spectrum, and chemical structures. Based on their unique chemical structure, beta-lactam antibiotics are divided into different groups [1]. Beta-lactam antibiotics have an antibacterial effect by blocking the synthesis of cell membranes in bacteria [1].

Pharmaceuticals that have been chosen from antibiotics in this research. Since antibiotics must be administered regularly due to their short half-lives [2] which otherwise can result in resistance of susceptible bacteria; this class of drugs is more appealing for polymeric drug development. Especially for antibiotic usage, when patients don't finish whole course of antibiotics, number of resistant bacteria increases and become more powerful as seen on figure a,b [3], [4]. According to Paterson's study; although most of the susceptible bacteria die within 3 days, resistant bacteria population will not end up till the 7th day of antibiotic usage as seen in Figure 1.1.

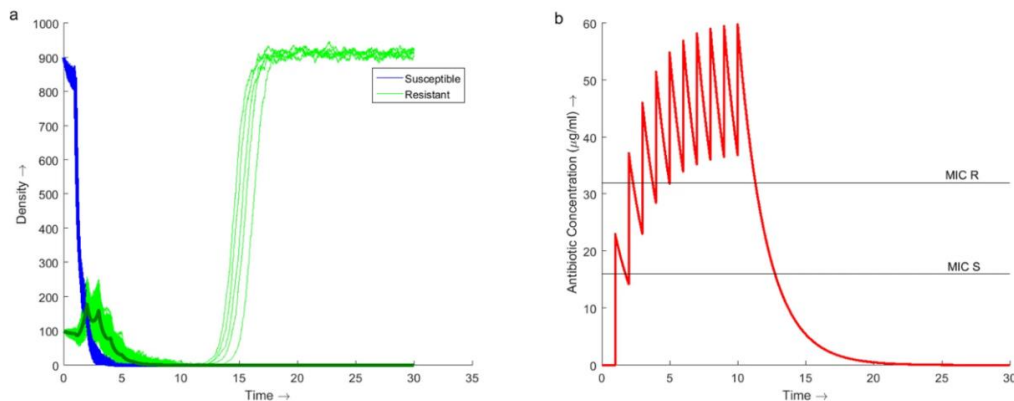


Figure 1.1 a.Bacterial density vs. Time, b. Antibiotic concentration vs. Time [4].

Additionally, antibiotic concentration just passes the threshold of minimum inhibitory concentration (MIC) for resistant bacteria at 5th day of drug usage [4]. Hence for the patients who are ceasing treatment earlier extended and sustained release of drug is a need. Drugs' physicochemical instability causes decomposition; biodegradable polymer conjugation can solve this problem [5].

Due to the benefits that they offer, such as prolonged release times, decreasing the required doses, prolonged half-lives, and enhanced stability in drug delivery systems, bioactive polymeric materials are the subject of interest. Recently, there are various methods to produce bioactive polymeric materials have been investigated for drug delivery systems.

Polymeric drug delivery systems, a type of formulation, enable the introduction of drugs into the body [6]. For drug delivery systems, polymers offer improved pharmacokinetic features such as longer circulation times [7]. The use of biocompatible polymers in drug delivery systems is a safe choice when it comes to complex biological mechanisms [7]. Longer-lasting localized medication administration is made possible by polymeric drug delivery systems [8].

Three key characteristics should exist in polymeric drug carriers to be effective;

- 1.It must not be immunogenic, toxic and should be easily metabolized,
- 2.It should be able to carry a sufficient amount of drugs,
- 3.It should be produced on a commercial scale at a reasonable cost [9].

By controlling the release of drugs, which can be made achievable by drug-delivery systems, physiological obstacles or problems with drug toxicity can be resolved with an enhancement in therapeutic impact [10].

Polymeric drug delivery systems fall into three main categories: hydrogels, colloidal carriers (micro, nanoparticles, micelles, and micro/nanogels, which have lately acquired importance), and polymer drug conjugates [11,12]. Even though it is unfeasible to select one of them as an ideal practice, the latter offers the benefits of prolonged circulation half-life, diminished clearance, and decreased drug immunogenicity and degradation [12]. Due to their biocompatibility and unique chemical structure, that enable the chemical attachment of numerous bioactive compounds, maleic anhydride derived polymers become excellent candidates for controlled drug delivery systems [13]. Thus, it is possible to alter and improve the characteristics and applications of this class of polymers [13]. Additionally, it has been seen that maleic anhydride-based copolymers have biomedical uses, such as serving as drug conjugates [14]. Despite not being natural polymers, maleic anhydride-derived polymers like other synthetic polymers with appropriate mechanical and chemical properties can be used for the development of new drug-delivery systems [15,16].

There are numerous examples of polymeric drug carriers derived from maleic anhydride that considerably prolong the half-lives of conjugated drugs in blood when compared to free drug molecules [17,18]. Therefore, maleic anhydride derived copolymers as drug-conjugates are appropriate options to extend circulation time in our proposed drug-delivery systems. There are numerous instances of drug delivery systems made up of different polymer chains and specific antibiotics as drugs in literature. In these researches, the polymer conjugates are either chosen from other polymers than ours or the first two approaches are employed.

According to the Kaplan and co-workers' study; Ampicillin and another antibiotic (Gentamycin) are utilized in designed drug delivery systems and antibacterial activities are compared. In this study; AMP loaded drug delivery system shows 7 mm and 10 mm inhibition zones against gram-negative (*E.coli*) and gram-positive (*S. aureus*) bacteria respectively, by agar disk diffusion method [19]. In our research; the selected formulation for AMP is PMVEMA-AMP conjugation,

exhibiting 15 mm and 19 mm inhibition zones against gram-negative (*E.coli*) and gram-positive (*S.aureus*), respectively, by disk-diffusion method.

According to a recent study, other Cefalexin-based drug delivery system is introduced with improved the oral administration of the drug. Pure Cefalexin and the drug delivery systems are compared in this study by various analytical methods, including zeta potential, and antibacterial activity. The selected formulation has a -24.37 mV zeta potential value, 0.25 PDI value, and shows 15.27 ± 1.52 mm inhibition zone against gram-positive (*S.aureus*) and 10.83 ± 1.37 mm inhibition zone against gram-negative (*E.coli*) by the disk diffusion method [20]. In our research, the selected formulation for the CEF drug is PMVEMA-CEF drug delivery system. In 0.9% isotonic sodium chloride solution; this PDDS has a -31.9 mV zeta potential value, 0.22 PDI value and shows 19 mm and 20 mm inhibition zones against *E.coli* and *S. aureus*, respectively.

1.2 Aim of the Study

Infectious diseases are commonly treated with antibiotics [21]. The most prominent defense worldwide for treating bacterial infections is beta-lactam antibiotics [22]. Beta-lactam (β -lactam) antibiotics that have four-membered β -lactam rings in common are classified into four different sub-groups: penicillins, cephalosporins, monobactams, and carbapenems [1]. The antibiotics chosen for this study were from the penicillin and cephalosporin sub-groups to gain insight into the behavior of β -lactam antibiotics in general. Cefalexin and Ampicillin were designated as essential medicines in the 'key access group antibiotics' by the World Health Organisation (WHO)'s Model List of Essential Medicines (2023) [23]. Although these antibiotics are commonly used world-wide, they have some drawbacks [24] like instability at certain pH values, or short half-lives and rapid clearance from body. The short half-lives [25] and systematic administration of antibiotics which otherwise can cause susceptible bacterial resistance makes this class of pharmaceuticals more attractive for polymeric drug delivery systems. To compensate for these drawbacks, polymeric drug delivery systems are the most suitable options. Since after the polymer conjugation of drugs, they exhibit similar characteristics like polymers [26]. Maleic anhydride derived copolymers are the great candidates for the drug conjugates due to their unique

properties like biocompatibility, reproducibility and low toxicity [27]. In literature, there are several examples of the usage of maleic anhydride copolymers as drug delivery systems in biomedical applications [28]. These copolymers have special chemical structure that can easily attach to β -lactam ring of the selected antibiotics. Our aim in this research is to improve the poor pharmacological properties of β -lactam antibiotics via chemical conjugation of maleic anhydride derived copolymers. Thus, we will be able to introduce novel drug delivery candidates for β -lactam antibiotics. In order to evaluate these novel polymer-drug conjugates, several characterizations such as proton ^1H NMR, FTIR/ATR spectroscopies and analysis methods like UV/VIS spectroscopy and Zeta-sizer measurements with antibacterial disk diffusion tests have been applied. To better and detailed evaluation of these conjugates; behaviors at very broad pH scale and wide period have been considered.

1.3 Hypothesis

β -lactam antibiotics are frequently prescribed and widely used drugs for over 50 years. Although it has been used for many types of infections there are some drawbacks like poor pharmacological properties. In order to improve these properties such as stability, activity, mobility etc., polymer conjugation is a good choice. Since drugs gain advantageous characteristics via polymer conjugation like bioavailability, low toxicity, better solubility etc... Maleic anhydride derived copolymers offer these properties with suitable chemical structure for the attachment of β -lactam antibiotics. In our research, we believe that the chemical conjugation of maleic anhydride copolymers with selected β -lactam antibiotics, lead the improvement in the pharmacological properties of these drugs. At the same time, we believe that anti-bacterial features of the drugs will be continued. Therefore, according to our expectation, β -lactam antibiotics functionalized maleic anhydride copolymers which are not present in literature will improve the poor pharmacological properties of these drugs and can be good drug delivery system candidates.

2

β -LACTAM ANTIBIOTICS & DRUG DELIVERY SYSTEMS

2.1 β -Lactam Antibiotics

Beta-lactam antibiotics which have four membered β -lactam ring in common are classified under four sub-groups: penicillins, cephalosporins, monobactams, and carbapenems [1].

In our project, selected antibiotics are from penicillins, cephalosporins sub-groups.

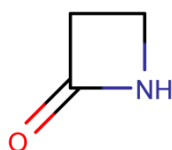


Figure 2.1 Chemical Structure of β -lactam ring

Under β -lactam antibiotic family; penicillin antibiotics are identified with penam structure which is β -lactam ring fused to a five membered sulphur containing ring; whereas cephalosporins are indicated with cephem structure which is β -lactam ring fused to a six membered sulphur containing ring [29].

β -lactam antibiotics have several characteristics depending on β -lactam ring. Firstly, ring strain makes these molecules very reactive. Thereby, these molecules open to nucleophilic attack due to the electrophilic carbonyl group of ring. It is possible to minimize nucleophilic attacks by another ring fused to β -lactam and even prevent these attacks by hydrolysis in certain mediums where salt of heavy metals, acids or bases are present [30].

Beta lactam antibiotics are easily attached to penicillin-binding proteins (PBPs) which are enzymes responsible for bacterial cell wall biosynthesis [31]. Beta-lactam

antibiotics inhibit the cell membranes synthesis of bacteria via PBP binding and with this mechanism; they show antibacterial effect [1].

2.1.1 Penicillins

Fleming discovered antibacterial activity of penicillin in 1929 [32]. In 1940; Florey and his co-workers did animal trials to test therapeutic effect of crude penicillin and continued with human clinical trials within a year [32]. There are four different penicillins known as “natural penicillins”; penicillin F (pentenyl penicillin), penicillin G (benzyl penicillin), penicillin K (heptylpenicillin) and penicillin X (p-hydroxybenzyl penicillin); within this group; penicillin G is the one mostly used in clinical studies [32].

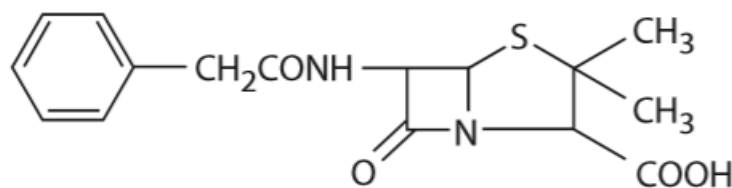


Figure 2.2 Chemical structure of penicillin G [32]

Since, Penicillin G is easily hydrolyzable in acidic and basic medium, it does not show stability under these conditions. Additionally, it shows antimicrobial activity only against Gram-positive bacteria (*staphylococcus*, *streptococcus*, and *pneumococci*) and *Bacillus anthracis* [1].

In 1957, 6-aminopenicillanic acid (6-APA) is classified as “penicillin nucleus” [32]. Semi-synthetic penicillins derived from this nucleus become successful by means of stability, pharmacokinetic properties and antibacterial activity compared to penicillin G and tens of thousands of tons of benzylpenicillin (penicillin G) produced for microbiological industry [1], [32].

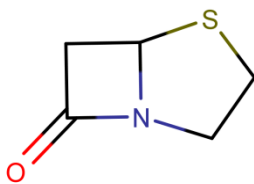


Figure 2.3 Chemical Structure of Penams

Penicillins and β -lactam antibiotics are seen as very safe therapeutic antibacterial agents since; their unpleasant side-effect is hypersensitivity can be in the form of skin eruption, itching or urticarial symptoms [32].

2.1.1.1 Ampicillin

Ampicillin belongs to semi-synthetic penicillin group. Ampicillin trihydrate is a specialized form for oral administration, and sodium salt of ampicillin is designed for parenteral (IV or IM) administration [32].

In 1961; semi-synthetic antibiotic ampicillin (a.k.a α -amino-benzylpenicillin) is introduced to the market with many advantages such as stability in acidic medium due to the primary amine group in structure, sensitivity to β -lactamases and having broader antimicrobial spectrum than original penicillins. As the pH values decrease; primary amine gets protonated and destabilizes iminium ion and this leads to stability of β -lactam antibiotic even in gastric media [30], [33], [34].

Among the aminopenicillins, ampicillin has the least absorption value and even be more reduced by food intake, additionally; about 34% of the active drug is discarded from body by urination [32].

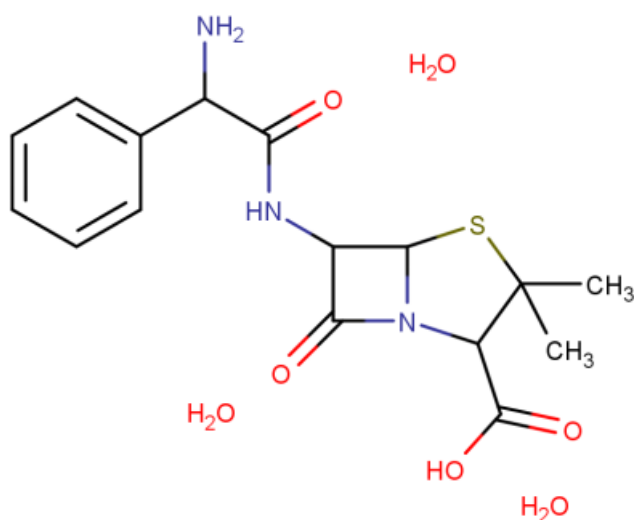


Figure 2.4 Chemical Structure of Ampicillin Trihydrate

Anti-bacterial molecules with β -lactam group is susceptible to hydrolysis reaction of β -lactamase enzyme produced by resistant bacteria in aqueous medium [1].

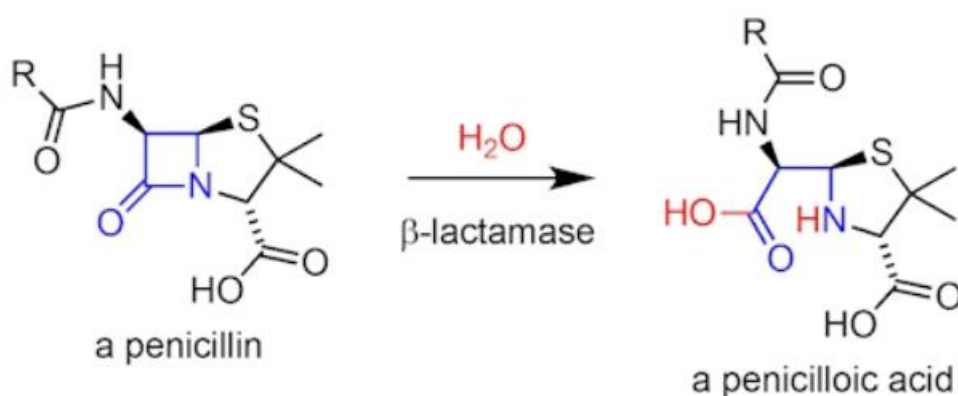


Figure 2.5 Degradation reaction of Ampicillin [1]

Ampicillin penicilloic acid is the product of degradation of ampicillin [35] and small proportion of drug is lost in this way [32]. Since bulky chemical groups provide steric hinderance, β -lactamase enzyme cannot reach to β -lactam ring [1]. Hence it is important to protect β -lactam ring for efficiency and effectivity of drug. In our study, with the design of drug-copolymer systems protection of β -lactam ring from the enzyme is aimed.

Penicillins are weak acids ($pK_a=2.65$) due to carboxyl groups, whereas ampicillin counted as amphoteric due to basic primary amine group in structure [30].

Distribution of ampicillin is reached mostly all over the body via both oral and parenteral administration of drug. Elimination of drug is provided mostly by tubal secretion in kidneys and minimally by glomerular filtration [36].

Diffusion across the porin channels is dependant on certain properties like lipophilicity, size and shape. Hence, moderately hydrophilic ampicillins due to their primary amine group can diffuse through the porin channels in outer membranes of most of the Gram-negative bacteria [37]. The underlying reason is that Gram-negative bacteria retain lipophilic compounds more than hydrophilic molecules [38].

2.1.2 Cephalosporins

In 1945, from *Cephalosporium acremonium* cultures, first cephalosporins which showed antibacterial activity against *Salmonella typhi* responsible for the typhoid fever were isolated [39].

Although cephalosporin C is natural compound, it doesn't show enough antimicrobial activity. Therefore, semi-synthetic cephalosporin antibiotics are synthesized from natural cephalosporin C and antimicrobial activity is improved [40].

Since cephalosporins have cephem scaffold with 6-membered ring (dihydrothiazine ring), the ring strain is much lesser than the penam of penicillins' have. Hence, cephalosporins are not as reactive as penicillins in the hydrolysis and nucleophilic addition reaction, also, showing more resistance for the opening of the β -lactam ring comparing to penicillins. There is experimental evidence of reactivity of carbonyl groups of β -lactam ring which shows absorption at 1760 cm^{-1} for cephalosporins whereas carbonyl absorption peak for penicillins comes at 1770 cm^{-1} in IR spectra [30].

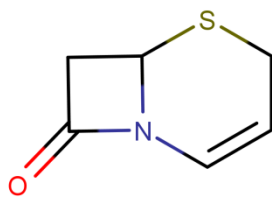


Figure 2.6 Chemical Structure of Cephems

Hydrophilicity and lipophilicity of antibiotics are so effective in penetration into bacterial cell wall. Quaresima and co-workers proved that lipophilic cephalosporins exhibit higher activity against Gram-positive bacteria with low lipid content. Gram-negative bacteria retain the lipophilic molecules, and they cannot reach the site of infection [38].

2.1.2.1 Cefalexin

In 1967; cefalexin which is one of the oral cephalosporins as first generation of cephalosporins, started to be used in medical practice. Among other cephalosporins, cefalexin exhibit less activity, very high concentration of drug in urine and blood, as well as stability of cephalalexin counterbalance each other [41].

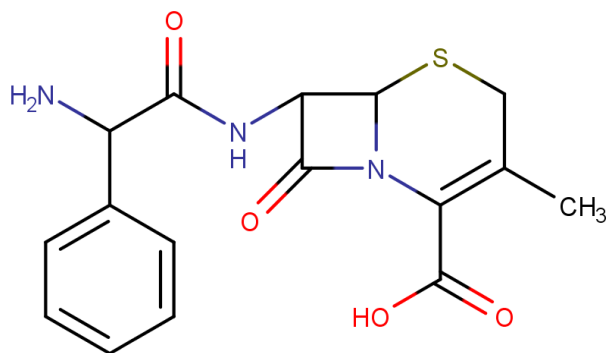


Figure 2.7 Chemical Structure of Cefalexin

Cefalexin and ampicillin has same side chains in their chemical structures, hence; they both are stable in acidic conditions [30], [39]. However, cefalexin differ from ampicillin by not having leaving group on dihydrothiazine ring and this leads to decreased antimicrobial activity [30], [39].

Semisynthetic antibiotics like ampicillin and cephalalexin are natural origin compounds that are partially changed by chemical reactions [1]. Pharmacologically,

half of the ampicillin can be recoverable from urine whereas almost all the cefalexin antibiotic can be recovered from urine [40].

Although cefalexin monohydrate and ampicillin trihydrate are placed under beta-lactam antibiotics, cephalixin monohydrate is located under cephalosporins and ampicillin trihydrate is located under penicillins. In our project, by working with these drugs under equal conditions, we will be able to project the applicability of same procedure to the rest of the β -lactam family.

A recent study shows that even though there are many studies in the literature, the pharmacokinetic properties of β -lactam antibiotics with antimicrobial activity are still required to be improved [24]. In addition, completing a drug course is a necessity for the oral administration of these antibiotics [41]. Specifically for the antibiotic usage, the number of resistant bacteria increases and becomes more powerful, when patients do not finish the entire course of antibiotics [3], [4].

In chronic infectious illnesses, patients are given very high doses are given very high doses, which can lead to undesirable outcomes such as cumulative toxicity, and antibiotic resistance due to poor pharmacological properties of drugs [8].

2.2 Polymeric Drug Delivery Systems

Despite their widespread use of Cefalexin [41] and Ampicillin as broad-spectrum antibiotics, their pharmacokinetics are poor.

By reducing the frequency of drug intake and extending the release time of the drug, patient compliance can be improved [11]. Hence, the distribution of antibiotics to infected sites is a requirement for effective drug delivery systems [8].

There is an increasing need for innovative drug delivery methods since enhancing the pharmacological and physical effects of pharmaceuticals is a more affordable, secure, and efficient method [42]. Drug delivery systems generally based on polymers since they can fulfill the specific requirements for reproducibility, biocompatibility, and biodegradability to adjust the features of drug delivery systems [43].

The purpose of polymeric drug delivery systems (PDDSs) is to enhance drug safety, efficacy, and patient compliance [8]. The local delivery of drugs for longer periods is made possible by these systems [8]. Bioactive polymeric materials are currently

the focus of biomaterials research, due to their desirable properties such as lower dosages, longer half-lives and better stability in drug delivery systems[44].

Several methods have been developed to prepare bioactive polymeric materials for drug delivery systems. Polymerization of the drug containing monomers is the first method [45]. The second approach involves the compression of drugs and polymers as polymer-drug blends (a.k.a. physical incorporation) [3,13] and the last method is to chemically conjugate drugs to polymers that already exist [3]. The capacity of drug loading is improved by chemical conjugation compared to polymer-drug blends that are physically incorporated (the second method) [3]. Therefore, the last method was more advantageous for our study.

Polymer-drug conjugates are macromolecular structures formed by covalent binding of drug to macromolecular structure like polymers. Polymer-drug conjugates are used to improve the pharmacological properties such as stability, activity, bioavailability etc. of chosen drugs [46]. The main building-blocks of macromolecular prodrugs are the polymeric backbone and active drug molecule [47].

According to Kamaly et.al.'s study [48]; micro-sized drug delivery systems provide certain advantages such as tuneable controlled release drugs up to months, standardized fabrication of polymeric microparticles, higher drug loading capacity, biodegradability, and in vivo degradability whereas small sized nanoparticles <10 nm, open to renal clearance, lead to accumulation in unwanted locations and can limit dosing amounts due to size dependent toxicity. Additionally, macro-sized drug delivery systems can cause foreign body reactions and lead to limited biological applications due to lack of circulation in the body [48]. Hence, the size of the drug delivery systems affects the pharmacological properties. Due to the disadvantageous features of macro and nano scale drug delivery systems, sub-micro scale drug delivery systems step forth.

The idea behind using PDDSs is that the pharmacokinetics of the polymer-drug conjugate will be similar to that of the polymer itself [25]. Polymer conjugation increases the molecular weight of the drug, which causes its half-life to increase and its elimination rate to decrease [49].

2.3 Maleic Anhydride Derived Copolymers

Candidate polymeric drug carriers must fulfill certain criteria, including biocompatibility and having functional groups like -COOH , -NH_2 , -OH , etc., that are suitable for covalent conjugation with drugs, water solubility, low PDI value and availability [50]. The biocompatibility and special chemical properties of polymers derived from maleic anhydride which allows chemical attachment of various bioactive materials make them excellent candidates for controlled drug delivery systems [13]. The properties and usage areas of this class of polymers can be modified and enhanced [13]. Also, it has been proven that maleic anhydride-based copolymers are suitable for biomedical uses, such as drug carriers [27]. There are also several examples of maleic anhydride-derived polymeric drug carriers that significantly prolong the half-life of conjugated drugs in the blood compared to free drugs [17,51]. Therefore, in order to prolong the circulation time for our designed drug delivery system, maleic anhydride-based polymers as drug conjugates are suitable options. Maleic anhydride copolymers have unique properties like biocompatibility, their pH-dependent solubility, reactivity of the anhydride group with nucleophilic agents (OH , NH) and reproducible structures [26]. Maleic anhydride derived polymers are known for their low cytotoxicity and thermal stability [50], [51].

Advantageous properties such as: biocompatibility, easily available and cheap monomers, variations of hydrophilicity and hydrophobicity depending on comonomer choice and water solubility of maleic copolymers step forth with pharmaceutical and medical applications [47]. In addition to these properties, maleic anhydrides have special chemical structures that allow mild reactions with drugs. In this way, drugs can easily attach maleic anhydride derived copolymers either through the comonomer reactive groups or through the anhydride cycle [47], [52]. With the conjugation of maleic copolymers, insoluble drugs become soluble or drug solubility is increased [47], [53].

The use of maleic anhydride or maleic acid copolymers can be diverse: as part of functionalization of hemodialysis membrane, as drugs, as a support of bioactive molecules or as conjugates in drug-controlled release systems [47]. Maleic copolymers' structure, properties and activity make them suitable for biomedical

applications. Maleic anhydride copolymers are commonly known to possess an alternating and well-defined structure that can be reproduced [47].

Alterations in comonomers leads to change in the hydrophilic/hydrophobic character and charge density of polymer [47]. The application of maleic copolymers as support for polymer-drug conjugates in controlled drug delivery systems are studied [47].

The anhydride cycle of maleic anhydride copolymers; make the the production of diverse drug-polymer conjugates through chemical reactions possible in mild conditions as well as avoid the contamination with impurities [47].

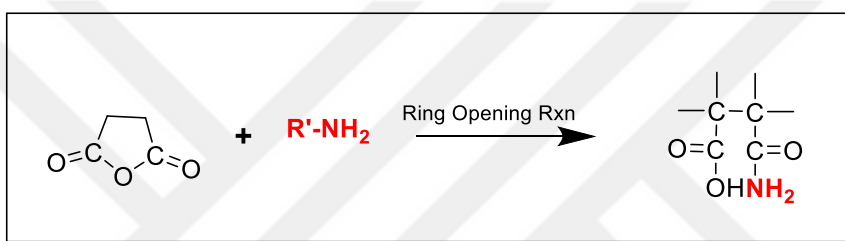


Figure 2.8 Representation of amide reaction for maleic anhydride

One of the maleic anhydride-derived copolymers (poly (styrene-alt-maleic anhydride)) was covalently conjugated with acetaminophen drug to improve the pharmacological properties [14].

Poly (methyl vinyl alt-maleic anhydride) (PMVEMA) is used in various health care and cosmetic applications due to its good safety profile [54], [55]. PMVEMA is known as pre-activated polymer, and very reactive to primary amines which are forming half-amides [55], [56]. In 2002; the first study is introduced by Arbós *et al.*, was about the preparation of PMVEMA based nanoparticles for oral delivery. PMVEMA is widely used and commercially available copolymer in various industries [55].

According to study conducted by Jin *et.al.*; the reaction between poly(maleic anhydride)-based polymers such as poly(isobutylene-alt-maleic anhydride) (PIBMA) and H_2N-R nucleophiles leads various multifunctional polymers and will have several usage areas as developing biocompatible materials in biotechnology and medicine [57]. In literature, there are several examples of materials based on

maleic anhydride such as adhesives, biomedical devices and drug delivery systems [58], [59], [60].

In our study, 3 different maleic anhydride derived copolymers are selected; Poly(methyl vinyl alt-maleic anhydride) (PMVEMA), Poly(isobutylene alt-maleic anhydride) (PIBMA) and Poly(ethylene alt-maleic anhydride) (PEMA) with different degrees of hydrophilicity. In Bettahar et.al.'s research; hydrophilicity of maleic anhydride copolymers results in differences in the thermal stability, mechanical and swelling properties [61]. Maleic anhydride copolymers can be ordered as PMVEMA>PEMA>PIBMA in terms of hydrophilicity.

According to Smith and co-workers' study; the need for improvement of PDDSs, in our research, we targeted to improve the pharmacological properties of antibiotics (AMP and CEF) from different sub-groups of the β -lactam family, via novel polymeric drug delivery systems and got better results compared to similar studies [19], [20]. In this research, synthesis of polymeric drug delivery systems are designed from 2 different beta-lactam antibiotics (ampicillin trihydrate-AMP and cefalexin monohydrate-CEF) and 3 different maleic anhydride derived copolymers. Different pH values and simulated body fluids as a function of time were determined with the Zetasizer and UV/VIS measurements, in addition to antibacterial tests resulting in determination of pharmacological properties such as stability, particle size, PDI and activity of PDDSs at *in vitro* conditions.

2.4 Characterization Techniques

2.4.1 FTIR/ATR

The invention of Fourier transform infrared (FTIR) spectroscopy in the 1960s has led to its widespread use in various fields and the growing interest as a potential instrument in drug discovery, quality control etc. [62], [63], [64], [65]. FT-IR imaging is great tool for use in the development of drug delivery systems because it can rapidly and easily extract useful information from the collected data [66]. FTIR can identify functional groups and obtain structural information in a qualitative or semi-quantitative way [46]. FTIR operates by detecting the absorption of infrared light that occurs due to vibrational transitions of covalent bonds, and different factors play a role in the signatures of FTIR spectra

making each spectrum unique [46], [67]. Carbon skeleton fingerprint vibrations and a high-wavenumber region which is associated with stretching vibrations, such as S-H, O-H, and C-H, are responsible for spectral variations in the lower-wavenumber region [68]. FTIR is used to identify functional groups and active pharmaceutical ingredients, which is essential for other research, including drug mechanism and design [69].

By analyzing the changes between FTIR spectra of complexes and each component, it is possible to detect the synthesis or reaction between drugs and polymers [70], [71], [72]. The functional groups are tested to ensure the effectiveness of drugs loaded into drug delivery systems. Due to its short determination time and high precision, along with its capacity for simultaneous, continuous, and non-damage properties of FTIR, many drug delivery studies have utilized from FTIR [46], [73], [74]. Most research on pharmaceutical preparations can generally be utilized from FTIR, which is an infrared absorption detection method. The scope of application can be broadened by combining FTIR with various sampling techniques or other detection techniques depending on different experimental needs [46]. The FTIR technique steps forth with properties like convenient, efficient, non-destructive, low cost, and label free, compared with other methods such as Raman, TEM and SEM techniques [46]. The rapid advancement of FTIR imaging enables it to accurately detect spatial distribution of chemical or physical structure in a complex system [46]. FTIR is a powerful tool in formulation optimization and screening, and it has a wide range of potential applications in both clinical and industrial research [46]. According to Wright and co-workers' study, they have proved that FT-IR imaging is applicable to the field of drug delivery [66]. Attenuated Total Reflection (ATR) mode of FTIR imaging is an effective way to evaluate biomedical samples and seen as a complementary mode to the transmission/reflection modes [75]. ATR is applicable to wide variety of different molecules and known as non-destructive, label-free method [76].

Sample preparation of spectral measurements can be minimized or eliminated by the ATR mode [75]. Although, there is a disbelief that ATR requires a pressure for solid samples, it has been disproven by literature [75]. In our study, we have utilized FTIR /ATR for the characterization of candidate drug delivery systems.

2.4.2 ^1H NMR

Suitable choice of polymer and drug molecule provides pharmacologically progress comparing to free drug molecule [77]. Molecular weight determination and molecular weight distribution have an importance in analysis and characterization of polymers and for this analysis; nuclear magnetic resonance (NMR) spectroscopy is well-recognized and suitable technique [78] has been used to understand the monomer sequence, molecular weight determination and polymer microstructure characterization such as tacticity (stereoregularity), composition of end-groups [78]. Especially for the determination of microstructure of polymers, proton NMR is a unique technique and frequently utilized from [78].

NMR gives information about isotropic chemical shift, conformation of chain and monomer distribution such as diads, triads etc. in copolymers [79]. It is possible to determine molecular weight of sample by considering that resonance peak areas and molar concentration are in proportion [78].

Although NMR is less preferred method comparing to gel permeation chromatography (GPC) for molecular weight determination of polymers, NMR is simpler, quite accurate and fast technique [77]. Additionally, Pinciroli and co-workers noted that; by fully utilizing from NMR methods, it is possible to understand even the cell/organ targeting properties of polymeric drugs [77]. In our research, we have utilized from proton NMR for chemical structure characterization and calculation of polymer-drug binding ratios.

2.5 Factors Affecting of β -Lactam Antibiotics

Infectious diseases are generally treated by antibiotics safely [21]. Especially for the chronic infections, very high dosages are administered to the patients, and it may cause unwanted results like cumulative toxicity, antibiotic resistance due to poor pharmacological properties of drugs [21]. Therefore, sustained distribution of antibiotics in the infected sites is a requirement for effective drug delivery systems [21].

In oral drug delivery systems, a drug can pass through 6 different types of body fluids such as: fasted-state/fed-state simulated gastric fluid, fasted-state/fed-state simulated intestinal fluid, fasted-state/fed-state simulated colonic fluid.

Composition of those body fluids affect possible in vivo behavior of a drug. Sodium chloride is present in fasted-state/fed-state simulated gastric fluid; fasted-state/fed-state simulated intestinal fluid. PBS (phosphate buffer saline) is used to mimic colonic fluid [80].

Additionally, gastric pH values are highly influenced by the food intake. pH values exhibit change from 2 to 7 with the transition from fasted-state to fed-state [81]. Several researches concluded that pH of the colon is between 5.7 and 8.4 [82]. Food intake may affect various physiological factors that have impact on drug absorption such as volume and composition of gastrointestinal fluids, the pH and buffer capacity of these fluids [83]. Absorption rate of ampicillin is only 62% and this value is decreased by food intake [84]. Since there is a limitation in dosage of ampicillin trihydrate by size, number of tablets and gastrointestinal side-effects, another way has to be found for the compensation of losses in absorption [85]. In order to increase bioavailability of candidate drug delivery systems, bioavailable copolymers are used.

According to the study conducted by Podcizek and co-workers, 5% dextrose solution can stay in gastric medium more than 20 minutes and gastric emptying time can exceed 100 minutes when the tablets are taken with this solution [80]. Hence, all drug delivery systems in this research will be analyzed considering time and pH change in various simulated body fluids such as 5% dextrose solution, phosphate buffered saline (PBS) solution and 0.9% dextrose solution.

2.6 Activity and Zeta-sizer Measurements

Surface charge, zeta potential and pH of particles make the understanding the state and stability of dispersions possible [86], [87]. In Zetasizer measurements, the polydispersity index, particle size, mobility and zeta potential of drugs and PDDSs were monitored in terms of homogeneity, charge of the particles and stability.

Following the gastrointestinal tract (GI) from the stomach to the colon, the pH value changes in the stomach is between 1.5-3.5, in the duodenum, pH value is around 5-6 and increases to pH 7-8 in the intestine and pH value reaches to 6 in the colon [88], [89]. Hence, drugs and drug delivery systems were studied under a broad pH scale.

2.6.1 Activity

UV/VIS spectroscopy is a non-destructive method and time, cost effective [90], it has been frequently used.

According to Asan and Seddiq's study; routine analysis of antibiotics (amoxicillin) in drug samples can be done by only using UV/VIS spectroscopy [91]. This technique can be even used for the determination of drug stability in samples of pharmaceutical dosage forms [92]. Activity of drug delivery systems can be evaluated by UV/VIS spectroscopy [93], [94]. In our research, based on the information in literature, we aimed to assess the activity of candidate drug delivery systems via UV/VIS spectroscopy.

2.6.2 Particle size

Particle size affects drug dissolution and absorption in solid drug delivery systems given orally [95]. Determination of rate determining-step in the absorption of drugs, is based on dissolution rate of drugs in the gastro-intestinal fluids [95].

Surface charge, zeta potential and pH of particles have an importance on understanding state and stability of dispersions [86], [87]. A study showed that surface charge of a nanoparticle has affected the permeability and integrity of blood-brain barrier [86], [87].

Definition of zeta potential is "the potential difference between the dispersion medium and the stationary layer of fluid attached to the particle" [96]. Physical stability of suspensions is dependent on zeta potential measurements [96], [97].

In 1940s, Derjaguin, Landau, Verwey and Overbeek established a theory called as "DLVO theory" explains the case that sum of the attractive forces due to Van der Waals interaction and repulsive forces between individual particles determines agglomeration and stability of particle dispersions [86], [98]. Higher value of zeta potential either positive or negative is the indicator of physically stable suspensions due to the repulsion of same charged particles [96], [97]. Below -30 mV and above +30 mV zeta potential values are enough for electrostatic repulsive forces which prevent aggregation and show physical stability of colloids [96], [97]. Van der Waals attractive forces are effective on particle aggregation and physical instability occurs where the zeta potential values are small [97], [99], [100], [101]. Particles with large negative or positive zeta potential values (above +30 mV and below -30

mV) are counted as stable due to the barrier that prevents aggregation of particles [96], [97].

Dynamic light scattering (DLS) can be known as photon correlation spectroscopy (PCS) and quasi-elastic light scattering (QELS) is a technique in Zeta-sizer instrument [86]. Solvent molecules and particles collide each other randomly called Brownian motion and results in light scatterings [86]. Scattered light intensities fluctuates in time and autocorrelation of these fluctuations enables to find “intensity weighted average hydrodynamic diameter with the help of Stokes-Einstein Equation shown below [86].

$$d_H = \frac{kT}{3\pi\eta D} \quad (2.1)$$

d_H = Hydrodynamic diameter(m)

k =Boltzmann constant (J/K=kg.m²/s².K)

T =Temperature(K)

η = Solvent viscosity (kg/ms)

D =Diffusion coefficient (m²/s)

2.6.3 PDI

The Polydispersity Index (PDI) has a definition as the distribution of size of the particles in a certain population. When PDI is at 0.0 value indicates perfect uniform particles or the beginning of the monodispersity and when PDI value reaches 1.0 that exhibits the presence of different particle sizes in a population namely, polydispersity[102]. PDI analyses determine the efficiency of the PDDSs. All drugs and drug delivery systems were interpreted in terms of PDI, particle size and stability analyses, respectively.

2.6.4 Mobility and Zeta Potential

It is described as most stable parameter for dynamic light scattering (DLS) method and mainly used in quality controls [103]. Due to the hydrodynamic sensitivity of parameters, it is only valid for the dispersed particles or molecules [103] . Particles in suspension show certain physical properties and one of them is zeta potential. Zeta potential is used to predict the stability of colloidal system and affected by pH,

conductivity and concentration of formulation [104]. Based on DLVO theory; stability of colloidal system is dependant on sum of the Van der Waals attractive and repulsive forces. In case of particles with enough repulsive forces resist coagulation, colloidal system become stable [104].

Negatively charged surfaces take place due to the dissociation of acidic groups. Ionisation of basic groups cause positively charged surfaces. pH of the solution and acidic or basic strength of surface groups affects the magnitude of the surface charge. For instance, charge of the surface can be zeroed with suppression of surface ionisation. Decrease in pH value for negatively charged particles and an increase in pH value for positively charged particles prevents surface ionization [104].

Classical electrophoresis is based on the movement of particles towards electrodes with an applied electrical field. Expression of the velocity of particles in unit electrical field is referred as mobility. For the particles with 0.1 μm diameter and bigger zeta potential can be calculated from mobility by Smulochowski model whereas for smaller particles dispersed in low dielectric constant media, Huckel model is more suitable [102], [104]. Determination of the stability of particle dispersion in a solution is made possible by zeta potential analysis. When the value of zeta potential is at ± 10 mV, solution is at incipient stability [105] and when the zeta potential is greater than ± 25 mV, then the solution is stable suspension [106]. The stability of dispersion is sustained by the electrostatic repulsion between the charged particles [106]. A higher value of absolute zeta potential is the indicator of physically stable suspensions [96], [97].

Huckel and Smoluchowski equations are used to measure the zeta potential. Huckel equation is valid when particles of the solution are in the nanosize region (<100 nm). Smoluchowski equation is proper for the particles larger than 100 nm in size. Equation 2.2 is known as the Smoluchowski equation for electrophoresis [107].

$$\mu_e = \frac{\epsilon_e \zeta}{\eta} \quad (2.2)$$

in which μ_e represents electrophoretic mobility, ϵ_e represents the dielectric constant of solvent, η represents the solvent viscosity, ζ represents zeta potential.

2.7 Anti-bacterial Susceptibility Tests

Antimicrobial susceptibility tests are utilized in drug discovery, epidemiology and estimation of therapeutic results [108]. Disk diffusion method steps forth with simplicity, low cost, the ability to test excessive number of microorganisms and ease in interpretation of results [108]. This method is only available for fastidious bacterial pathogens [108] and used to determine the effectivity of antimicrobial drug delivery systems.

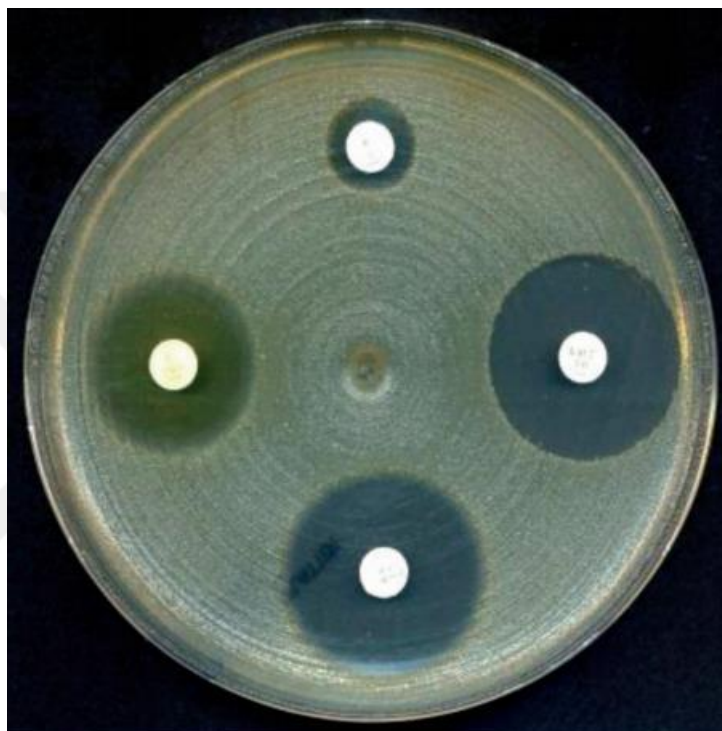


Figure 2.9 Disk Diffusion test- Inhibition zones [109]

In fig. 2.9; there is a example of disk diffusion test. White zones represent anti-bacterial agents and transparent zones exhibit inhibition of bacteria zones. The larger the zones mean the more anti-bacterial effect of these agents. In our study, disk diffusion tests are done to compare anti-bacterial efficiency of drug delivery systems.

In our project, the optimum stability and activity conditions of the designed drug delivery systems in various pH values and simulated body fluids as a function of time are determined to obtain new drug delivery system candidates for two different antibiotics.

3

EXPERIMENTAL PART

3.1 Materials

Poly(methyl vinyl ether-alt-maleic anhydride) (PMVEMA) ($M_w \approx 210,000$, $M_n \approx 80,000$, Sigma-Aldrich), Poly(isobutylene-alt-maleic anhydride) (PIBMA) (average $M_w \sim 6,000$, 12-200 mesh, Sigma-Aldrich), Poly(ethylene-alt-maleic anhydride) (PEMA) (average M_w 100,000-500,000, Sigma-Aldrich), Ampicillin Trihydrate (AMP) (Bilim Pharmaceuticals) (%99.5 purity), Cefalexin Monohydrate (CEF) (Bilim Pharmaceuticals) (%99.6 purity), N,N-Dimethylformamide (DMF) ($\geq$ %99.8, Merck), Toluene (Merck) and Dimethyl Sulfoxide (DMSO) ($\geq$ %99.9, Merck) are used in chemical reactions.

3.2 Synthesis of Drug Delivery Systems

The anhydride ring of maleic anhydride is prone to the nucleophilic attacks by amino or hydroxyl groups [94]. It is expected that amino group of ampicillin trihydrate/cephalexin monohydrate attacks to the anhydride ring and ring-opening reaction occurs, theoretically.

As a reaction procedure, ring opening reaction took place by dissolving the copolymer and the drug in dimethyl formamide (DMF) with 1:1 molar ratio and mixing them under inert conditions for 7 days at 70 °C degrees. After the reaction, toluene is added onto crude solution with 3:1 ratio to establish azeotropic mixture and to lower the boiling point. The mixture is then rota-evaporated at 110 °C under vacuum and drug delivery system is obtained. This procedure has been applied for all the copolymer (PMVEMA, PEMA, PIBMA) and drug candidates (AMP, CEF). This procedure has been applied for all the copolymer (PMVEMA, PEMA, PIBMA) and drug candidates (AMP, CEF).

Amino groups of drugs are reacted with the anhydride group of maleic anhydride derived polymers and six different drug delivery systems are obtained under pre-determined reaction conditions. Color change is observed with the completion of reactions, photos showing the final physical appearances of samples are given in figure 3.1.

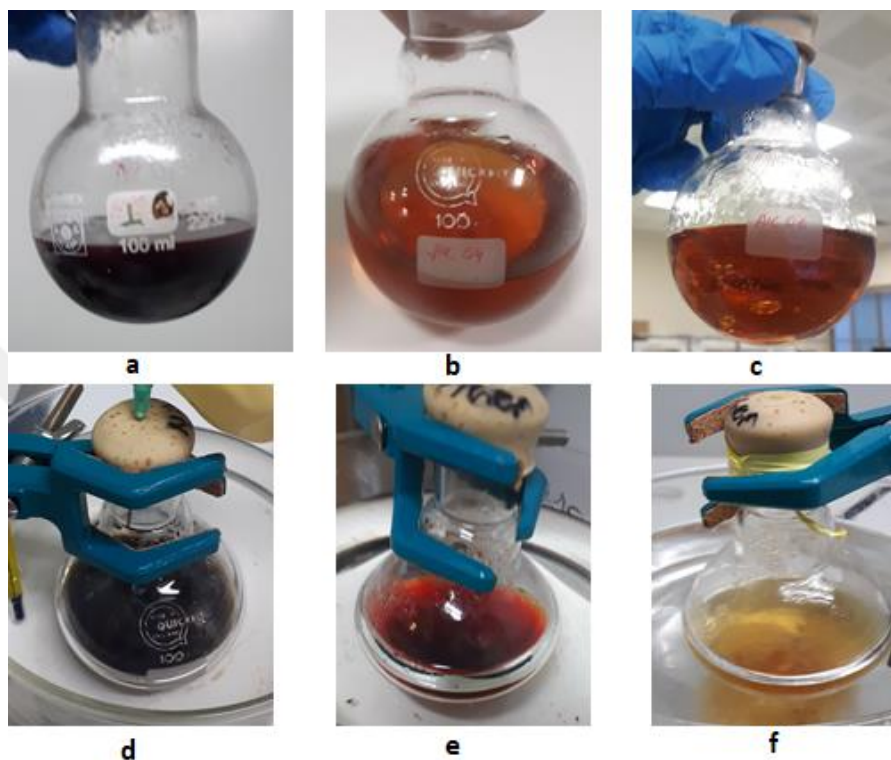


Figure 3.1 Final physical appearances of PMVEMA-AMP DDS (a), PIBMA-AMP DDS (b), PEMA-AMP DDS (c), PMVEMA-CEF DDS (d), PIBMA-CEF DDS (e), PEMA-CEF DDS (f)

3.2.1 Synthesis of PMVEMA-AMP DDS

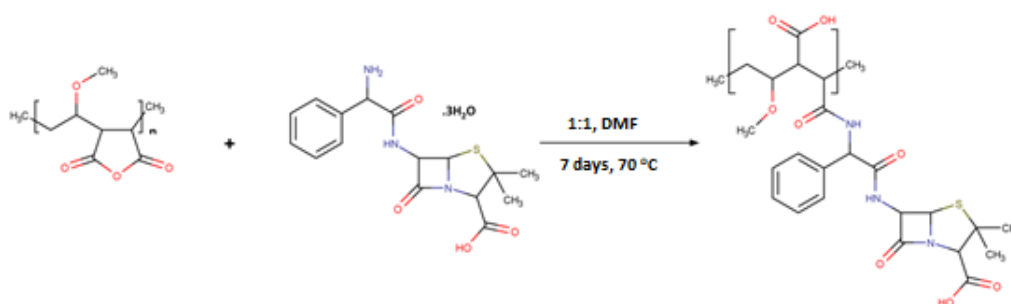


Figure 3.2 Synthesis of PMVEMA-AMP drug delivery system

Amino group of AMP is reacted with the anhydride group of PMVEMA in fig. 3.2. and PMVEMA-AMP DDS is obtained.

3.2.2 Synthesis of PIBMA-AMP DDS

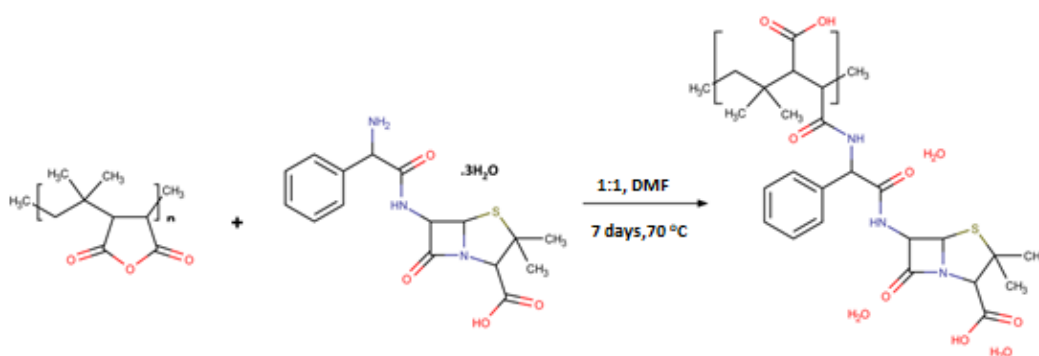


Figure 3.3 Synthesis of PIBMA-AMP drug delivery system

Amino group of AMP is reacted with the anhydride group of PIBMA in figure 3.3. and PIBMA-AMP DDS is obtained.

3.2.3 Synthesis of PEMA-AMP DDS

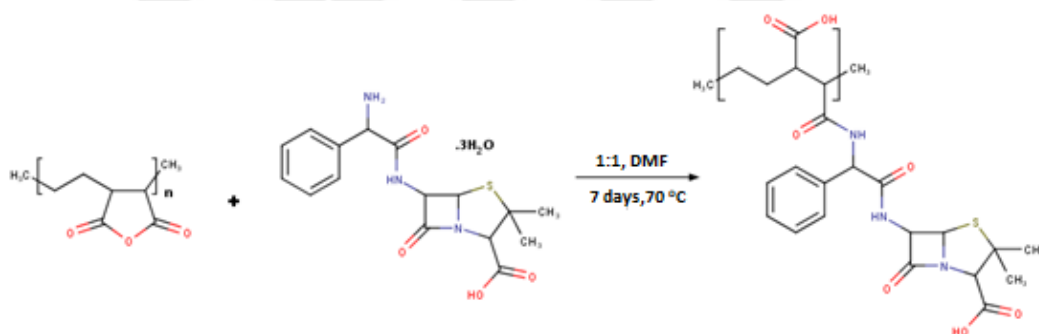


Figure 3.4 Synthesis of PEMA-AMP drug delivery system

Amino group of AMP is reacted with the anhydride group of PEMA in figure 3.4. and PEMA-AMP DDS is obtained.

3.2.4 Synthesis of PMVEMA-CEF DDS

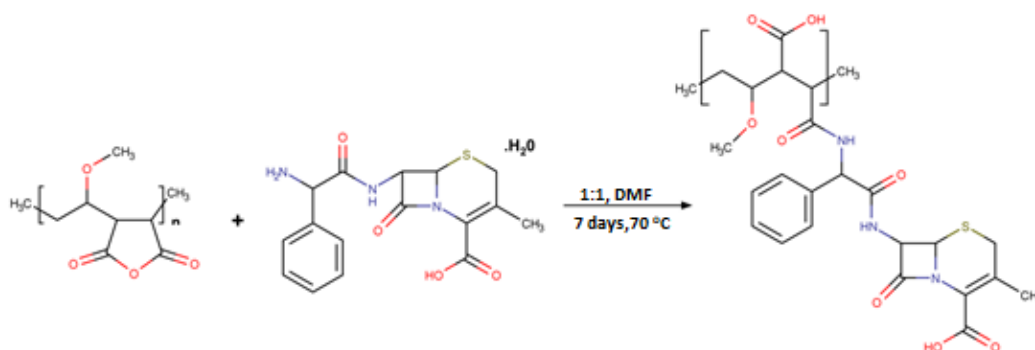


Figure 3.5 Synthesis of PMVEMA-CEF drug delivery system

Amino group of CEF is reacted with the anhydride group of PMVEMA in figure 3.5. and PMVEMA-CEF DDS is obtained.

3.2.5 Synthesis of PIBMA-CEF DDS

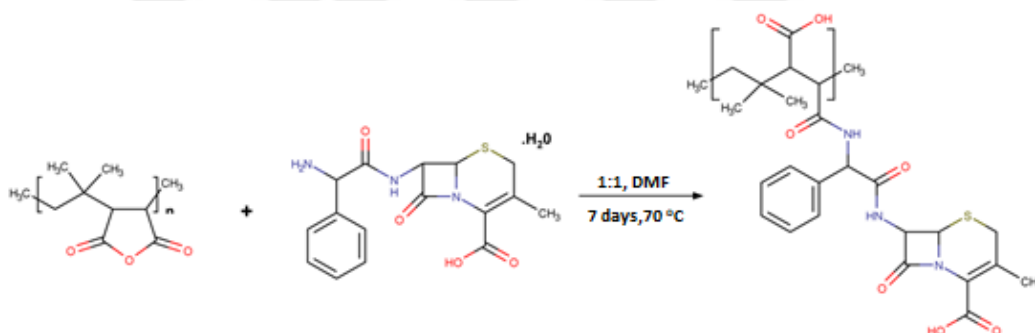


Figure 3.6 Synthesis of PIBMA-CEF drug delivery system

Amino group of CEF is reacted with the anhydride group of PIBMA in fig 3.6 and PIBMA-CEF DDS is obtained.

3.2.6 Synthesis of PEMA-CEF DDS

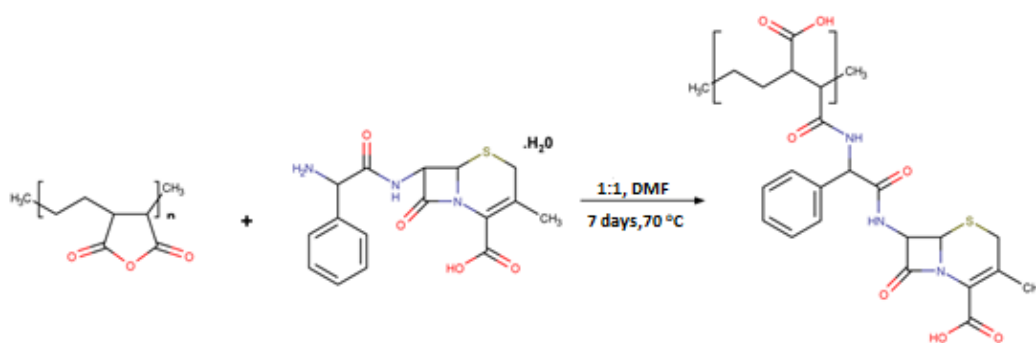


Figure 3.7 Synthesis of PEMA-CEF drug delivery system

Amino group of CEF is reacted with the anhydride group of PEMA in figure 3.7. and PEMA-CEF DDS is obtained.

3.3 Structural Characterization

3.3.1 FTIR/ATR Spectroscopic Analyses

Infrared (IR) analyses were done with Thermo Fisher Scientific branded Nicolet iS10 model The Fourier Transform Infrared Spectrometer instrument with Smart iTR model ATR (Attenuated Total Reflectance) apparatus. Analyses are taken in between 4000 cm^{-1} and 600 cm^{-1} wave numbers with 32 scanings. Baseline is measured against air. Samples are prepared as solid, granulated form. Results are saved in the form of %Transmittance- Wavenumber (cm^{-1}). All analyses are done at $20\text{ }^{\circ}\text{C}$ degrees.

3.3.2 ^1H NMR Characterization

Agilent 400 MHz Varian VNMRS instrument is used for proton NMR characterization. Samples are prepared by dissolving compounds in DMSO- d_6 solvent. Analyses are taken with 32 scanning. All analyses are done at $20\text{ }^{\circ}\text{C}$ degrees.

3.4 Stability And Activity Measurements

3.4.1 Particle Size and Zeta Potential Measurement Conditions

Brookhaven Instruments 90 Plus Particle Size Analyzer instrument is used for particle size and zeta potential measurements. Smoluchowski method is preferred,

and SR-542 probe is used. Sentek P14/BNC electrode is utilized for pH measurements. All analyses are conducted at 37 °C degrees to mimic the body temperature.

3.4.2 UV/VIS Measurement Conditions

Shimadzu UV mini-1240 model UV/VIS spectrophotometer instrument is used with Hellma 100-QS quartz cuvette. Analyses are taken in between 600 nm and 190 nm wavelength range with single scan. Baseline is taken against one of ultra pure water, PBS (Phosphate buffer saline), 0.9% isotonic sodium chloride solution and 5% dextrose solution depending on the type of sample. Samples are prepared as homogenous, clear liquid solutions. Results are saved in the form of Absorbance - Wavelength (nm). All samples are analyzed at 37 °C degrees to mimic the body temperature.

3.4.3 Sample Preparation For Zetasizer and UV/VIS Measurements

Standard sample solutions of PMVEMA, PEMA, PIBMA, AMP, CEF, PMVEMA-AMP, PMVEMA-CEF, PEMA-AMP, PEMA-CEF, PIBMA-AMP, PIBMA-CEF are prepared as dissolving samples in 5% (v/v) DMSO and mixed with distilled water for pH measurements and pH values are adjusted by various concentrations of NaOH and HCl solutions.

To mimick body fluids, these samples are dissolved in 5% (v/v) DMSO, are transferred and mixed into artificial body fluids such as 5% dextrose solution, 0.9% isotonic NaCl solution and PBS solution seperately. These standard solutions are mixed well and sonicated to obtain clear, homogenous solutions for more accurate measurements. Thereafter, solutions are incubated at 37°C and analyzed as a function of time. Sample solution concentrations are given in Table 3.1.

Table 3.1 Solution concentrations (mg/mL) of samples for zetasizer and UV/VIS measurements

S	AMP	CEF	I	I-A	I-C	II	II-A	II-C	III	III-A	III-C
pH	0.12	0.003	0.3	0.012	0.003	0.003	0.3	0.003	0.03	0.12	0.006
5% D	0.12	0.003	0.3	0.012	0.003	0.003	0.3	0.003	0.03	0.12	0.006
PBS	0.12	0.003	0.3	0.012	0.003	0.003	0.3	0.003	0.03	0.12	0.006
0.9% NaCl	0.12	0.003	0.3	0.012	0.003	0.003	0.3	0.003	0.03	0.12	0.006

3.4.3.1 Artificial Body Fluids

Phosphate-buffered saline, 5% dextrose and 0.9% isotonic sodium chloride solutions are used as artificial body fluids.

- **Phosphate Buffer Saline Solution (PBS)**

Phosphate buffered saline (PBS) tablet is provided from Sigma Aldrich and each tablet dissolved in 200 ml ultra pure water. pH is adjusted to 7.4. Freshly prepared for each analysis. Tablets are kept at room temperature and after prepared for analysis, solution is kept under 37 °C degrees to mimic the body temperature.

- **5% Dextrose Solution**

Polifleks 5% Dextrose 100 ml solution is provided from pharmacies in polypropylene bags. IV infusion specialized sterilized solution used for particle size measurements and each bag is single time used. pH is 4.3. Closed bags are kept at room temperature and after opened for analysis, the solution is kept under 37 °C degrees to mimic the body temperature.

- **0.9% Isotonic Sodium Chloride Solution**

Polifleks 100 ml 0.9% isotonic NaCl solution is provided from pharmacies in polypropylene bags. IV infusion specialized sterilized solution used for particle size measurements and each bag is single time used. pH is 6.5. Closed bags are kept at room temperature and after opened for analysis, the solution is kept under 37 °C degrees to mimic the body temperature.

3.5 Anti-Bacterial Tests

In order to prepare agar medium, 28 grams of nutrient agar and 1 liter of distilled water is mixed with magnetic stirrer for 10 minutes and dissolved. This solution is autoclaved at 121 °C degrees for 15 minutes for sterilization. It has been cooled down to 45 °C degrees and transferred into sterilized petri dishes and solidified afterwards.

In order to prepare broth, 13 grams of nutrient broth is dissolved in 1 liter of distilled water. This solution is autoclaved at 121 °C degrees for 15 minutes for sterilization. Bacterial strains that will be used in anti-microbial activity tests are incubated in nutrient broth at 37 °C degrees for 48 hours. Growing colony of bacteria is diluted with physiological saline solution and adjusted to 10⁸ CFU/ml according to McFarland 0.5 turbidity standard.

In this study, both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria are used.

3.5.1 Disk Diffusion Method

Anti-bacterial activities of samples are tested by agar disk diffusion method. 20 µL of suspensions of bacteria (10⁸ CFU/mL) that is prepared according to 0.5 McFarland turbidity standard are taken and planted into previously prepared feed-lots in petri dishes. After that, 6 mm diameter sized wells are created. 25 µL of solution where samples are dissolved in DMSO (8 mg/ml) with 200 µg of antibiotics are added in these wells and incubated at 37 °C degrees for 24 hour

4.1 Structural Characterization of PMVEMA, AMP, PMVEMA-AMP Copolymer-Drug Delivery Systems

FTIR/ATR spectroscopy and ^1H NMR spectroscopies are used to characterize the synthesized drug delivery systems and gain detailed information and comparison based on chemical structure, functional groups and binding ratios of copolymer to drug. In figure 4.1; comparative FTIR/ATR spectra of PMVEMA, AMP, PMVEMA-AMP DDS are given.

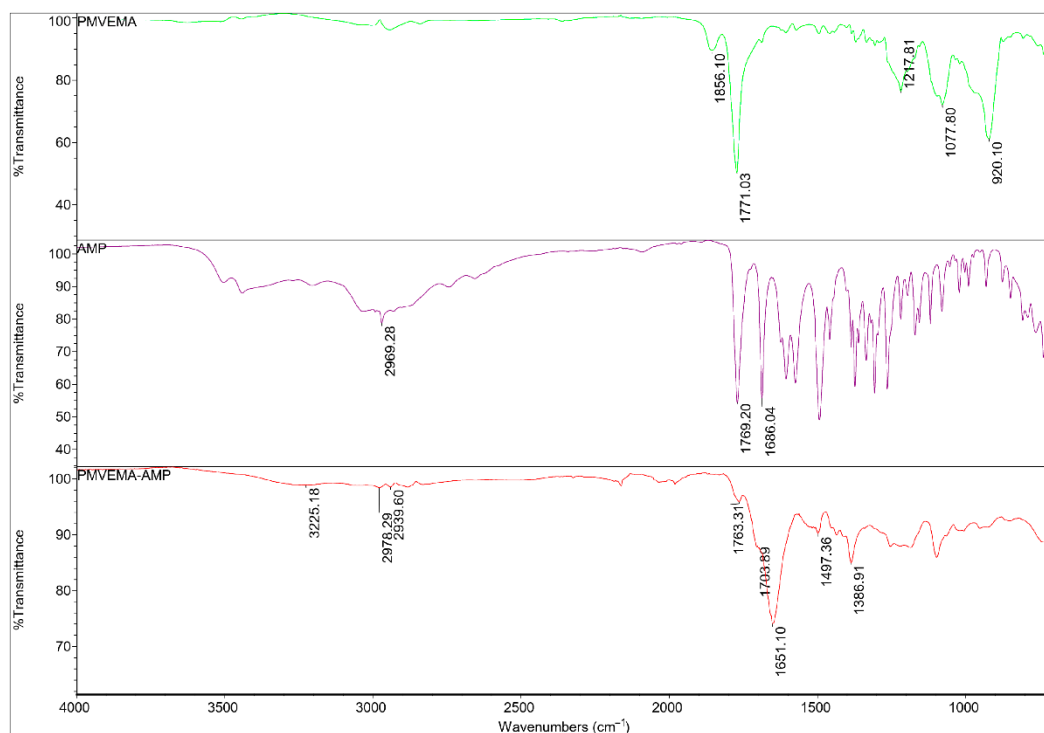


Figure 4.1 FTIR/ATR spectra of PMVEMA, AMP, PMVEMA-AMP DDS

According to FTIR/ATR spectrum of PMVEMA in Fig.4.1; peaks at $\nu = 1856 \text{ cm}^{-1}$ (C=O stretching-anhydride) and 1771 cm^{-1} (C=O stretching-cyclopentanone), 1218 cm^{-1} (-O-CH₃ stretching), 1078 cm^{-1} (O=C-O-C=O stretching-anhydride), 920 cm^{-1} (-C-H bending) were observed.

The carbonyl group of beta-lactam ring in vibration mode appeared around $\nu = 1770 \text{ cm}^{-1}$ [110] as seen on the FTIR/ATR spectrum of AMP in Figure 4.1. Carbonyl group absorption for ampicillin trihydrate appeared around $\nu = 1769 \text{ cm}^{-1}$, the peak for the side amide link on the beta-lactam ring appeared at $\nu = 1686 \text{ cm}^{-1}$. A very broad O-H group peak belonging to carboxylic acid was seen around $\nu = 2969 \text{ cm}^{-1}$.

According to FTIR/ATR spectrum of PMVEMA-AMP PDDS in Fig. 4.1; broad carboxylic acid at $\nu = 3225 \text{ cm}^{-1}$ (O-H stretching), alkane at $\nu = 2978 \text{ cm}^{-1}$ (CH₃ (a)symmetric stretching), cyclobutane at $\nu = 2939 \text{ cm}^{-1}$ CH₂ symmetric stretching), peaks at $\nu = 1763 \text{ cm}^{-1}$ (C=O group of beta-lactam) and 1651 cm^{-1} carboxylic acid peak at $\nu = 1704 \text{ cm}^{-1}$ (C=O stretching), 3 substituted alkene peaks at $\nu = 1651 \text{ cm}^{-1}$ (C=C str.), 2° amide (of β -lactam) at $\nu = 1651 \text{ cm}^{-1}$ (C=O stretching), mono substituted benzene $\nu = 1497 \text{ cm}^{-1}$ (C-C stretching) peaks were observed.

The characteristic symmetric and asymmetric C=O stretching vibration peaks of anhydride of PMVEMA at 1856 and 1771 cm^{-1} disappeared. The carbonyl group peak of the beta-lactam ring of AMP is shifted from 1770 cm^{-1} to 1763 cm^{-1} . After the AMP-PMVEMA conjugation new peaks at 1763 cm^{-1} ($-\text{N}-\text{C}=\text{O}$), 1651 cm^{-1} ($\text{CO}-\text{N}-\text{H}$) due to amide formation.

^1H NMR spectrum of PMVEMA-AMP DDS is given in figure 4.2.

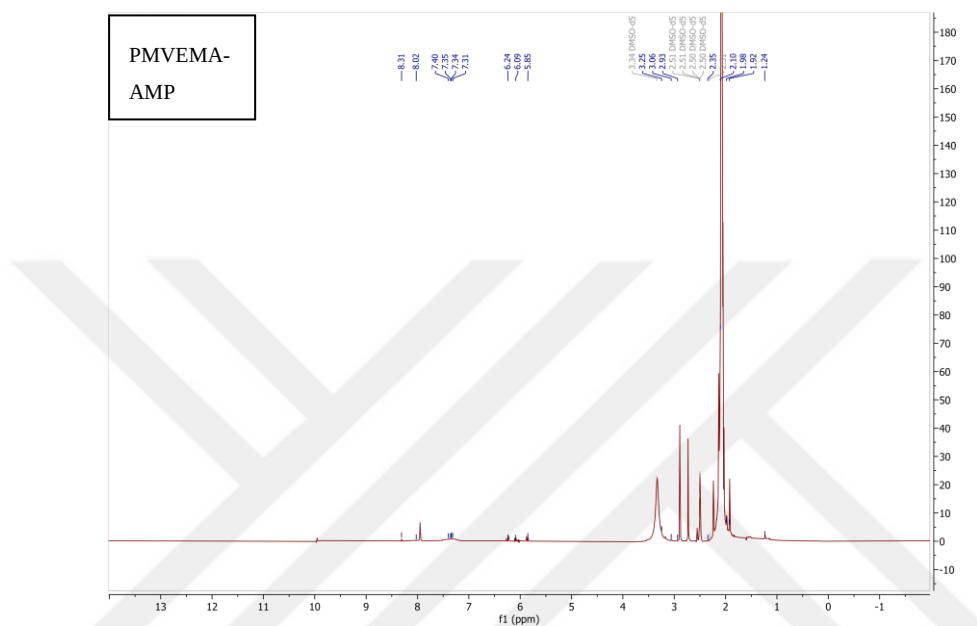


Figure 4.2 ^1H NMR spectrum of PMVEMA-AMP DDS

According to ^1H NMR spectrum of PMVEMA-AMP PDDS in Figure 4.2 ; ^1H NMR (400 MHz, $\text{dmso}-d_6$) δ : 8.31 ppm (1H,N-H), 8.02 ppm (1H, N-H), 7.40-7.31 ppm (5H,C-H), 6.24 ppm (1H,C-H), 6.09 ppm (1H,C-H), 5.85 ppm (1H,C-H), 3.24 ppm (3H, CH_3), 3.05 ppm (1H,C-H), 2.93 ppm (1H,C-H), 2.34 ppm (1H,C-H), 1.98 ppm (1H,C-H), 1.92 ppm (6H, CH_3), 1.23 ppm (2H, CH_2).

4.2 Structural Characterization of PIBMA, AMP, PIBMA-AMP Copolymer-Drug Delivery Systems

Comparative FTIR/ATR spectra of PIBMA, AMP, PIBMA-AMP DDS are given in figure 4.3.

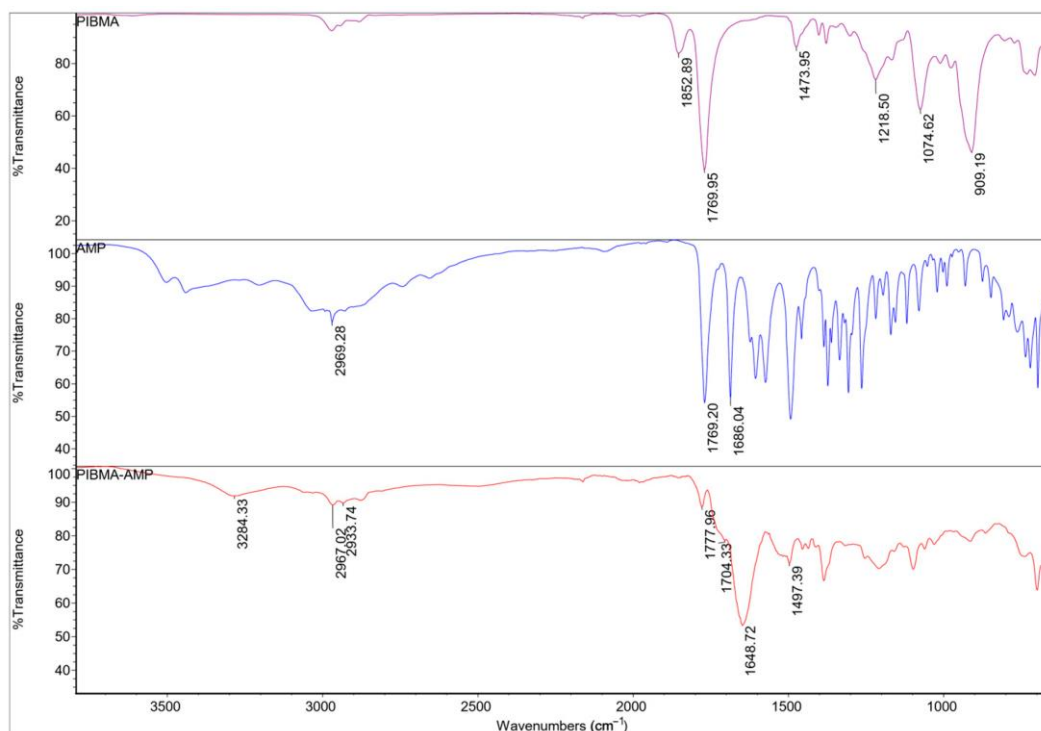


Figure 4.3 FTIR/ATR spectra of PIBMA, AMP, PIBMA-AMP DDS

According to the FTIR/ATR spectrum of PIBMA in Figure 4.3.; peaks at $\nu = 1853 \text{ cm}^{-1}$ (C=O stretching-anhydride) and 1770 cm^{-1} (C=O stretching-cyclopentanone), 1474 cm^{-1} (C-H bending-dimethyl), 1219 cm^{-1} (-O-CH₃ stretching), 1075 cm^{-1} (O=C-O-C=O stretching-anhydride), 909 cm^{-1} (-C-H bending) were observed.

According to FTIR/ATR spectrum of PIBMA-AMP PDDS in Figure 4.3.; broad carboxylic acid peaks at $\nu = 3284 \text{ cm}^{-1}$ (O-H stretching), alkane at $\nu = 2967 \text{ cm}^{-1}$ (CH₃ (a)symmetric stretching), cyclobutane at $\nu = 2933 \text{ cm}^{-1}$ CH₂ symmetric stretching), beta-lactam ring peak $\nu = 1778 \text{ cm}^{-1}$ (C=O stretching) and carboxylic acid peak at $\nu = 1704 \text{ cm}^{-1}$ (C=O stretching), 3 substituted alkene peaks at $\nu = 1649 \text{ cm}^{-1}$ (C=C str.), 2° amide(of β -lactam) at $\nu = 1649 \text{ cm}^{-1}$ (C=O stretching), monosubstituted benzene $\nu = 1497 \text{ cm}^{-1}$ (C-C stretching) peaks were observed.

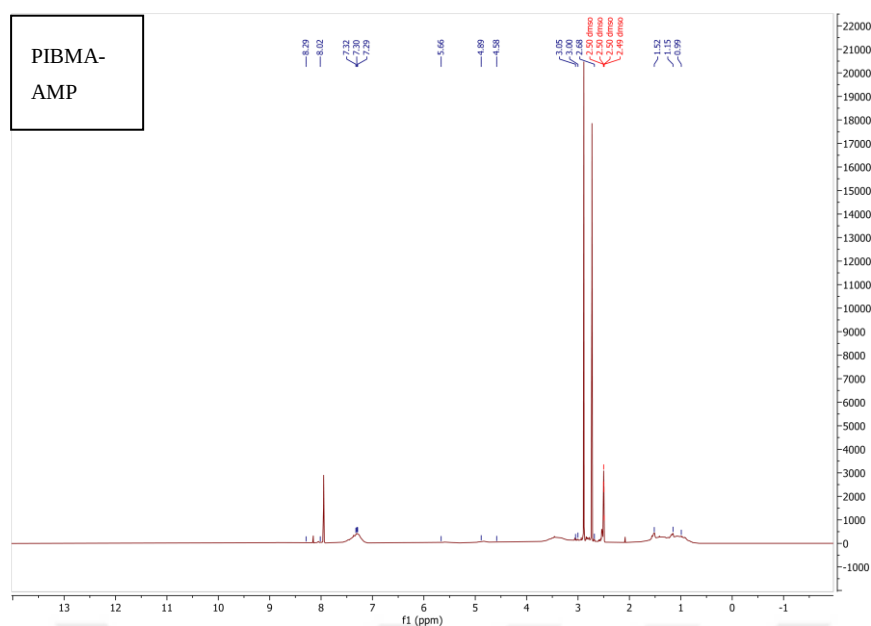


Figure 4.4 ^1H NMR spectrum of PIBMA-AMP DDS

According to ^1H NMR spectrum of PIBMA-AMP PDDS in Figure 4.4.; ^1H NMR (400 MHz, dmso-d_6) δ : 8.26 ppm (1H, N-H), 7.99 ppm (1H, N-H), 7.30-7.26 ppm (5H, C-H), 5.64 ppm (1H, C-H), 4.86 ppm (1H, C-H), 4.56 ppm (1H, C-H), 3.02 ppm (1H, C-H), 2.97 ppm (1H, C-H), 2.65 ppm (1H, C-H), 1.49 ppm (1H, C-H), 1.12 ppm (6H, CH_3), 0.96 ppm (2H, CH_2).

4.3 Structural Characterization of PEMA, AMP, PEMA-AMP Copolymer-Drug Delivery Systems

FTIR/ATR spectra of PEMA, AMP, PEMA-AMP DDS comparatively are given in figure 4.5.

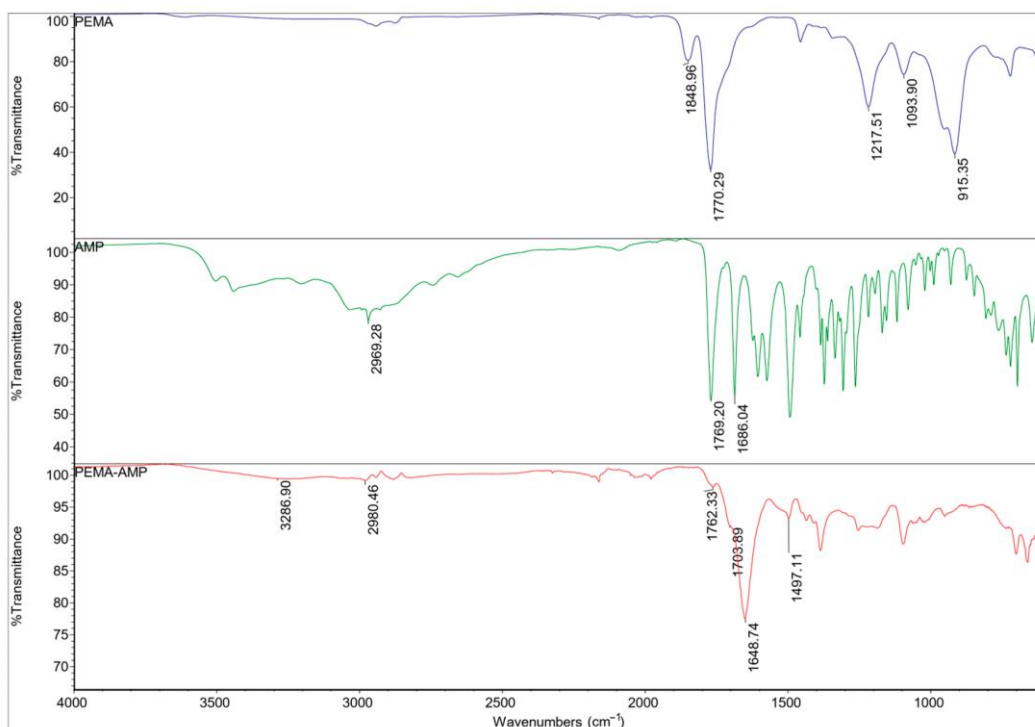


Figure 4.5 FTIR/ATR spectra of PEMA, AMP, PEMA-AMP DDS

According to the FTIR/ATR spectrum of PEMA in Figure 4.5; peaks at $\nu = 1849 \text{ cm}^{-1}$ (C=O stretching-anhydride) and 1770 cm^{-1} (C=O stretching-cyclopentanone), 1218 cm^{-1} (-O-CH₃ stretching), 1094 cm^{-1} (O=C-O-C=O stretching-anhydride), 915 cm^{-1} (-C-H bending) were observed.

According to FTIR/ATR spectrum of PEMA-AMP PDDS in Figure 4.5; peaks at $\nu = 3287 \text{ cm}^{-1}$ (O-H stretching of broad carboxylic acid), alkane at $\nu = 2980 \text{ cm}^{-1}$ (CH₃ (a)symmetric stretching), peaks at $\nu = 1762 \text{ cm}^{-1}$ (C=O group of beta-lactam) and carboxylic acid peak at $\nu = 1704 \text{ cm}^{-1}$ (C=O stretching), 3 substituted alkene peaks at $\nu = 1649 \text{ cm}^{-1}$ (C=C str.), 2° amide (of β -lactam) at $\nu = 1649 \text{ cm}^{-1}$ (C=O stretching), monosubstituted benzene $\nu = 1497 \text{ cm}^{-1}$ (C-C stretching) peaks were observed.

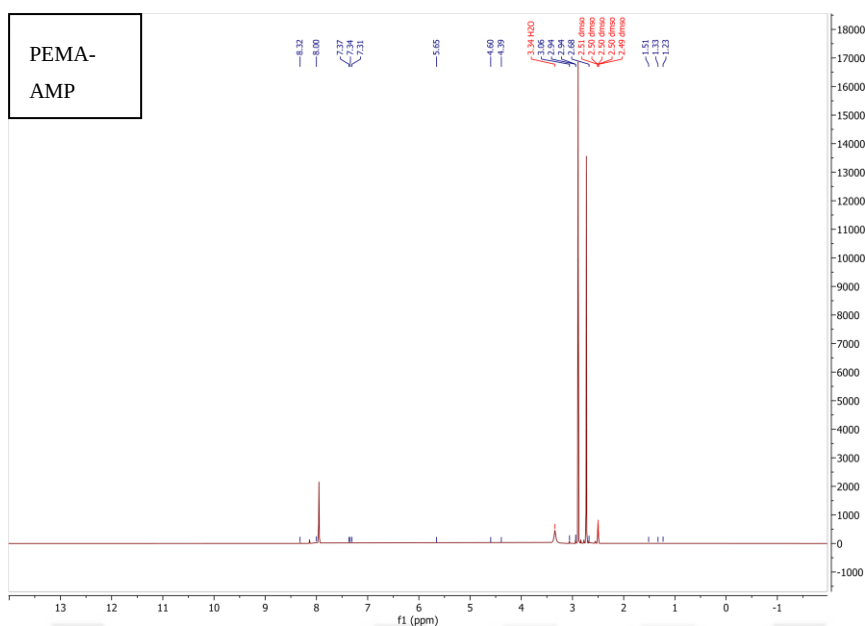


Figure 4.6 ^1H NMR spectrum of PEMA-AMP DDS

According to ^1H NMR spectrum of PEMA-AMP PDDS in Figure 4.6; ^1H NMR (400 MHz, dmso-d_6) δ : 8.32 ppm (1H, N-H), 8.00 ppm (1H, N-H), 7.37-7.31 ppm (5H, C-H), 5.65 ppm (1H, C-H), 4.60 ppm (1H, C-H), 4.39 ppm (1H, C-H), 3.06 ppm (1H, C-H), 2.94 ppm (1H, C-H), 2.68 ppm (1H, C-H), 1.51 ppm (6H, CH_3), 1.33 ppm (1H, C-H), 1.23 ppm (2H, CH_2).

4.4 Structural Characterization of PMVEMA, CEF, PMVEMA-CEF Copolymer-Drug Delivery Systems

Comparative FTIR/ATR spectra of PMVEMA, CEF, PMVEMA-CEF DDS are given in figure 4.7.

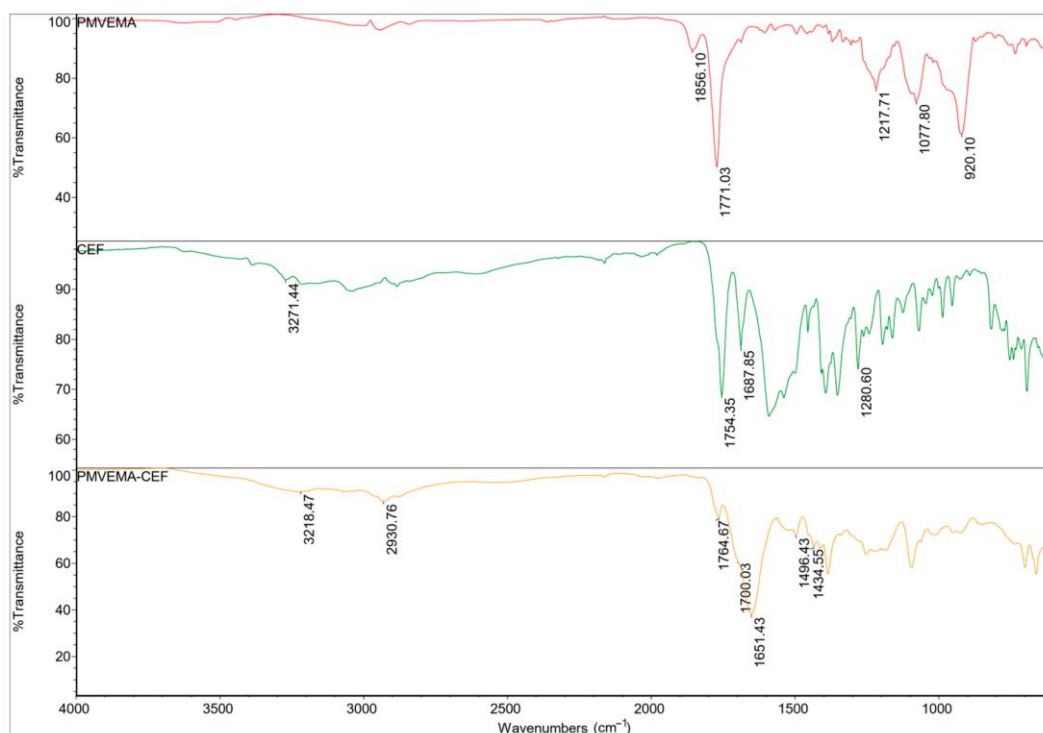


Figure 4.7 FTIR/ATR spectra of PMVEMA-CEF DDS, CEF, PMVEMA

Theoretically, the carbonyl group of the beta-lactam ring in vibration mode should appear around $\nu = 1770 \text{ cm}^{-1}$ [110]. As seen on the FTIR/ATR spectrum of CEF in Figure 4.7.; carbonyl group absorption for cephalexin monohydrate appeared around $\nu = 1764 \text{ cm}^{-1}$ and the peak for side amide link on beta-lactam ring appeared at $\nu = 1687 \text{ cm}^{-1}$.

According to the FTIR/ATR spectrum of PMVEMA-CEF PDDS in Figure 4.7.; peaks at $\nu = 3218 \text{ cm}^{-1}$ (O-H stretching of broad c. acid), alkane at $\nu = 2930 \text{ cm}^{-1}$ (CH_3) symmetric stretching), peaks at $\nu = 1765 \text{ cm}^{-1}$ (C=O group of beta-lactam) and carboxylic acid peak at $\nu = 1700 \text{ cm}^{-1}$ (C=O stretching), 3 substituted alkene peaks at $\nu = 1651 \text{ cm}^{-1}$ (C=C str.), 2° amide (of β -lactam) at $\nu = 1651 \text{ cm}^{-1}$ (C=O stretching), monosubstituted benzene $\nu = 1496 \text{ cm}^{-1}$ (C-C stretching) peaks were observed.

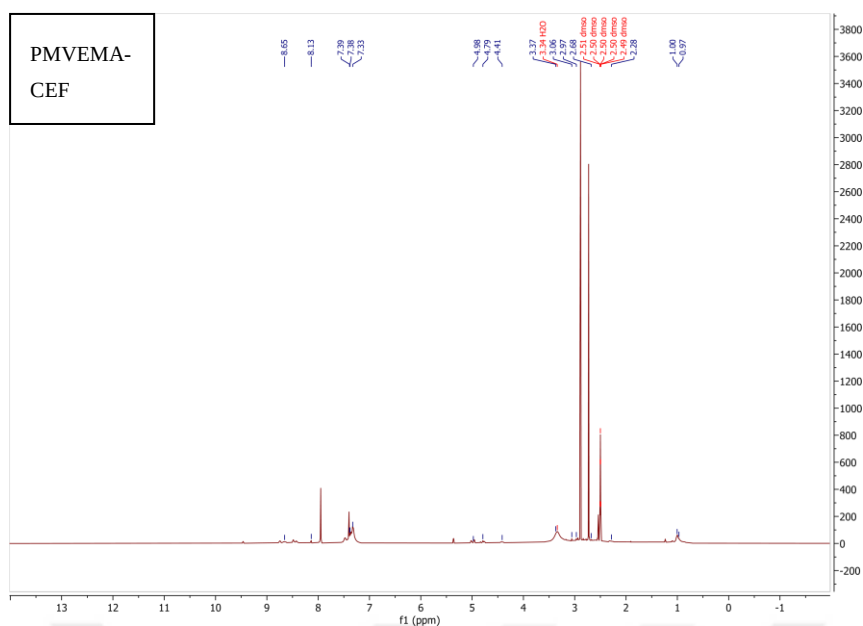


Figure 4.8 ^1H NMR spectrum of PMVEMA-CEF DDS

According to ^1H NMR spectrum of PMVEMA-CEF in Figure 4.8., ^1H NMR (400 MHz, dmso-d_6) δ : 8.65 ppm (1H, N-H), 8.13 ppm (1H, N-H), 7.39-7.33 ppm (5H, C-H), 4.98 ppm (1H, C-H), 4.79 ppm (1H, C-H), 4.41 ppm (1H, C-H), 3.37 ppm (3H, CH_3), 3.06 ppm (1H, C-H), 2.97 ppm (1H, C-H), 2.68 ppm (1H, C-H), 2.28 ppm (3H, CH_3), 1.00 ppm (2H, CH_2), 0.97 ppm (2H, CH_2).

4.5 Structural Characterization of PIBMA, CEF, PIBMA-CEF Copolymer-Drug Delivery Systems

Comparative FTIR/ATR spectra of PIBMA, CEF, PIBMA-CEF DDS are given in figure 4.9.

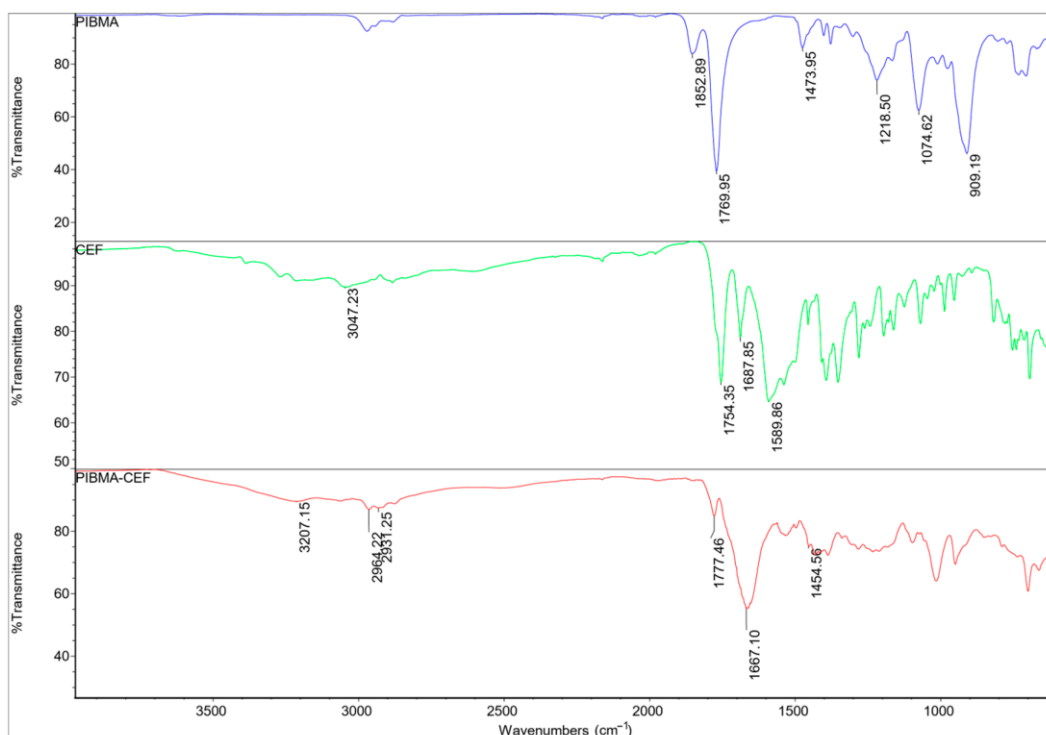


Figure 4.9 FTIR/ATR spectra of PIBMA, CEF, PIBMA-CEF DDS

In the FTIR/ATR spectrum of PIBMA-CEF conjugate in Figure 4.9; broad c. acid at $\nu = 3207 \text{ cm}^{-1}$ (O-H stretching), alkane at $\nu = 2964 \text{ cm}^{-1}$ (CH_3 (a)symmetric stretching), cyclobutane at $\nu = 2931 \text{ cm}^{-1}$ (CH_2 symmetric stretching), beta lactam ring peak $\nu = 1777 \text{ cm}^{-1}$ (C=O stretching), 3 substituted alkene peaks at $\nu = 1667 \text{ cm}^{-1}$ ($\text{C}=\text{C}$ str.), 2° amide (of β -lactam) at $\nu = 1667 \text{ cm}^{-1}$ (C=O stretching), monosubstituted benzene $\nu = 1455 \text{ cm}^{-1}$ (C-C stretching) peaks were observed.

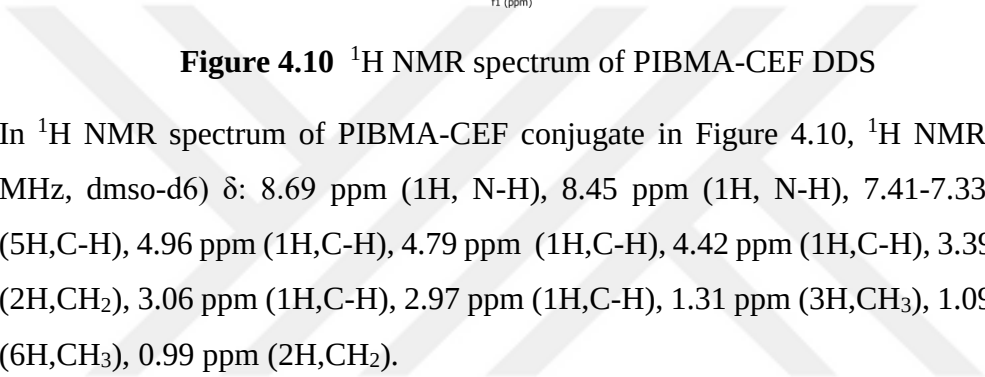


Figure 4.10 ^1H NMR spectrum of PIBMA-CEF DDS

In ^1H NMR spectrum of PIBMA-CEF conjugate in Figure 4.10, ^1H NMR (400 MHz, $\text{dms}\text{-d}_6$) δ : 8.69 ppm (1H, N-H), 8.45 ppm (1H, N-H), 7.41-7.33 ppm (5H, C-H), 4.96 ppm (1H, C-H), 4.79 ppm (1H, C-H), 4.42 ppm (1H, C-H), 3.39 ppm (2H, CH_2), 3.06 ppm (1H, C-H), 2.97 ppm (1H, C-H), 1.31 ppm (3H, CH_3), 1.09 ppm (6H, CH_3), 0.99 ppm (2H, CH_2).

4.6 Structural Characterization of PEMA, CEF, PEMA-CEF Copolymer-Drug Delivery Systems

FTIR/ATR spectra of PEMA, CEF, PEMA-CEF DDS are given in figure 4.11 comparatively.

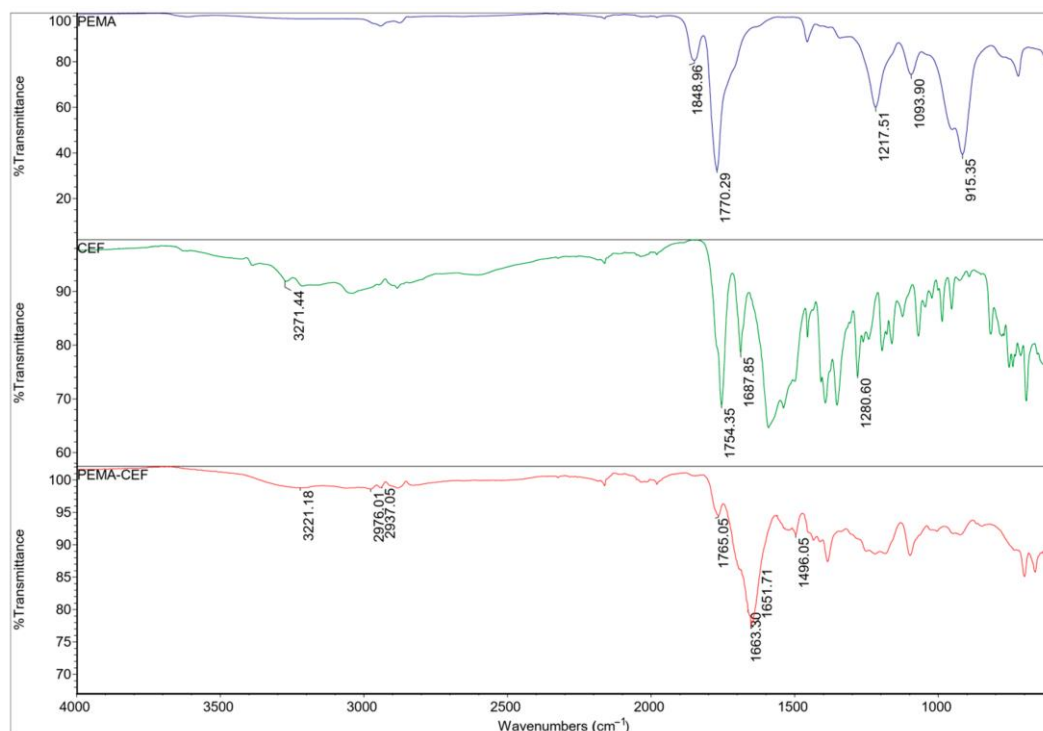


Figure 4.11 FTIR/ATR spectra of PEMA, CEF and PEMA-CEF DDS

In FTIR/ATR spectrum of PEMA-CEF PDDS at Figure 4.11; broad carboxylic acid at $\nu = 3222 \text{ cm}^{-1}$ (O-H stretching), alkane at $\nu = 2976 \text{ cm}^{-1}$ (CH_3 (a)symmetric stretching), cyclobutane at $\nu = 2937 \text{ cm}^{-1}$ CH_2 symmetric stretching), beta-lactam ring peak $\nu = 1765 \text{ cm}^{-1}$ (C=O stretching), 3 substituted alkene peaks at $\nu = 1652 \text{ cm}^{-1}$ (C=C str.), 2° amide (of β -lactam) at $\nu = 1663 \text{ cm}^{-1}$ (C=O stretching), monosubstituted benzene $\nu = 1496 \text{ cm}^{-1}$ (C-C stretching) peaks were observed. Proton NMR spectrum of PEMA-CEF DDS is given in Figure 4.12.

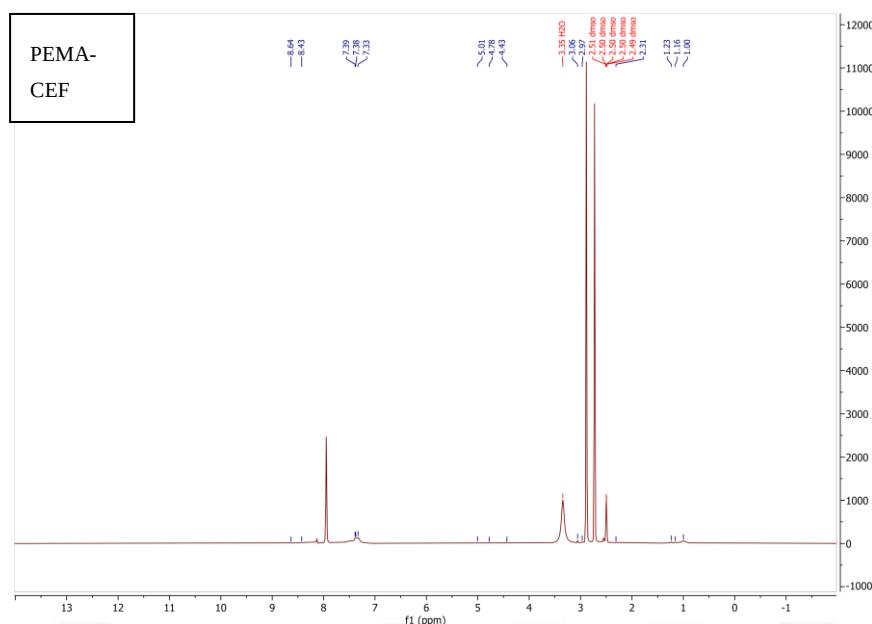


Figure 4.12 ^1H NMR spectrum of PEMA-CEF DDS

In ^1H NMR spectrum of PEMA-CEF PDDS in Figure 4.12; ^1H NMR (400 MHz, $\text{dms}\text{-}d_6$) δ : 8.64 ppm (1H, N-H), 8.43 ppm (1H, N-H), 7.39-7.33 ppm (5H, C-H), 5.01 ppm (1H, C-H), 4.78 ppm (1H, C-H), 4.43 ppm (1H, C-H), 3.06 ppm (1H, C-H), 2.97 ppm (1H, C-H), 2.31 ppm (2H, CH_2), 1.23 ppm (2H, CH_2), 1.16 ppm (3H, CH_3), 1.00 ppm (2H, CH_2).

4.7 Calculation Of Drug Binding Percentage To Copolymers, DP_w and $\overline{M}_w$

Binding ratio of all drugs to copolymer is calculated as the ratio of the integrals of proton of newly formed amide to the proton of a carbon on polymeric backbone.

The ampicillin content in the AMP-conjugated DDSs are calculated by integration of the ^1H -NMR spectra. The signal intensities of newly formed amide and the signal intensity of the proton of the carbon on polymeric backbone are compared and percentage of binding ratio is calculated.

Binding ratio of AMP-drug to PMVEMA copolymer: $(h/l) \times 100 = 67\%$

Binding ratio of AMP-drug to PIBMA copolymer: $(h/l) \times 100 = 72\%$

Binding ratio of AMP drug to PEMA copolymer: $(h/l) \times 100 = 65\%$

The cephalexin content in the CEF-conjugated DDS are calculated by integration of the ^1H -NMR spectra. The signal intensities of newly formed amide and the signal

intensity of the proton of the carbon on polymeric backbone are compared and percentage of binding ratio is calculated.

Binding ratio of CEF-drug to PMVEMA copolymer: $(h/l)*100= 72\%$

Binding ratio of CEF-drug to PIBMA copolymer: $(h/l)*100= 64\%$

Binding ratio of CEF-drug to PEMA copolymer: $(h/l)*100= 79\%$

Molecular weight of the repeating unit for PMVEMA is $156.14 \text{ g.mol}^{-1}$ and average molecular weight of PMVEMA ($\overline{M}_w$) is $216000 \text{ g.mol}^{-1}$.

$$DP_w = \frac{\overline{M}_w}{M_p} = \frac{216000}{156.14} = 1383.37 \quad (4.1)$$

Molecular weight of the repeating unit for PIBMA is $154.17 \text{ g.mol}^{-1}$ and average molecular weight of PIBMA ($\overline{M}_w$) is 6000 g.mol^{-1} .

$$DP_w = \frac{\overline{M}_w}{M_p} = \frac{6000}{154.17} = 38.91 \quad (4.2)$$

Molecular weight of the repeating unit for PEMA is $126.11 \text{ g.mol}^{-1}$ and average molecular weight of PEMA ($\overline{M}_w$) is $100000 \text{ g.mol}^{-1}$.

$$DP_w = \frac{\overline{M}_w}{M_p} = \frac{100000}{126.11} = 792 \quad (4.3)$$

Equation for the calculation of average molecular weight of repeating unit in PMVEMA-AMP DDS is given at equation 4.4. Molecular weight of repeating opened ring unit is $174.15 \text{ g.mol}^{-1}$ and molecular weight of drug conjugated ring unit is $505.54 \text{ g.mol}^{-1}$.

$$M_1 = \frac{(67 \times 505.54) + (33 \times 174.15)}{100} = 396.18 \text{ g/mol} \quad (4.4)$$

Equation for the calculation of average molecular weight of repeating unit in PMVEMA-CEF DDS is given at equation 4.5. Molecular weight of repeating opened ring unit is $174.15 \text{ g.mol}^{-1}$ and molecular weight of drug conjugated ring unit is $503.52 \text{ g.mol}^{-1}$.

$$M_1 = \frac{(72 \times 503.52) + (28 \times 174.15)}{100} = 411.30 \text{ g/mol} \quad (4.5)$$

Equation for the calculation of average molecular weight of repeating unit in PIBMA-AMP DDS is given at equation 4.6. Molecular weight of repeating opened

ring unit is 172.18 g. mol⁻¹ and molecular weight of drug conjugated ring unit is 503.57 g. mol⁻¹.

$$M_1 = \frac{(72 \times 503.57) + (28 \times 172.18)}{100} = 410.78 \text{ g/mol} \quad (4.6)$$

Equation for the calculation of average molecular weight of repeating unit in PIBMA-CEF DDS is given at equation 4.7. Molecular weight of repeating opened ring unit is 172.18 g. mol⁻¹ and molecular weight of drug conjugated ring unit is 501.55 g. mol⁻¹.

$$M_1 = \frac{(64 \times 501.55) + (36 \times 172.18)}{100} = 382.98 \text{ g/mol} \quad (4.7)$$

Equation for the calculation of average molecular weight of repeating unit in PEMA-AMP DDS is given at equation 4.8. Molecular weight of repeating opened ring unit is 144.13 g. mol⁻¹ and molecular weight of drug conjugated ring unit is 475.51 g. mol⁻¹.

$$M_1 = \frac{(65 \times 475.51) + (35 \times 144.13)}{100} = 359.53 \text{ g/mol} \quad (4.8)$$

Equation for the calculation of average molecular weight of repeating unit in PEMA-CEF DDS is given at equation 4.9. Molecular weight of repeating opened ring unit is 144.13 g. mol⁻¹ and molecular weight of drug conjugated ring unit is 473.50 g. mol⁻¹.

$$M_1 = \frac{(79 \times 473.50) + (21 \times 144.13)}{100} = 404.33 \text{ g/mol} \quad (4.9)$$

Since the degree of polymerization has not been changed during the synthesis, new molecular weight of repeating unit of PMVEMA-AMP drug delivery system is calculated with DP_w and $\overline{M}_1$ in equation (4.10)

$$\overline{M}_w = DP_w \times M_1 = 1383.37 \times 396.18 = 548015.67 \text{ g/mol} \quad (4.10)$$

New molecular weight of repeating unit of PMVEMA-CEF drug delivery system is calculated with DP_w and $\overline{M}_1$ in equation (4.11)

$$\overline{M}_w = DP_w \times M_1 = 1383.37 \times 411.30 = 568980.08 \text{ g/mol} \quad (4.11)$$

New molecular weight of repeating unit of PIBMA-AMP drug delivery system is calculated with DP_w and $\overline{M}_1$ in equation (4.12)

$$\overline{M}_w = DP_w \times M_1 = 38.91 \times 410.78 = 15983.45 \text{ g/mol} \quad (4.12)$$

New molecular weight of repeating unit of PIBMA-CEF drug delivery system is calculated with DP_w and $\overline{M}_1$ in equation (4.13)

$$\overline{M}_w = DP_w \times M_1 = 38.91 \times 382.98 = 14901.75 \text{ g/mol} \quad (4.13)$$

New molecular weight of repeating unit of PEMA-AMP drug delivery system is calculated with DP_w and $\overline{M}_1$ in equation (4.14)

$$\overline{M}_w = DP_w \times M_1 = 792 \times 359.53 = 284747.76 \text{ g/mol} \quad (4.14)$$

New molecular weight of repeating unit of PEMA-CEF drug delivery system is calculated with DP_w and $\overline{M}_1$ in equation (4.15)

$$\overline{M}_w = DP_w \times M_1 = 792 \times 404.33 = 320150.16 \text{ g/mol} \quad (4.15)$$

In Table 4.1; drug binding ratios of copolymers and degree of polymerization values are given.

Table 4.1 Percentage of drug binding to copolymers, $\overline{M}_w$ (g/mol), DP_w , M_1 (g/mol) values belong to copolymers and DDSs

Copolymer& DDS	Percentage of drug binding (%)	DP_w	M_1	$\overline{M}_w$
PMVEMA	-	1383.37	-	216000
PMVEMA-AMP	67	-	396.18	548064
PMVEMA-CEF	72	-	411.3	568980
PIBMA	-	38.91	-	6000
PIBMA-AMP	72	-	410.78	15983
PIBMA-CEF	64	-	382.98	14902
PEMA	-	792	-	100000
PEMA-AMP	65	-	359.53	284748
PEMA-CEF	79	-	404.33	320150

The highest binding of AMP to copolymer is obtained with PIBMA-AMP as %72 and for CEF is obtained with PEMA-CEF as %79, as seen in Table 4.1.

4.8 Stability of PMVEMA, PIBMA, PEMA, AMP, AMP Conjugated DDSs at Different pH Values

The stability of drugs, copolymers and drug delivery systems is monitored Zetasizer measurements such as polydispersity index, particle size, mobility and zeta potential. In table 4.2; polydispersity index (PDI) values of AMP, copolymers and AMP-conjugated DDSs are given with changing pH values.

Table 4.2 The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles at the different pH values

pH	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
2	0.32	0.28	0.29	0.32	0.39	0.11	0.35
3	0.34	0.54	0.31	0.34	0.38	0.30	0.32
4	0.30	0.97	0.31	0.31	0.29	0.28	0.33
5	0.31	0.35	0.27	0.30	0.34	0.26	0.33
6	0.67	0.24	0.27	0.31	0.35	0.29	0.32
7	0.33	0.35	0.53	0.36	0.33	0.30	0.30
8	0.31	0.38	0.26	0.32	0.36	0.24	0.44
9	0.33	0.39	0.24	0.34	0.37	0.31	0.36
10	0.31	0.35	0.36	0.33	0.39	0.31	0.38
11	0.31	0.42	0.30	0.34	0.34	0.31	0.34
12	0.33	0.36	0.27	0.36	0.33	0.32	0.42
13	0.34	0.34	0.28	0.30	0.34	0.36	0.36

The polydispersity index belonging to copolymer-drug delivery systems in solution should be as low as possible and the size distribution unimodal for monodispersed [111] . Particles of AMP-conjugated DDSs in this pH range are all monodispersed in this pH range.

Particle size values of drug, copolymers and DDS particles in studied pH ranges are listed in table 4.3.

Table 4.3 The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles at the different pH values

pH	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
2	870	190	440	973	990	672	960
3	850	205	226	741	890	396	765
4	750	245	239	641	300	207	679
5	710	580	193	586	420	196	523
6	685	320	179	533	460	218	506
7	680	640	157	639	460	222	590
8	680	740	186	695	580	288	615
9	670	745	177	695	590	364	670
10	690	765	181	724	660	362	680
11	745	785	215	724	750	486	727
12	900	850	227	735	775	728	738
13	990	885	284	969	800	857	875

At both extremes of studied pH range, particles of drug and drug delivery systems are clustered. Mobility values of AMP, copolymers and AMP-conjugated DDSs with changing pH values are given in Table 4.4.

Table 4.4 The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles at the different pH values

pH	AMP	PMVEMA	PIBMA	PEMA	PMVEMA – AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
2	-0.1	-0.1	-1.0	-0.9	-1.3	-0.2	-0.9
3	-0.2	-0.2	-1.2	-1.5	-1.5	-1.1	-1.0
4	-0.4	-1.2	-1.3	-1.5	-1.9	-1.6	-1.6
5	-0.9	-1.8	-2.0	-2.2	-3.0	-2.3	-1.6
6	-0.5	-3.3	-2.6	-2.0	-3.5	-2.3	-1.9
7	-1.0	-2.3	-1.9	-2.0	-3.6	-2.1	-2.0
8	-1.1	-4.1	-2.7	-1.9	-2.7	-2.1	-2.1
9	-1.2	-4.3	-1.8	-1.8	-2.6	-1.4	-2.0
10	-1.2	-3.6	-1.6	-1.7	-2.6	-2.2	-2.0
11	-1.4	-3.1	-1.5	-1.7	-2.4	-2.0	-1.8
12	-2.3	-3.0	-1.5	-1.5	-2.2	-1.5	-1.8
13	-1.5	-2.4	-1.0	-1.4	-1.9	-1.4	-1.7

Absolute mobility values of DDSs are higher than the mobility values of AMP and all particles are negatively charged in table 4.4.

Zeta potential values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS are given in table 4.5.

Table 4.5 The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS at the different pH values

pH	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
2	-2.1	-1.3	-11.0	-9.3	-13.7	-10.9	-9.9
3	-2.4	-1.9	-12.6	-15.8	-15.9	-11.9	-10.1
4	-4.5	-12.6	-13.7	-16.2	-20.0	-17.8	-16.5
5	-9.8	-19.1	-20.7	-24.2	-32.1	-25.2	-17.3
6	-5.1	-34.0	-26.4	-21.8	-36.8	-25.2	-20.0
7	-10.5	-23.4	-19.8	-21.3	-38.5	-22.9	-21.2
8	-11.9	-44.4	-27.6	-20.5	-29.0	-22.8	-22.2
9	-12.6	-44.2	-18.6	-19.5	-27.8	-15.6	-21.5
10	-12.6	-38.6	-17.0	-18.5	-27.7	-23.8	-21.4
11	-14.4	-33.9	-16.1	-17.9	-25.8	-21.4	-19.4
12	-24.2	-32.9	-15.6	-16.2	-23.8	-16.7	-19.6
13	-15.5	-25.6	-10.5	-14.6	-20.6	-14.9	-18.4

In table 4.5; zeta potential values of copolymers and DDSs become larger regardless of charge and shows more stability in the middle of pH range, whereas drug is way more stable in basic conditions. It is understood that zeta potential of AMP increases conjugation with maleic anhydride copolymers and as a result that the stability of AMP increases in studied pH ranges.

4.9 Stability of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated DDSs at Different pH Values

In table 4.6; polydispersity index values of CEF, copolymers and CEF-conjugated copolymers at different pH values are given.

Table 4.6 The PDI values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles at the different pH values.

pH	CEF	PMVEMA	PIBMA	PEMA	PMVEMA-CEF DDS	PIBMA-CEF DDS	PEMA-CEF DDS
2	0.34	0.28	0.29	0.32	0.33	0.31	0.30
3	0.35	0.54	0.31	0.34	0.33	0.33	0.34
4	0.32	0.97	0.31	0.31	0.33	0.33	0.30
5	0.33	0.35	0.27	0.30	0.35	0.36	0.30
6	0.37	0.24	0.27	0.31	0.37	0.35	0.37
7	0.41	0.35	0.53	0.36	0.35	0.33	0.32
8	0.34	0.38	0.26	0.32	0.45	0.32	0.36
9	0.60	0.39	0.24	0.34	0.42	0.34	0.32
10	0.36	0.35	0.36	0.33	0.36	0.33	0.36
11	0.36	0.42	0.30	0.34	0.33	0.35	0.37
12	0.32	0.36	0.27	0.36	0.35	0.31	0.67
13	0.32	0.34	0.28	0.30	0.39	0.34	0.46

As far as polydispersity values of these particles in table 4.6 are concerned, all the particles are monodispersed. In table 4.7; particle sizes of the CEF, copolymers and CEF-conjugated copolymers at different pH values are given.

Table 4.7 The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles at the different pH values.

pH	CEF	PMVEMA	PIBMA	PEMA	PMVEMA-CEF DDS	PIBMA-CEF DDS	PEMA-CEF DDS
2	560	190	440	973	756	767	851
3	485	205	226	741	494	708	764
4	490	245	239	641	85	657	530
5	668	580	193	586	105	627	566
6	867	320	179	533	110	541	577
7	868	640	157	639	130	544	582
8	831	740	186	695	379	571	585
9	763	745	177	695	394	580	620
10	776	765	181	724	740	587	698
11	627	785	215	724	780	651	741
12	577	850	227	735	865	722	795
13	571	885	284	969	874	843	878

According to the table 4.7; particles of drug are accumulated in the middle of pH range, whereas; particles of DDSs are dispersed in solution. DDSs show aggregation of particles at both extremes of pH range. In table 4.8; mobility values of CEF, copolymers and CEF-conjugated copolymers at different pH values are given.

Table 4.8 The mobility values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles at the different pH values

pH	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
2	-0.5	-0.1	-1.0	-0.9	-1.3	-0.9	-1.1
3	-0.8	-0.2	-1.2	-1.5	-1.4	-1.5	-1.3
4	-1.7	-1.2	-1.3	-1.5	-1.4	-1.7	-1.4
5	-1.8	-1.8	-2.0	-2.2	-2.3	-1.9	-1.6
6	-1.8	-3.3	-2.6	-2.0	-2.1	-2.3	-2.0
7	-1.5	-2.3	-1.9	-2.0	-2.1	-1.9	-2.1
8	-1.5	-4.1	-2.7	-1.9	-2.1	-1.7	-2.1
9	-2.0	-4.3	-1.8	-1.8	-2.0	-1.6	-1.9
10	-1.9	-3.6	-1.6	-1.7	-1.8	-1.6	-1.7
11	-2.0	-3.1	-1.5	-1.7	-1.8	-1.6	-1.5
12	-1.7	-3.0	-1.5	-1.5	-1.7	-1.6	-1.2
13	-1.5	-2.4	-1.0	-1.4	-1.4	-1.2	-1.0

Absolute mobility values of all particles are higher in intermediate pH values and all particles are negatively charged in Table 4.8. In table 4.9; zeta potential values of particles of CEF, copolymers and CEF-conjugated copolymers at different pH values are given.

Table 4.9 The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS at the different pH values

pH	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
2	-4.8	-1.3	-11.0	-9.3	-14.1	-10.2	-11.9
3	-8.5	-1.9	-12.6	-15.8	-14.6	-16.4	-13.4
4	-18.9	-12.6	-13.7	-16.2	-14.9	-17.9	-14.9
5	-19.4	-19.1	-20.7	-24.2	-24.3	-20.5	-17.2
6	-19.7	-34.0	-26.4	-21.8	-22.7	-24.2	-20.9
7	-16.2	-23.4	-19.8	-21.3	-22.4	-20.5	-22.4
8	-16.7	-44.4	-27.6	-20.5	-22.3	-17.9	-21.9
9	-21.2	-44.2	-18.6	-19.5	-21.2	-17.7	-19.7
10	-20.1	-38.6	-17.0	-18.5	-19.4	-17.6	-18.2
11	-21.9	-33.9	-16.1	-17.9	-19.2	-17.4	-16.2
12	-18.2	-32.9	-15.6	-16.2	-17.7	-16.9	-12.3
13	-16.0	-25.6	-10.5	-14.6	-14.8	-12.9	-10.8

In table 4.9; zeta potential values of all the particles, regardless of charge, shrinks in the very low pH values and shows less stability in the very high pH values.

4.10 Temporal In Vitro Stability Tests of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In Simulated Body Fluids

4.10.1 Zetasizer Measurements of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In 5% Dextrose Solution

In table 4.10; polydispersity index values of AMP, copolymers and AMP-conjugated DDSs in 5% dextrose solution are given.

Table 4.10 The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 5% Dextrose solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	0.28	0.24	0.32	0.38	0.34	0.35	0.42
15	0.28	0.20	0.42	0.38	0.32	0.34	0.53
30	0.27	0.15	0.37	0.38	0.33	0.33	0.39
60	0.27	0.21	0.39	0.37	0.35	0.34	0.37
120	0.27	0.44	0.41	0.33	0.33	0.33	0.37
180	0.27	0.36	0.35	0.33	0.42	0.34	0.36
1440(1d)	0.35	0.37	0.91	0.43	0.40	0.34	0.33
2880(2d)	0.34	0.48	0.37	0.34	0.38	0.34	0.36
4320(3d)	0.32	0.34	0.39	0.47	0.39	0.33	0.45
10080(1w)	0.27	0.39	0.32	0.35	0.38	0.34	0.44
20160(2w)	0.34	0.55	0.35	0.48	0.37	0.34	0.31
30240(3w)	0.35	0.88	0.33	0.36	0.38	0.33	0.42

According to Table 4.10; all particles are monodispersed, there are homogeneity in sample solutions. The particle size values of AMP, PMVEMA, PIBMA, PEMA and AMP conjugated copolymers in 5% dextrose solution are given in Table 4.11.

Table 4.11 The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 5% Dextrose solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	550	100	476	508	345	300	603
15	570	145	552	646	365	330	620
30	515	220	613	509	385	335	626
60	520	360	781	582	390	335	640
120	530	670	820	598	435	335	647
180	530	675	904	665	490	340	680
1440(1d)	590	800	961	761	500	400	684
2880(2d)	1010	994	974	760	500	440	654
4320(3d)	1020	1070	997	760	525	515	703
10080(1w)	1520	1200	1179	713	550	495	784
20160(2w)	1550	1360	1247	750	590	465	792
30240(3w)	1570	1365	1325	782	610	440	853

Table 4.11 indicates that particles of AMP, PMVEMA, PIBMA form clusters whereas DDSs aggregated very little at the end of the 3-week period. The average particle size of DDSs is smaller than pure AMP particles. In table 4.12; mobility values of AMP, copolymers and AMP-conjugated DDSs in 5% dextrose solution are given.

Table 4.12 The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 5% Dextrose solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	-2.8	-1.8	-1.8	-2.6	-2.6	-2.6	-2.3
15	-2.5	-2.1	-1.8	-2.7	-2.6	-2.1	-2.3
30	-2.4	-2.0	-1.7	-2.9	-2.3	-2.0	-2.2
60	-2.3	-2.2	-1.7	-2.9	-2.2	-1.8	-2.1
120	-2.2	-2.2	-1.7	-2.8	-2.0	-1.8	-2.1
180	-2.1	-1.6	-1.7	-2.4	-1.9	-1.8	-2.1
1440(1d)	-2.1	-1.6	-1.5	-2.3	-2.0	-1.8	-2.0
2880(2d)	-1.0	-2.2	-1.0	-2.0	-2.0	-1.8	-1.9
4320(3d)	-0.6	-2.1	-0.8	-2.0	-2.0	-1.6	-2.0
10080(1w)	-0.3	-2.1	-0.9	-1.9	-1.8	-1.2	-1.7
20160(2w)	-0.3	-1.9	-0.7	-2.1	-1.7	-1.0	-1.4
30240(3w)	-0.3	-1.8	-0.7	-1.7	-1.7	-0.9	-1.5

The absolute average mobility values of DDSs are greater than the values of drug. All particles are negatively charged in table 4.12. After second day, the particles of drug become almost immobile that is explained as the formation of coagulation of particles. Zeta potential values of copolymers, AMP and AMP-conjugated DDSs in 5% Dextrose are given in Table 4.13.

Table 4.13 The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS in 5% Dextrose solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	-30.5	-18.4	-13.5	-27.9	-28.2	-27.2	-24.5
15	-26.6	-21.3	-14.1	-28.7	-28.0	-22.0	-24.0
30	-26.2	-21.6	-18.0	-31.4	-24.8	-21.4	-23.7
60	-24.4	-23.0	-18.3	-30.9	-24.3	-19.0	-22.8
120	-23.4	-23.5	-18.2	-29.8	-21.9	-19.2	-22.6
180	-22.8	-17.3	-18.6	-25.9	-20.8	-19.1	-22.5
1440(1d)	-22.5	-16.7	-15.9	-24.6	-21.7	-19.6	-21.5
2880(2d)	-11.3	-23.9	-10.4	-21.1	-21.2	-18.7	-20.3
4320(3d)	-7.5	-23.3	-8.8	-20.8	-21.1	-17.4	-20.9
10080(1w)	-3.6	-23.0	-9.9	-20.6	-19.4	-12.7	-18.6
20160(2w)	-3.3	-20.2	-7.8	-22.3	-18.0	-10.5	-15.3
30240(3w)	-3.1	-19.9	-7.3	-19.6	-17.6	-9.2	-15.4

In table 4.13; zeta potential values regardless of sign, show a decreasing trend for drug and DDSs. Very low zeta potential values of drug have been compensated by copolymer conjugation after 2nd day. DDSs show stability for at least for two week period.

4.10.2 Zetasizer Measurements of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In PBS Solution

In table 4.14; polydispersity index values of AMP, copolymers and AMP-conjugated DDSs in PBS solution are given.

Table 4.14 The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in PBS solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	0.32	0.29	0.36	0.39	0.40	0.38	0.33
15	0.36	0.26	0.34	0.27	0.45	0.47	0.45
30	0.43	0.31	0.29	0.30	0.43	0.33	0.36
60	0.32	0.29	0.36	0.25	0.36	0.31	0.37
120	0.31	0.29	0.34	0.26	0.37	0.32	0.39
180	0.35	0.29	0.84	0.20	0.33	0.34	0.36
1440(1d)	0.32	0.28	0.97	0.26	0.39	0.37	0.41
2880(2d)	0.29	0.28	0.36	0.20	0.53	0.33	0.36
4320(3d)	0.33	0.31	0.78	0.23	0.43	0.32	0.42
10080(1w)	0.39	0.28	0.39	0.23	0.33	0.32	0.33
20160(2w)	0.32	0.26	0.35	0.24	0.43	0.36	0.33
30240(3w)	0.33	0.29	0.37	0.28	0.36	0.31	0.29

According to table 4.14; all particles are monodispersed and homogenously dispersed in sample solutions. In table 4.15; particle size values of AMP, copolymers and AMP-conjugated DDSs in PBS solution are given.

Table 4.15 The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in PBS solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	610	490	180	294	683	789	742
15	640	433	180	348	688	837	754
30	790	411	185	343	746	970	773
60	810	405	200	333	756	868	783
120	825	390	210	333	758	860	795
180	840	375	210	343	778	866	797
1440(1d)	860	359	805	315	833	701	806
2880(2d)	840	342	840	339	845	711	868
4320(3d)	800	325	860	322	916	703	881
10080(1w)	765	332	890	337	925	825	905
20160(2w)	740	320	900	394	932	945	918
30240(3w)	725	292	920	385	963	992	957

Table 4.15 indicates that size of particles of DDSs exhibit formation of clusters over 3 week period. However, clusters of particles of AMP accumulate and particle size of the solutions become smaller. In table 4.16; mobility values values of AMP, copolymers and AMP-conjugated DDSs in PBS solution are given.

Table 4.16 The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in PBS solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	-1.4	-1.5	-1.3	-2.5	-2.9	-1.6	-3.1
15	-1.4	-2.5	-1.6	-2.2	-2.5	-1.9	-3.1
30	-1.5	-2.5	-1.5	-2.3	-2.2	-1.8	-2.8
60	-1.4	-2.4	-2.2	-2.7	-2.1	-1.7	-2.6
120	-1.7	-3.2	-2.1	-2.2	-2.0	-1.3	-2.5
180	-1.7	-2.4	-1.8	-2.6	-1.8	-1.3	-2.5
1440(1d)	-1.4	-3.1	-1.9	-2.9	-1.8	-1.3	-2.5
2880(2d)	-1.2	-2.8	-1.5	-2.9	-1.7	-1.3	-2.4
4320(3d)	-1.3	-2.5	-2.9	-2.7	-1.7	-1.3	-2.1
10080(1w)	-0.8	-2.3	-1.5	-2.8	-1.5	-1.2	-2.1
20160(2w)	-0.7	-2.2	-1.4	-2.4	-1.5	-1.2	-2.1
30240(3w)	-0.7	-2.2	-1.5	-2.7	-1.4	-1.2	-1.9

According to table 4.16; the average absolute mobility values of DDSs are much higher than the values of drug. All the particles in the table are negatively charged. Zeta potential values of AMP, PMVEMA, PIBMA, PEMA and AMP-conjugated DDSs in PBS solution is given in Table 4.17.

Table 4.17 The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS in PBS solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	-14.8	-15.6	-13.1	-27.0	-31.3	-16.8	-32.5
15	-14.8	-25.6	-16.2	-22.4	-27.1	-20.3	-32.7
30	-15.8	-26.0	-15.1	-24.7	-23.0	-19.7	-29.4
60	-15.5	-25.0	-22.9	-27.4	-22.7	-18.6	-27.2
120	-18.3	-33.4	-22.0	-22.7	-21.2	-14.0	-26.3
180	-18.7	-24.9	-19.1	-27.2	-19.5	-14.0	-26.6
1440(1d)	-14.8	-31.9	-19.5	-30.3	-18.6	-14.4	-26.3
2880(2d)	-12.7	-29.1	-15.6	-30.2	-18.0	-13.7	-25.3
4320(3d)	-13.9	-25.7	-29.7	-28.4	-17.7	-13.8	-22.7
10080(1w)	-8.3	-23.4	-15.6	-28.9	-15.6	-13.6	-22.5
20160(2w)	-7.5	-23.1	-14.5	-25.1	-15.4	-12.6	-22.2
30240(3w)	-8.0	-23.1	-15.6	-27.6	-14.5	-12.5	-20.5

In table 4.17; zeta potential values of drug and DDSs regardless of sign, show a decreasing trend over 3-week period. Very low zeta potential values of drug have been compensated by copolymer conjugation after 2nd day. Zeta potential values of all DDSs regardless of sign, show stability in 3-week period.

4.10.3 Zetasizer Measurements of AMP, PMVEMA, PIBMA, PEMA, AMP-Conjugated Copolymer-Drug Delivery Systems In 0.9% Isotonic NaCl Solution

In table 4.18.; polydispersity index values of AMP, copolymers and AMP-conjugated DDSs in 0.9% NaCl solution are given.

Table 4.18 The PDI values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 0.9% NaCl solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	0.52	0.26	0.31	0.25	0.26	0.18	0.45
15	0.35	0.28	0.32	0.26	0.23	0.20	0.34
30	0.35	0.41	0.29	0.22	0.28	0.18	0.33
60	0.35	0.27	0.28	0.34	0.30	0.25	0.31
120	0.35	0.33	0.30	0.54	0.29	0.21	0.33
180	0.31	0.39	0.28	0.74	0.29	0.22	0.36
1440(1d)	0.57	0.31	0.30	0.33	0.37	0.23	0.33
2880(2d)	1.10	0.33	0.32	0.68	0.30	0.23	0.39
4320(3d)	0.77	0.32	0.48	0.38	0.35	0.24	0.38
10080(1w)	0.43	0.32	0.32	0.34	0.36	0.32	0.32
20160(2w)	0.33	0.31	0.35	0.35	0.34	0.42	0.13
30240(3w)	0.33	0.26	0.37	0.26	0.35	0.33	0.40

According to table 4.18; all particles of DDSs in sample solutions are monodispersed and more homogenously dispersed than the particles of drug itself. The particle size values of AMP, copolymers and AMP–conjugated DDSs in 0.9% isotonic NaCl solution is given in Table 4.19.

Table 4.19 The particle size values (nm) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 0.9% NaCl solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	520	330	352	110	281	313	545
15	555	315	364	130	285	295	550
30	545	220	412	174	293	300	551
60	582	250	410	367	316	357	553
120	561	290	392	491	320	336	575
180	550	260	386	513	332	347	582
1440(1d)	528	265	413	569	338	341	593
2880(2d)	591	235	427	540	341	372	622
4320(3d)	570	275	439	534	357	392	656
10080(1w)	855	330	823	503	518	715	668
20160(2w)	846	335	882	723	703	830	709
30240(3w)	748	365	960	864	746	887	856

Table 4.19 indicates that particles of drug and DDSs form clusters at the end of the 3-week period. Clusters of particles of AMP aggregate and size of the particles get smaller after 1st week. In table 4.20; mobility values of AMP, copolymers and AMP-conjugated DDSs in 0.9% NaCl solution are given.

Table 4.20 The mobility values of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS particles in 0.9% NaCl solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	-0.9	-2.6	-2.3	-2.7	-2.4	-1.4	-2.5
15	-0.9	-2.4	-2.0	-2.2	-2.4	-1.3	-2.4
30	-1.3	-2.4	-1.8	-2.3	-2.2	-1.7	-2.4
60	-1.3	-2.1	-1.7	-2.6	-2.2	-1.7	-2.3
120	-1.3	-2.1	-1.8	-2.3	-2.0	-1.9	-2.2
180	-1.2	-2.0	-1.8	-2.0	-2.0	-1.4	-2.1
1440(1d)	-1.0	-1.6	-2.2	-1.8	-2.0	-1.3	-2.1
2880(2d)	-0.9	-1.5	-1.8	-1.9	-1.9	-1.1	-2.0
4320(3d)	-0.9	-1.5	-1.6	-1.6	-1.8	-0.9	-1.9
10080(1w)	-0.9	-1.4	-1.2	-1.6	-1.6	-1.0	-1.9
20160(2w)	-0.8	-1.4	-1.3	-1.4	-1.5	-1.0	-1.7
30240(3w)	-0.6	-0.9	-1.2	-1.3	-1.0	-0.9	-1.6

Mobility of particles of drug, copolymers and DDSs are all negatively charged, and particles of DDSs are more mobile than the particles of drug in 0.9% NaCl solution, in table 4.20. In Table 4.21; time-dependant zeta potential values in 0.9% isotonic NaCl solution of AMP, copolymers and AMP-conjugated DDSs are given.

Table 4.21 The zeta potential values (mV) of AMP, PMVEMA, PIBMA, PEMA and PMVEMA-AMP DDS, PIBMA-AMP DDS and PEMA-AMP DDS in 0.9% NaCl solution

Time (min)	AMP	PMVEMA	PIBMA	PEMA	PMVEMA-AMP DDS	PIBMA-AMP DDS	PEMA-AMP DDS
0	-9.6	-27.9	-23.7	-27.8	-25.6	-15.6	-27.1
15	-10.1	-26.3	-20.6	-22.7	-25.3	-14.4	-25.3
30	-15.1	-25.4	-18.6	-24.0	-23.6	-17.9	-25.1
60	-15.4	-23.0	-17.6	-26.4	-23.1	-18.3	-24.1
120	-14.9	-22.8	-19.1	-23.9	-21.7	-20.3	-23.3
180	-14.1	-20.6	-18.7	-20.4	-21.2	-15.0	-22.9
1440(1d)	-10.6	-17.3	-22.2	-18.5	-21.0	-14.1	-22.1
2880(2d)	-9.5	-16.0	-18.5	-19.2	-20.1	-12.4	-21.3
4320(3d)	-9.6	-15.8	-18.1	-16.4	-19.2	-10.1	-20.3
10080(1w)	-9.8	-15.3	-14.1	-16.5	-17.7	-11.3	-19.8
20160(2w)	-8.2	-14.6	-13.9	-16.6	-15.8	-10.8	-18.3
30240(3w)	-6.6	-10.7	-12.4	-16.3	-10.9	-10.1	-16.9

In table 4.21; zeta potential values of all regardless of sign, show a decreasing trend in general. Very low zeta potential values of drug have been compensated and stabilized by copolymer conjugation especially after 2nd day.

4.11 Temporal In Vitro Stability Tests of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In Simulated Body Fluids

4.11.1 Zetasizer Measurements of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In 5% Dextrose Solution

In table 4.22; polydispersity index values of CEF, copolymers and CEF-conjugated DDSs in 5% dextrose solution are given.

Table 4.22 The PDI values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 5% Dextrose solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA-CEF DDS	PIBMA-CEF DDS	PEMA-CEF DDS
0	0.43	0.24	0.32	0.38	0.39	0.37	0.35
15	0.61	0.20	0.42	0.38	0.35	0.34	0.33
30	0.39	0.15	0.37	0.38	0.38	0.33	0.36
60	0.38	0.21	0.39	0.37	0.36	0.38	0.36
120	0.43	0.44	0.41	0.33	0.39	0.39	0.43
180	0.47	0.36	0.35	0.33	0.39	0.32	0.36
1440(1d)	0.43	0.37	0.91	0.43	0.37	0.38	0.31
2880(2d)	0.33	0.48	0.37	0.34	0.37	0.37	0.33
4320(3d)	0.34	0.34	0.39	0.47	0.43	0.35	0.35
10080(1w)	0.34	0.39	0.32	0.35	0.40	0.56	0.43
20160(2w)	0.60	0.55	0.35	0.48	0.42	0.47	0.29
30240(3w)	0.35	0.88	0.33	0.36	0.38	0.47	0.32

According to table 4.22; all particles are monodispersed in sample solutions. The particle size values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in 5% Dextrose solutions are given in Table 4.23.

Table 4.23 The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 5% Dextrose solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA-CEF DDS	PIBMA-CEF DDS	PEMA-CEF DDS
0	773	100	476	508	542	333	678
15	783	145	552	646	558	340	621
30	921	220	613	509	607	356	611
60	1055	360	781	582	660	411	661
120	1038	670	820	598	692	534	627
180	1046	675	904	665	714	680	617
1440(1d)	1156	800	961	761	786	759	622
2880(2d)	1347	994	974	760	801	786	624
4320(3d)	1371	1070	997	760	843	793	633
10080(1w)	1396	1200	1179	713	850	905	645
20160(2w)	1379	1360	1247	750	852	906	674
30240(3w)	1469	1365	1325	782	864	949	748

Table 4.23 indicates that particles of copolymers, drug and DDSs form clusters at the end of the 3-week period. Drug forms micron-sized clusters which makes travelling in the body more difficult after 30 minutes. The mobility values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in 5% Dextrose solutions are given in Table 4.24.

Table 4.24 The mobility values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 5% Dextrose solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	-0.7	-1.8	-1.8	-2.6	-1.8	-1.7	-2.2
15	-0.9	-2.1	-1.8	-2.7	-1.8	-1.6	-2.2
30	-1.0	-2.0	-1.7	-2.9	-1.7	-1.5	-2.1
60	-0.8	-2.2	-1.7	-2.9	-1.8	-1.4	-2.5
120	-1.1	-2.2	-1.7	-2.8	-1.9	-1.4	-2.2
180	-1.3	-1.6	-1.7	-2.4	-1.9	-1.3	-2.0
1440(1d)	-1.0	-1.6	-1.5	-2.3	-1.8	-1.2	-2.0
2880(2d)	-0.9	-2.2	-1.0	-2.0	-1.7	-1.0	-1.8
4320(3d)	-0.6	-2.1	-0.8	-2.0	-1.6	-1.0	-1.7
10080(1w)	-0.3	-2.1	-0.9	-1.9	-1.5	-1.0	-1.5
20160(2w)	-0.3	-1.9	-0.7	-2.1	-1.3	-1.0	-1.4
30240(3w)	-0.2	-1.8	-0.7	-1.7	-1.3	-0.9	-1.3

According to mobility measurements in table 4.24; particles of all drug, copolymers and DDSs are negatively charged. Drug become almost immobile after third day, however; the DDSs are still mobile at the end of the 3-week period. Zeta potential values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in 5% Dextrose is given in Table 4.25.

Table 4.25 The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS in 5% Dextrose solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	-7.9	-18.4	-13.5	-27.9	-19.0	-18.7	-23.6
15	-9.8	-21.3	-14.1	-28.7	-18.8	-17.4	-23.3
30	-10.6	-21.6	-18.0	-31.4	-18.3	-16.0	-22.4
60	-8.3	-23.0	-18.3	-30.9	-18.6	-15.4	-26.3
120	-12.2	-23.5	-18.2	-29.8	-19.6	-14.8	-23.2
180	-14.3	-17.3	-18.6	-25.9	-20.3	-13.7	-20.9
1440(1d)	-10.6	-16.7	-15.9	-24.6	-19.4	-12.3	-20.9
2880(2d)	-9.9	-23.9	-10.4	-21.1	-17.6	-11.9	-19.1
4320(3d)	-6.1	-23.3	-8.8	-20.8	-16.3	-10.9	-17.9
10080(1w)	-3.2	-23.0	-9.9	-20.6	-15.8	-10.7	-16.2
20160(2w)	-2.7	-20.2	-7.8	-22.3	-14.0	-10.4	-15.4
30240(3w)	-2.5	-19.9	-7.3	-19.6	-14.1	-9.1	-14.3

In table 4.25; zeta potential values of drug and DDSs, regardless of sign, show a decreasing trend. Instability of drug have been compensated by copolymer conjugation after 1st day. This decreasing trend in absolute zeta potential can be easily seen in Figure 4.13.

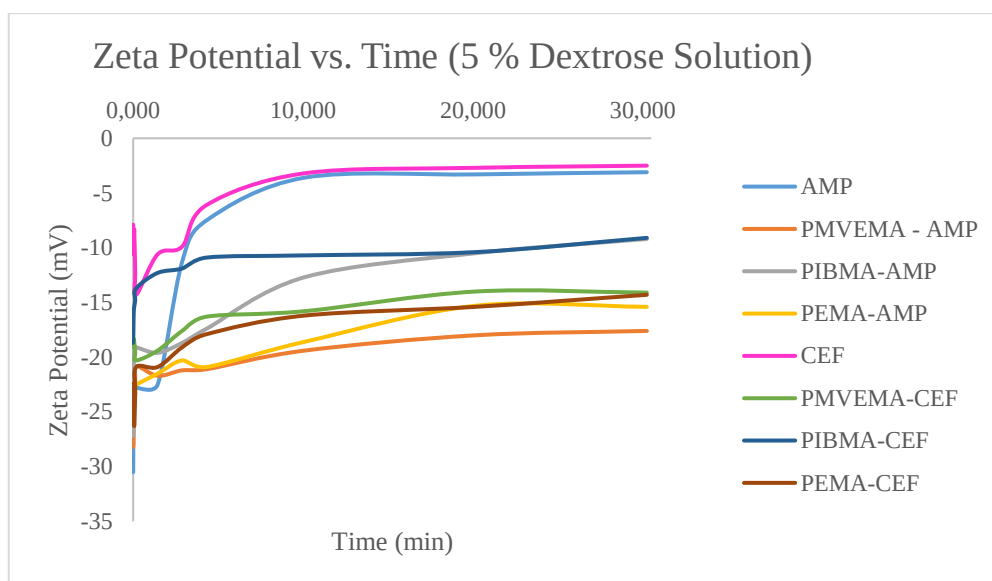


Figure 4.13 Zeta Potential vs. Time (5% Dextrose Solution)

4.11.2 Zetasizer Measurements of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In PBS Solution

In table 4.26; polydispersity index values of CEF, copolymers and CEF-conjugated DDSs in PBS solution are given.

Table 4.26 The PDI of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in PBS solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA-CEF DDS	PIBMA-CEF DDS	PEMA-CEF DDS
0	0.37	0.29	0.36	0.39	0.35	0.41	0.34
15	0.31	0.26	0.34	0.27	0.33	0.38	0.30
30	0.33	0.31	0.29	0.30	0.36	0.57	0.38
60	0.30	0.29	0.36	0.25	0.33	0.42	0.36
120	0.34	0.29	0.34	0.26	0.38	0.35	0.31
180	0.37	0.29	0.84	0.20	0.39	0.34	0.39
1440(1d)	0.32	0.28	0.97	0.26	0.34	0.35	0.35
2880(2d)	0.38	0.28	0.36	0.20	0.29	0.35	0.36
4320(3d)	0.34	0.31	0.78	0.23	0.37	0.41	0.35
10080(1w)	0.28	0.28	0.39	0.23	0.34	0.42	0.26
20160(2w)	0.33	0.26	0.35	0.24	0.34	0.39	0.27
30240(3w)	0.30	0.29	0.37	0.28	0.43	0.41	0.24

In table 4.26; all the particles are monodispersed and homogenously distributed in sample solutions. The particle size values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in PBS solution are given in Table 4.27.

Table 4.27 The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in PBS solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	785	490	180	294	689	643	700
15	850	433	180	348	699	691	712
30	862	411	185	343	748	650	713
60	811	405	200	333	823	733	734
120	832	390	210	333	851	765	725
180	806	375	210	343	876	736	736
1440(1d)	830	359	805	315	904	785	735
2880(2d)	819	342	840	339	913	793	738
4320(3d)	862	325	860	322	910	852	738
10080(1w)	890	332	890	337	915	894	744
20160(2w)	789	320	900	394	945	892	780
30240(3w)	553	292	920	385	952	977	785

Table 4.27 indicates the particles of polymer, drug and DDS form clusters at the end of the 3-week period. At the end of the period particle size of drug shrinks due to the precipitation of formed clusters in sample solution, whereas there is no precipitation observed in solutions of DDSs. The mobility values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in PBS solution are given in Table 4.28.

Table 4.28 The mobility of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in PBS solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	-1.2	-1.5	-1.3	-2.5	-2.9	-2.1	-2.2
15	-1.4	-2.5	-1.6	-2.2	-2.6	-1.8	-2.1
30	-1.4	-2.5	-1.5	-2.3	-2.4	-1.8	-2.0
60	-1.2	-2.4	-2.2	-2.7	-2.2	-1.8	-1.6
120	-1.3	-3.2	-2.1	-2.2	-2.3	-1.8	-1.7
180	-1.1	-2.4	-1.8	-2.6	-2.3	-1.7	-1.6
1440(1d)	-1.4	-3.1	-1.9	-2.9	-2.2	-1.6	-1.5
2880(2d)	-1.2	-2.8	-1.5	-2.9	-2.2	-1.5	-1.5
4320(3d)	-1.0	-2.5	-2.9	-2.7	-2.1	-1.5	-1.5
10080(1w)	-0.9	-2.3	-1.5	-2.8	-1.9	-1.4	-1.5
20160(2w)	-0.9	-2.2	-1.4	-2.4	-1.8	-1.3	-1.4
30240(3w)	-0.9	-2.2	-1.5	-2.7	-1.7	-1.3	-1.4

According to mobility measurements in table 4.28; particles of all drug, copolymers and DDSs are negatively charged and the absolute average mobility values of DDSs are greater than the mobility values of drug. Zeta potential values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in PBS is given in Table 4.29.

Table 4.29 The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS in PBS solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	-13.4	-15.6	-13.1	-27.0	-30.4	-22.1	-23.3
15	-15.2	-25.6	-16.2	-22.4	-27.5	-18.9	-22.2
30	-14.7	-26.0	-15.1	-24.7	-25.6	-18.6	-21.3
60	-14.9	-25.0	-22.9	-27.4	-23.4	-19.1	-18.3
120	-13.1	-33.4	-22.0	-22.7	-24.4	-18.8	-17.1
180	-14.5	-24.9	-19.1	-27.2	-24.0	-18.0	-17.1
1440(1d)	-14.7	-31.9	-19.5	-30.3	-23.7	-17.2	-16.1
2880(2d)	-13.4	-29.1	-15.6	-30.2	-23.2	-15.8	-16.0
4320(3d)	-10.9	-25.7	-29.7	-28.4	-22.9	-15.6	-15.5
10080(1w)	-10.2	-23.4	-15.6	-28.9	-20.1	-15.3	-15.8
20160(2w)	-10.1	-23.1	-14.5	-25.1	-18.9	-14.1	-14.9
30240(3w)	-9.6	-23.1	-15.6	-27.6	-18.4	-13.8	-14.6

In table 4.29; zeta potential values of drug and DDS, regardless of sign, show a decreasing trend in 3-week period. Very low zeta potential values of drug have been compensated by copolymer conjugation after 2nd day. This table is visualized by Figure 4.14.

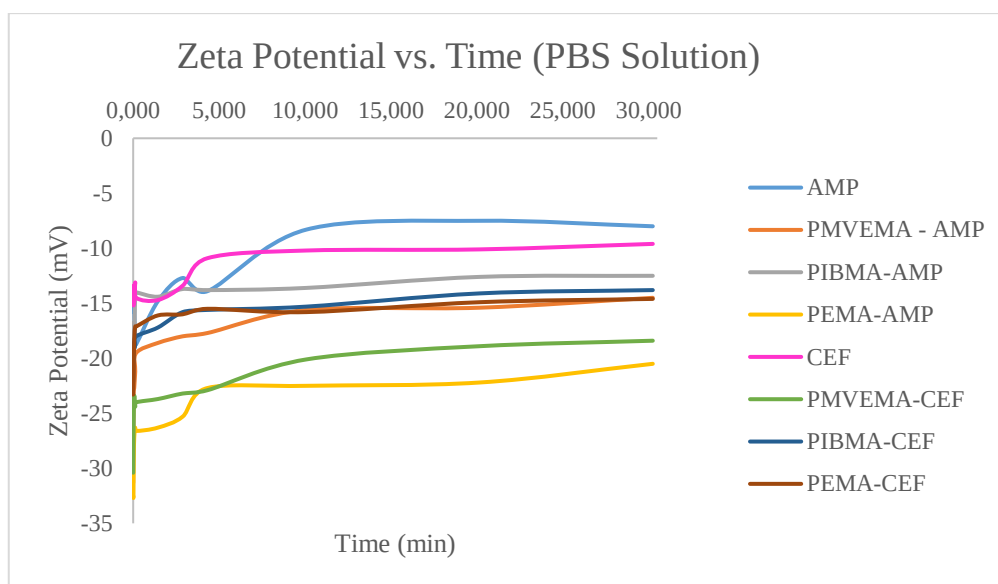


Figure 4.14 Zeta Potential vs. Time (PBS Solution)

4.11.3 Zetasizer Measurements of CEF, PMVEMA, PIBMA, PEMA, CEF-Conjugated Copolymer-Drug Delivery Systems In 0.9% Isotonic NaCl Solution

The PDI values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in 0.9% isotonic NaCl solution are given in Table 4.30.

Table 4.30 The PDI values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 0.9% NaCl solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA-CEF DDS	PIBMA-CEF DDS	PEMA-CEF DDS
0	0.47	0.26	0.31	0.25	0.22	0.31	0.34
15	0.34	0.28	0.32	0.26	0.26	0.32	0.34
30	0.31	0.41	0.29	0.22	0.27	0.31	0.35
60	0.32	0.27	0.28	0.34	0.24	0.29	0.33
120	0.32	0.33	0.30	0.54	0.19	0.35	0.35
180	0.32	0.39	0.28	0.74	0.29	0.33	0.35
1440(1d)	0.37	0.31	0.30	0.33	0.26	0.34	0.51
2880(2d)	0.32	0.33	0.32	0.68	0.31	0.33	0.39
4320(3d)	0.34	0.32	0.48	0.38	0.35	0.32	0.59
10080(1w)	0.32	0.32	0.32	0.34	0.38	0.35	0.35
20160(2w)	0.34	0.31	0.35	0.35	0.38	0.30	0.34
30240(3w)	0.32	0.26	0.37	0.26	0.35	0.34	0.41

According to PDI measurements in table 4.30; particles of all drug, copolymers and DDSs are monodispersed. The particle size values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in 0.9% isotonic NaCl solution are given in Table 4.31.

Table 4.31 The particle size values (nm) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 0.9% NaCl solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	351	330	352	110	153	357	624
15	422	315	364	130	180	363	625
30	502	220	412	174	189	383	659
60	507	250	410	367	207	419	636
120	517	290	392	491	245	483	768
180	519	260	386	513	247	487	782
1440(1d)	526	265	413	569	261	502	796
2880(2d)	535	235	427	540	281	512	826
4320(3d)	611	275	439	534	326	531	912
10080(1w)	616	330	823	503	714	647	917
20160(2w)	653	335	882	723	728	695	923
30240(3w)	660	365	960	864	738	719	962

Table 4.31 indicates that particles of drug and DDSs start to aggregate at the end of the 3-week period. The mobility values of CEF, PMVEMA, PIBMA, PEMA and CEF-conjugated DDSs in 0.9% isotonic NaCl solution are given in Table 4.32.

Table 4.32 The mobility values of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS particles in 0.9% NaCl solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	-1.6	-2.6	-2.3	-2.7	-3.0	-2.1	-2.5
15	-1.6	-2.4	-2.0	-2.2	-2.8	-1.8	-2.2
30	-1.4	-2.4	-1.8	-2.3	-2.9	-1.7	-2.3
60	-1.0	-2.1	-1.7	-2.6	-2.6	-1.6	-1.9
120	-1.0	-2.1	-1.8	-2.3	-2.3	-1.7	-1.8
180	-0.9	-2.0	-1.8	-2.0	-2.4	-1.7	-1.6
1440(1d)	-0.8	-1.6	-2.2	-1.8	-2.3	-1.8	-1.6
2880(2d)	-0.9	-1.5	-1.8	-1.9	-2.3	-1.8	-1.4
4320(3d)	-0.9	-1.5	-1.6	-1.6	-2.2	-1.7	-1.4
10080(1w)	-0.9	-1.4	-1.2	-1.6	-2.3	-1.5	-1.4
20160(2w)	-0.8	-1.4	-1.3	-1.4	-2.0	-1.5	-1.3
30240(3w)	-0.8	-0.9	-1.2	-1.3	-2.0	-1.5	-1.3

According to table 4.32; the average absolute mobility values of DDS are grater than the values of drug. All the particles in sample solutions in table 4.32; are negatively charged. Zeta potential values of CEF, copolymers and CEF-conjugated DDSs in 0.9% isotonic NaCl solution are given in Table 4.33.

Table 4.33 The zeta potential values (mV) of CEF, PMVEMA, PIBMA, PEMA and PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS in 0.9% NaCl solution

Time (min)	CEF	PMVEMA	PIBMA	PEMA	PMVEMA- CEF DDS	PIBMA- CEF DDS	PEMA-CEF DDS
0	-16.9	-27.9	-23.7	-27.8	-31.9	-22.4	-26.9
15	-16.7	-26.3	-20.6	-22.7	-30.2	-19.2	-23.8
30	-15.0	-25.4	-18.6	-24.0	-30.3	-17.9	-24.4
60	-10.5	-23.0	-17.6	-26.4	-27.6	-17.0	-20.7
120	-10.5	-22.8	-19.1	-23.9	-24.6	-17.9	-19.4
180	-9.9	-20.6	-18.7	-20.4	-25.0	-18.4	-17.3
1440(1d)	-9.0	-17.3	-22.2	-18.5	-24.0	-19.0	-16.9
2880(2d)	-9.3	-16.0	-18.5	-19.2	-23.9	-19.1	-15.3
4320(3d)	-9.1	-15.8	-18.1	-16.4	-23.4	-17.3	-15.4
10080(1w)	-9.6	-15.3	-14.1	-16.5	-23.9	-16.5	-14.4
20160(2w)	-8.4	-14.6	-13.9	-16.6	-21.7	-16.2	-13.8
30240(3w)	-8.0	-10.7	-12.4	-16.3	-20.9	-16.1	-13.7

In table 4.33; zeta potential values of copolymers, drug and DDSs regardless of sign, show a decreasing trend. Instability of drug have been compensated by copolymer conjugation after 2nd hour. The significant difference in absolute zeta potential values belong to drugs and DDSs can be better seen in Figure 4.15.

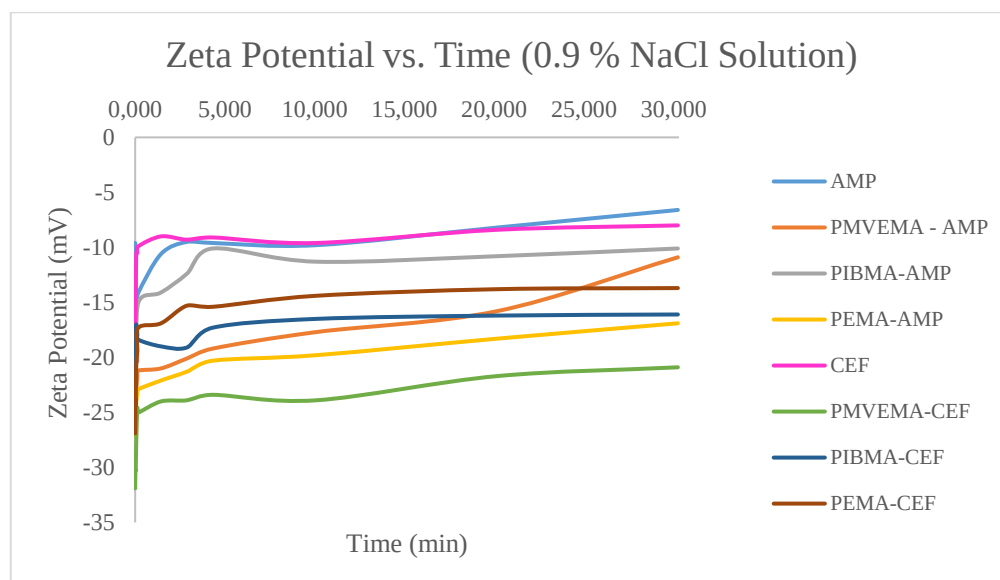


Figure 4.15. Zeta Potential vs. Time (0.9% NaCl Solution)

It is seen that from figs.4.13, 4.14 and 4.15, AMP and CEF stability in simulated body fluids are increased after copolymer conjugation.

4.12 UV/VIS Spectra Of Copolymers At Different pH Values

pH-dependant activity change of drugs, copolymers and drug delivery systems is decided with UV/VIS spectrums. UV/VIS spectra of polymers at different pH values are given in figure 4.16.

UV/Vis spectra of AMP and AMP-conjugated DDSs depending on pH values are given in figure 4.16. The pH-dependant maximum and minimum activity of AMP, PMVEMA, PIBMA, PEMA, AMP-conjugated DDSs are summarized in Table 4.34. In activity measurements, wavelength at maximum value of ampicillin is selected for the comparison of ampicillin-conjugated DDSs.



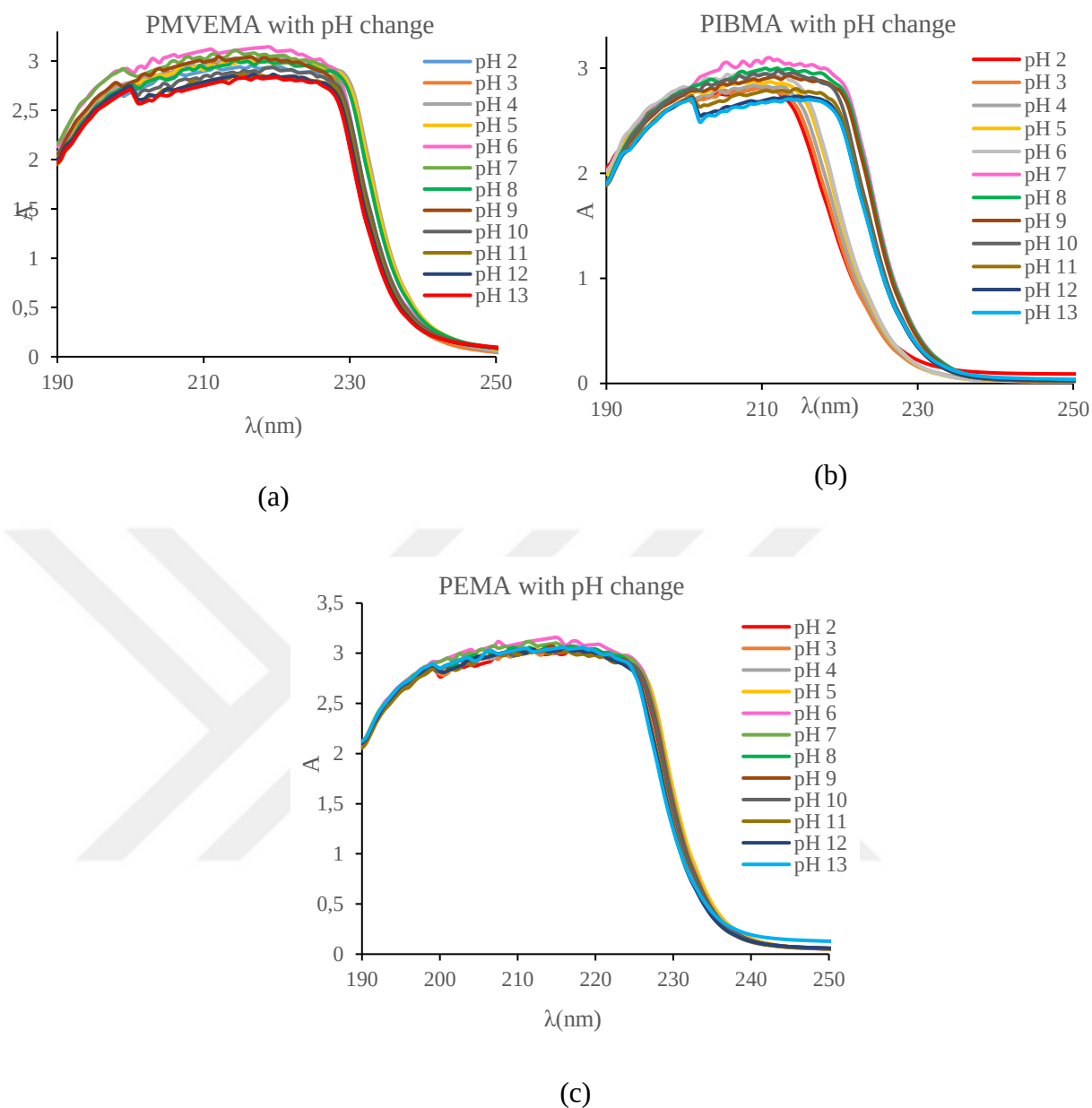
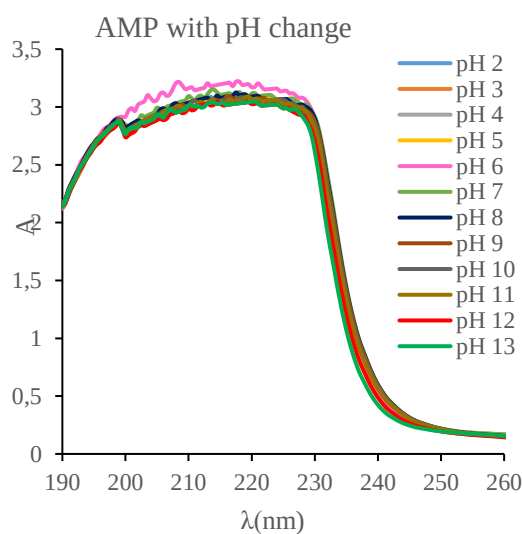


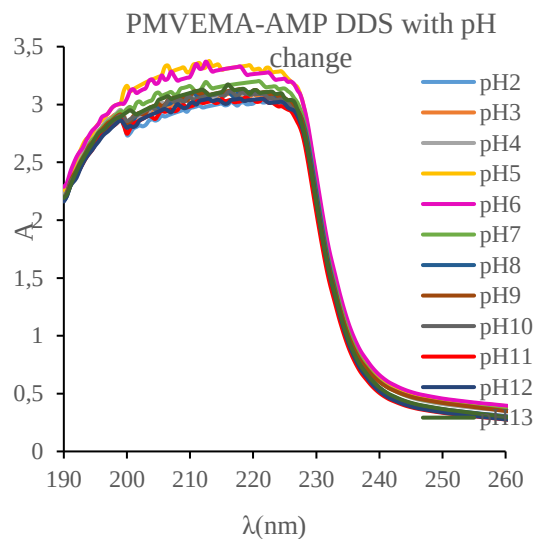
Figure 4.16 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at different pHs

4.13 UV/VIS Spectra Of AMP And AMP-Conjugated DDSs At Different pH Values

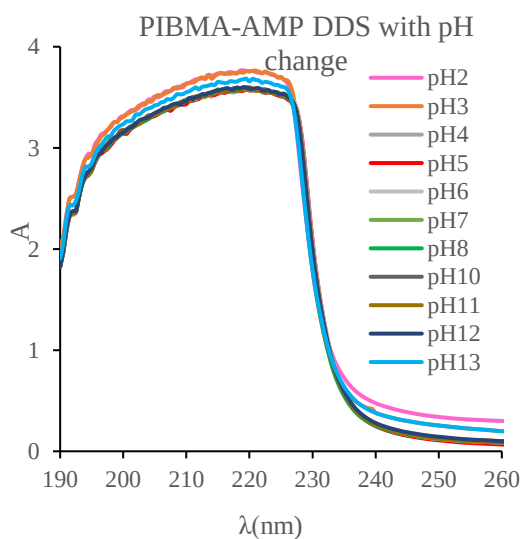
In figure 4.17; AMP and AMP-conjugated DDSs are compared at different pH values in terms of activity.



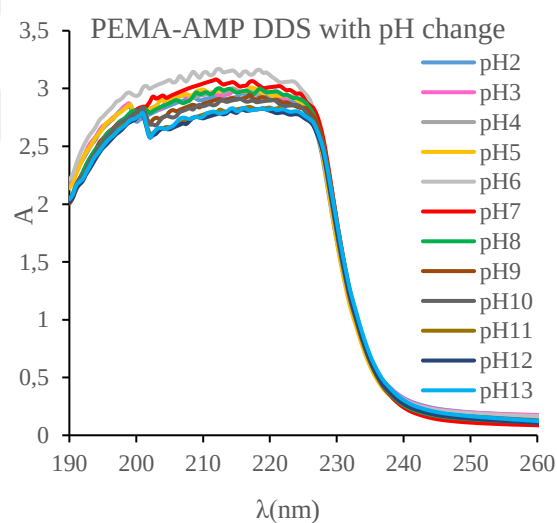
(a)



(b)



(c)

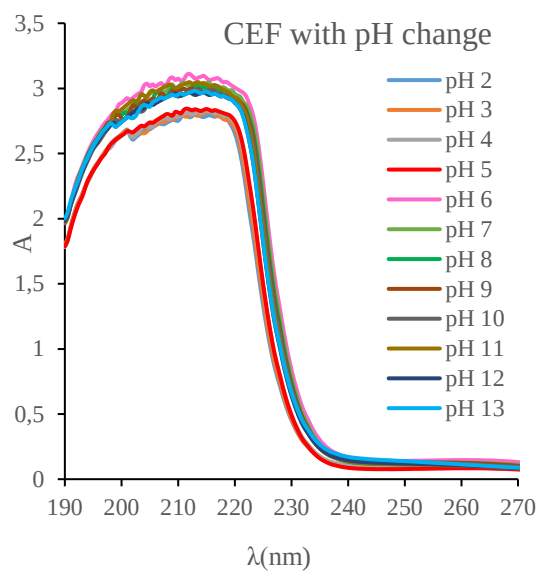


(d)

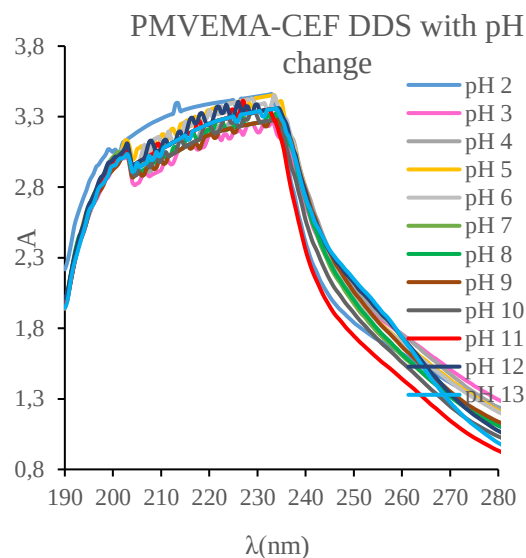
Figure 4.17 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at different pHs

4.14 UV/VIS Spectra of CEF and CEF-Conjugated DDSs at Different pH Values

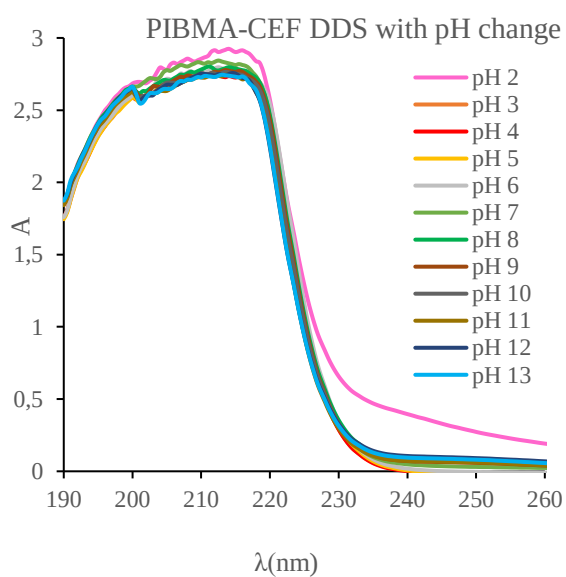
In figure 4.18; CEF and CEF-conjugated DDSs are compared at different pH values in terms of activity.



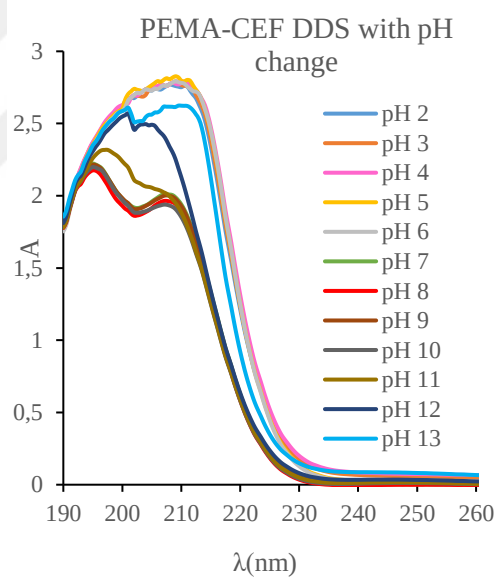
(a)



(b)



(c)



(d)

Figure 4.18 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at different pH values

4.15 UV/VIS Spectra of Copolymers at Different Body Fluids

4.15.1 UV/VIS Spectra of Copolymers in 5% Dextrose Solution

In figure 4.19; polymer at 5% dextrose solution over 3-week period are analyzed by UV/VIS spectroscopy.

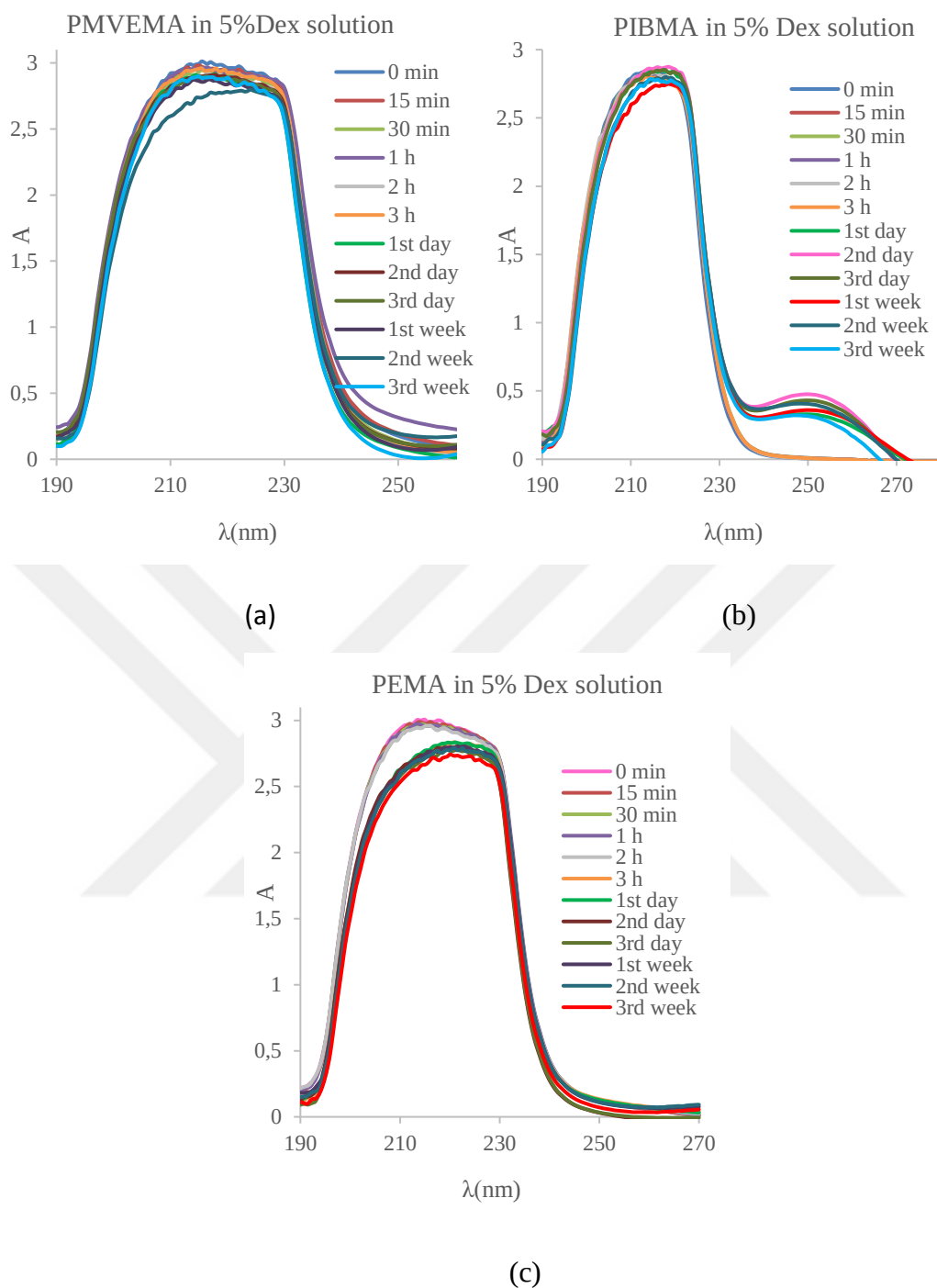


Figure 4.19 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at 5% Dextrose solution

4.15.2 UV/VIS Spectra of Copolymers in PBS Solution

In figure 4.20; polymers at PBS solution over 3-week period are analyzed by UV/VIS spectroscopy.

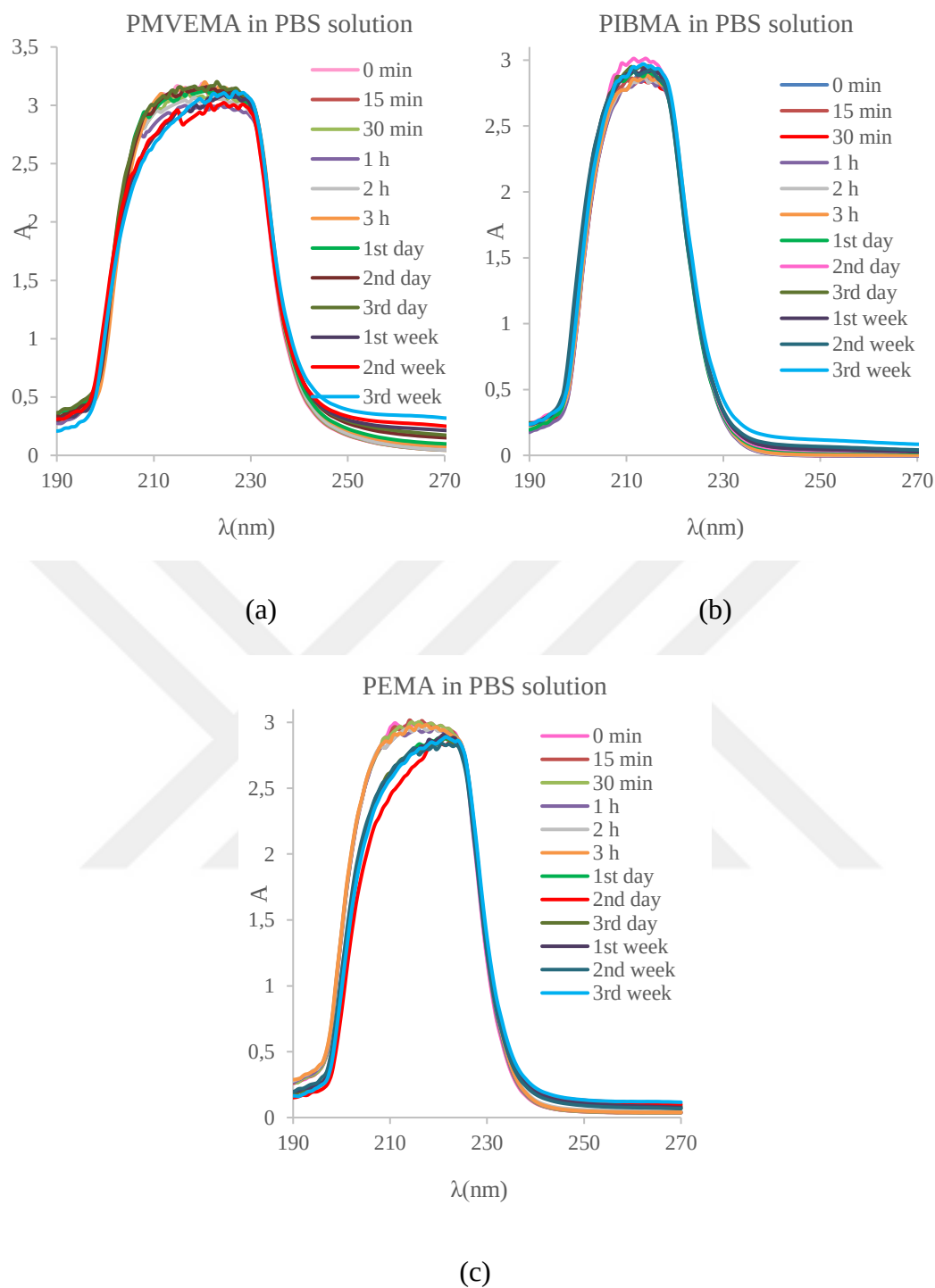


Figure 4.20 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at PBS solution

4.15.3 UV/VIS Spectra of Copolymers in 0.9% Isotonic NaCl Solution

In figure 4.21; polymers at 0.9% NaCl solution over 3-week period are analyzed by UV/VIS spectroscopy.

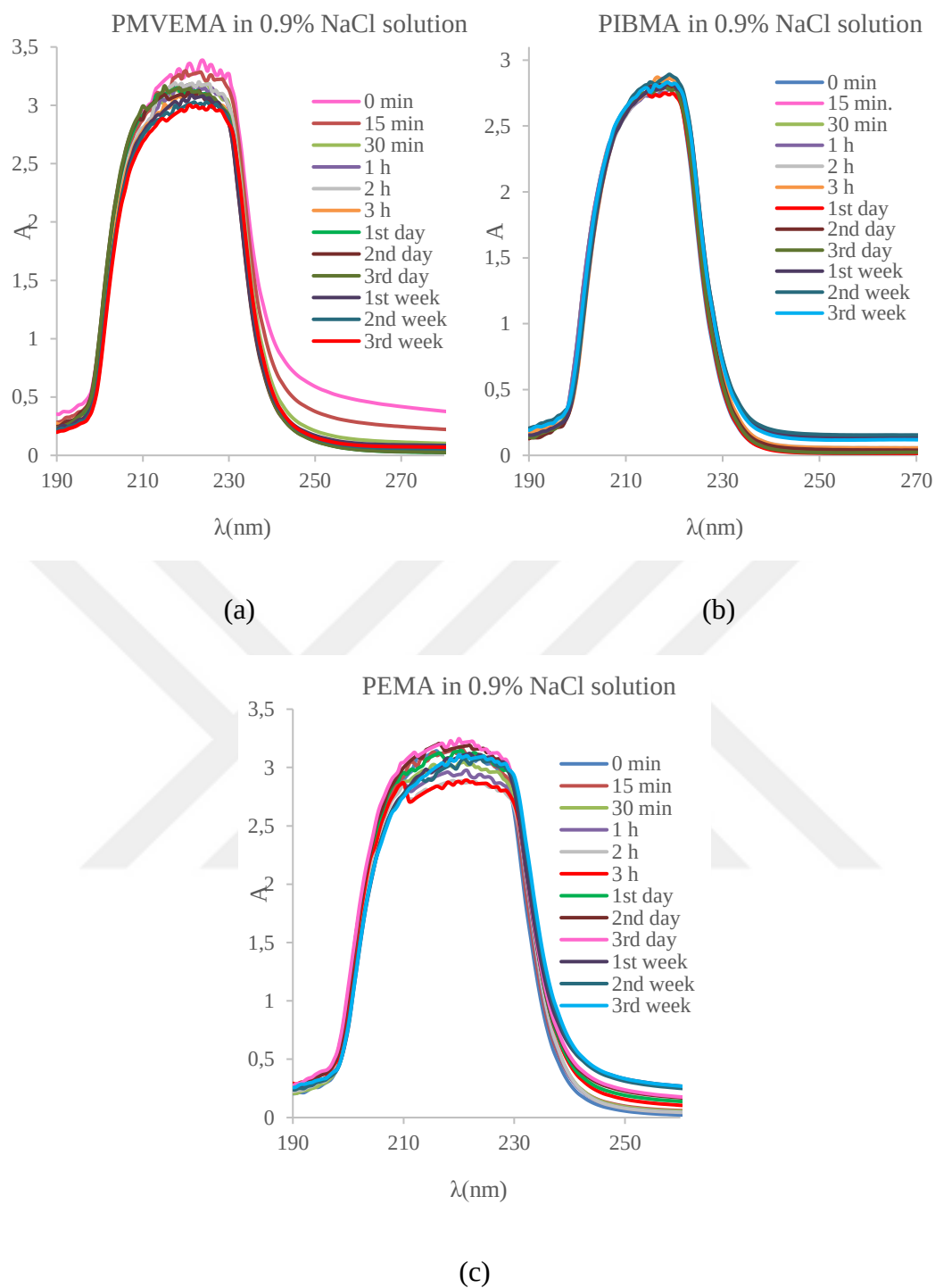


Figure 4.21 Absorption spectra of PMVEMA (a), PIBMA (b) and PEMA (c) at 0.9% NaCl solution

4.16 UV/VIS Spectra of AMP and AMP-Conjugated DDSs at Different Body Fluids

4.16.1 UV/VIS Spectra of AMP and AMP-Conjugated DDSs in 5% Dextrose Solution

In figure 4.22; AMP and AMP-conjugated DDSs in 5% Dextrose solution are analyzed by UV/VIS spectroscopy at certain time intervals.



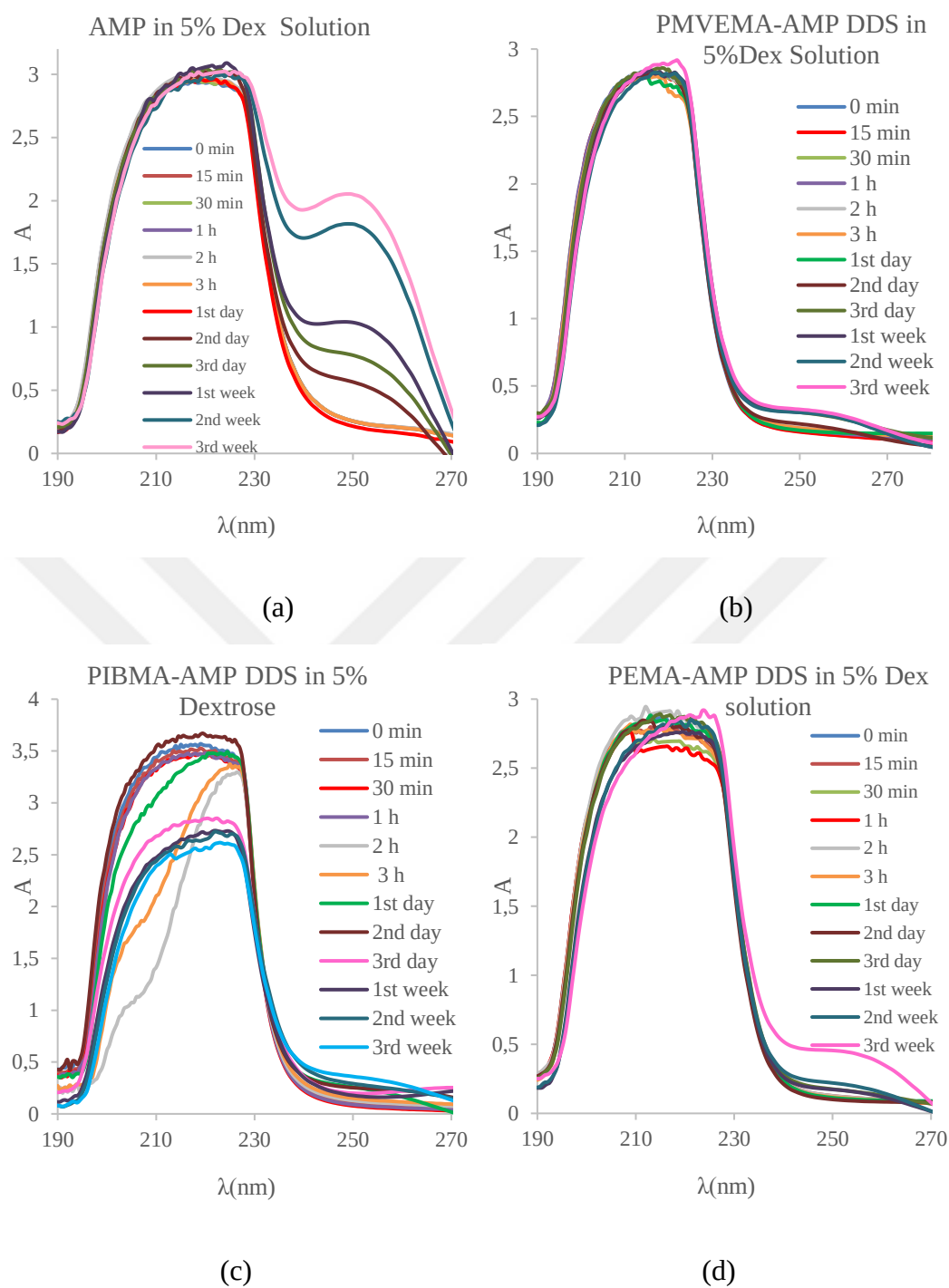


Figure 4.22 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at 5% dextrose solution

4.16.2 UV/VIS Spectra of AMP and AMP-Conjugated DDSs in PBS Solution

In figure 4.23; AMP and AMP-conjugated DDSs in PBS solution are analyzed by UV/VIS spectroscopy at certain time intervals.

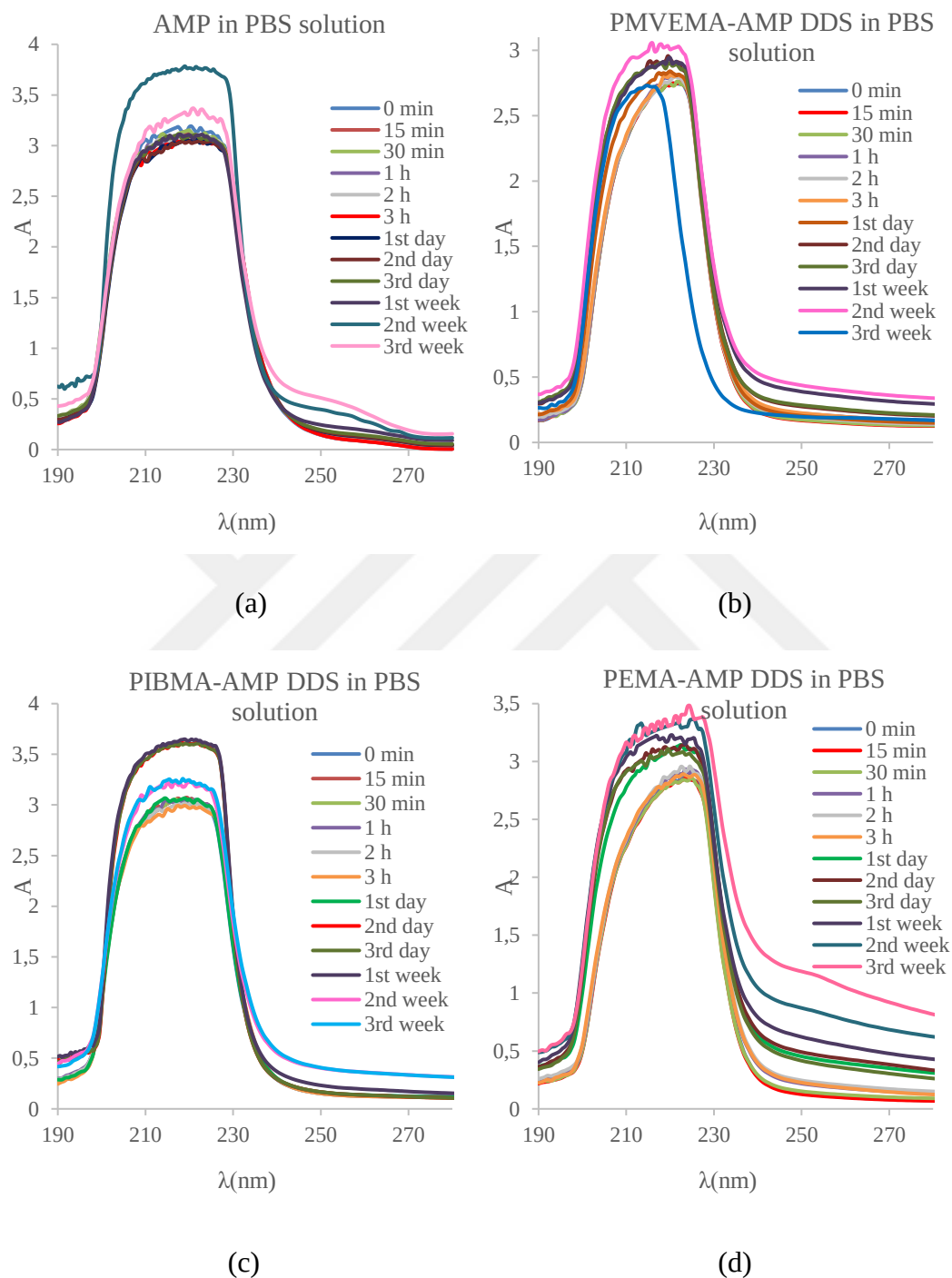


Figure 4.23 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at PBS solution

4.16.3 UV/VIS Spectra of AMP and AMP-Conjugated DDSs in 0.9%

Isotonic NaCl Solution

In figure 4.24; AMP and AMP-conjugated DDSs in 0.9% NaCl solution are analyzed by UV/VIS spectroscopy at certain time intervals.

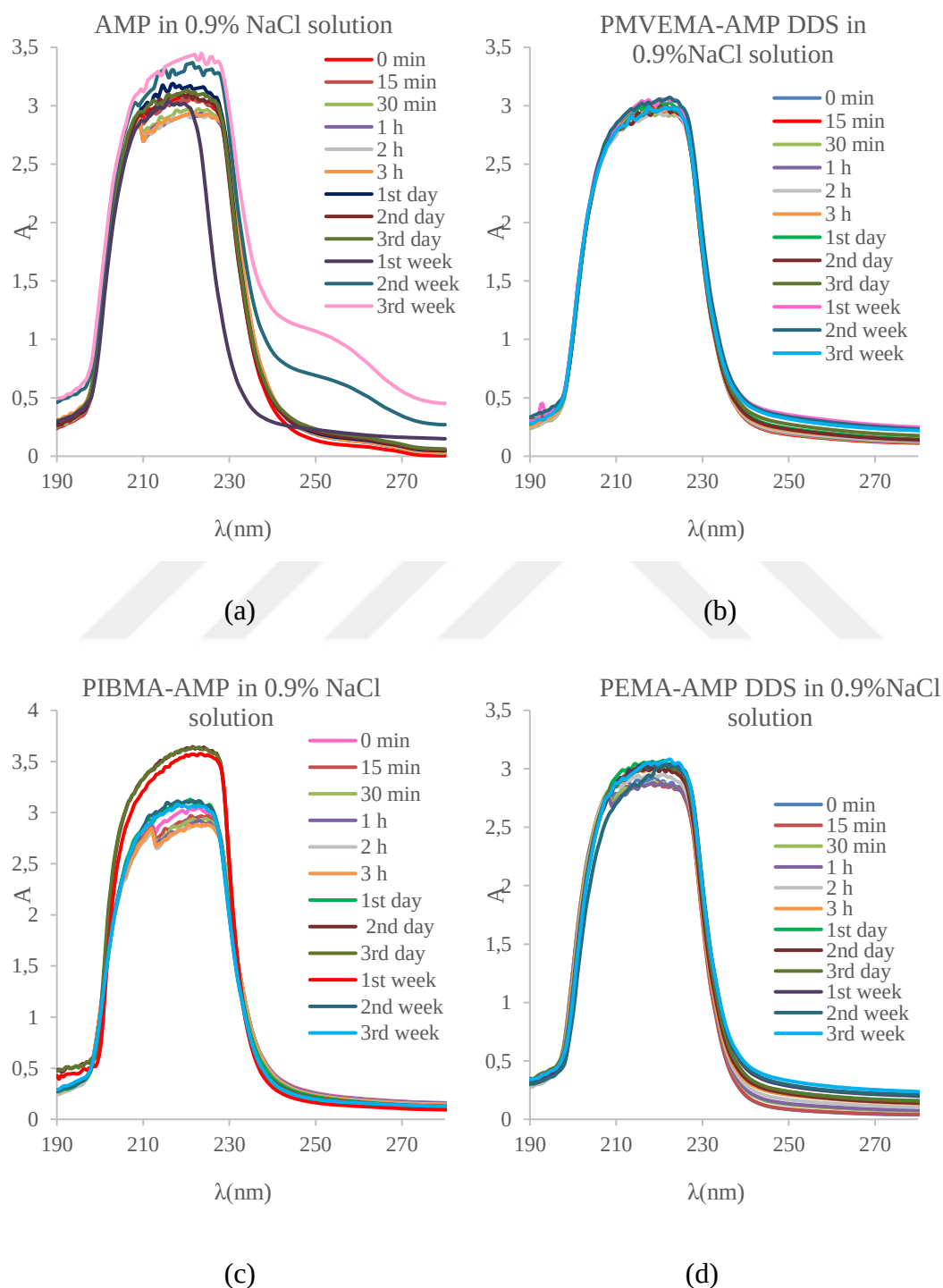


Figure 4.24 Absorption spectra of AMP (a), PMVEMA-AMP DDS (b), PIBMA-AMP DDS (c) and PEMA-AMP DDS (d) at 0.9% NaCl solution

4.17 UV/VIS Spectra of CEF and CEF-Conjugated DDSs at Different Body Fluids

4.17.1 UV/VIS Spectra of CEF and CEF-Conjugated DDSs in 5% Dextrose Solution

In figure 4.25; CEF and CEF-conjugated DDSs in 5% Dextrose solution are analyzed by UV/VIS spectroscopy at certain time intervals.



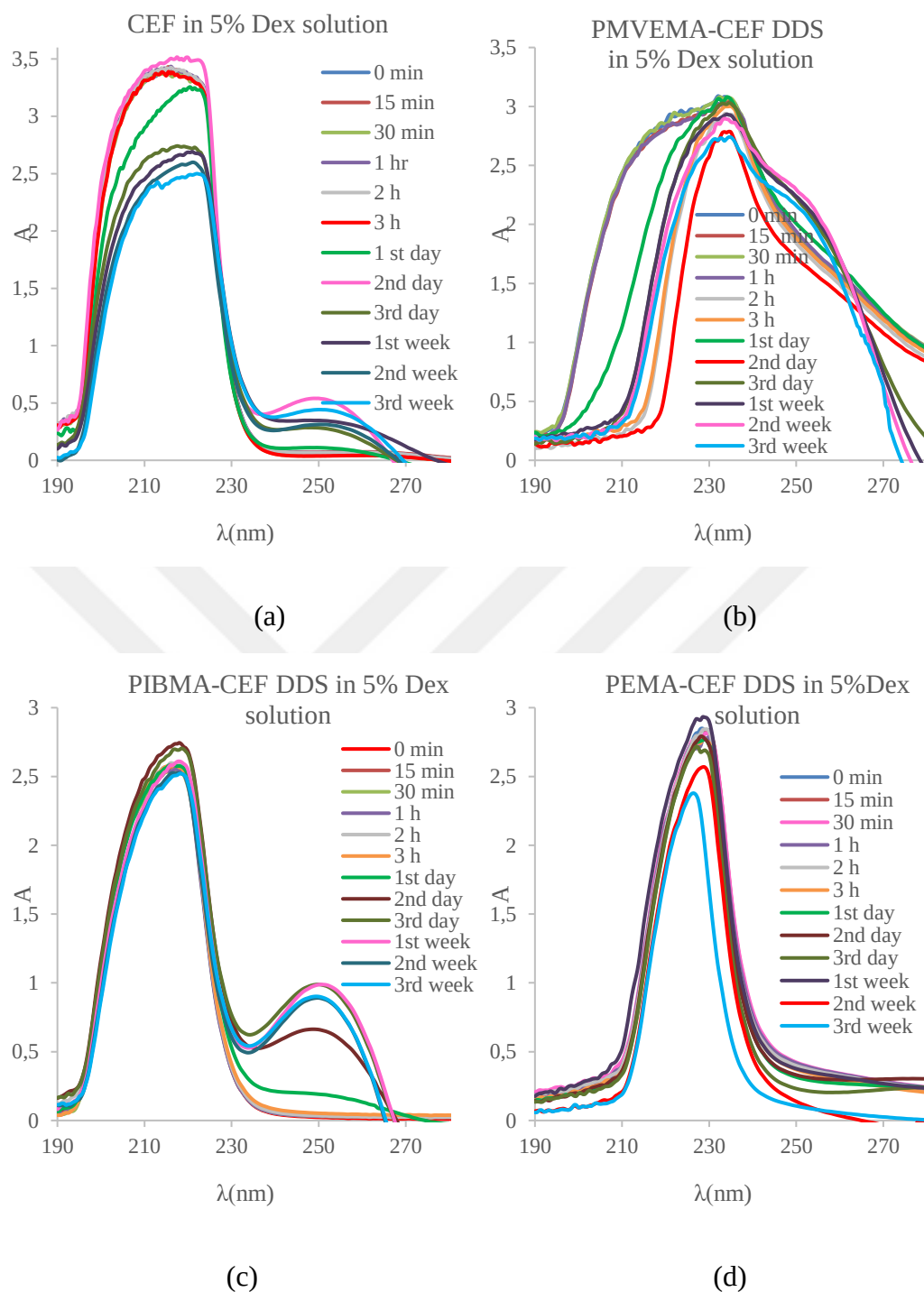


Figure 4.25 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at 5% Dextrose solution

4.17.2 UV/VIS Spectra of CEF and CEF-Conjugated DDSs in PBS Solution

In figure 4.26; CEF and CEF-conjugated DDSs in PBS solution are analyzed by UV/VIS spectroscopy at certain time intervals.

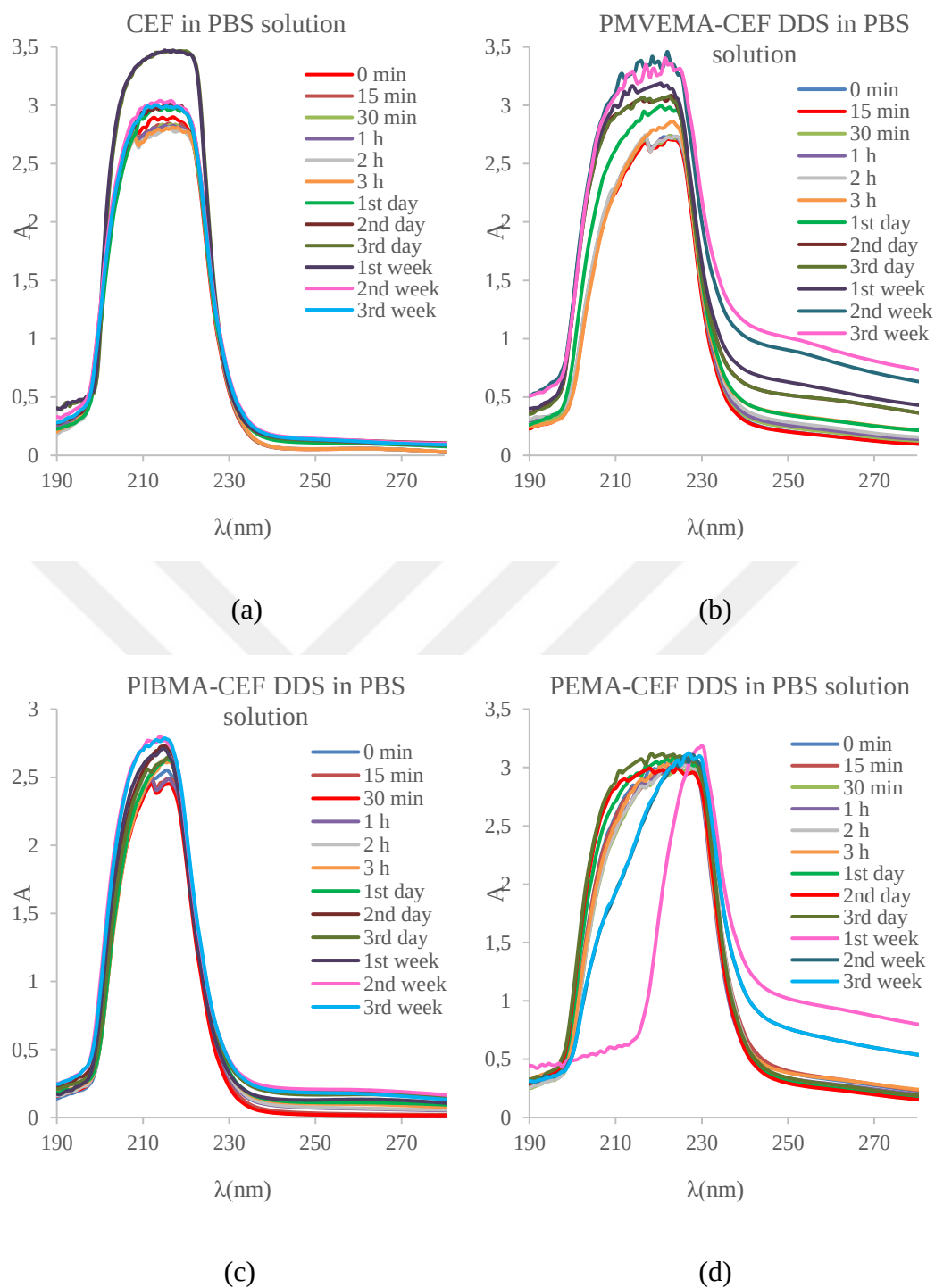


Figure 4.26 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at PBS solution

4.17.3 UV/VIS Spectra of CEF and CEF-Conjugated DDSs in 0.9% Isotonic NaCl Solution

In figure 4.27; CEF and CEF-conjugated DDSs in 0.9% NaCl solution are analyzed by UV/VIS spectroscopy at certain time intervals.

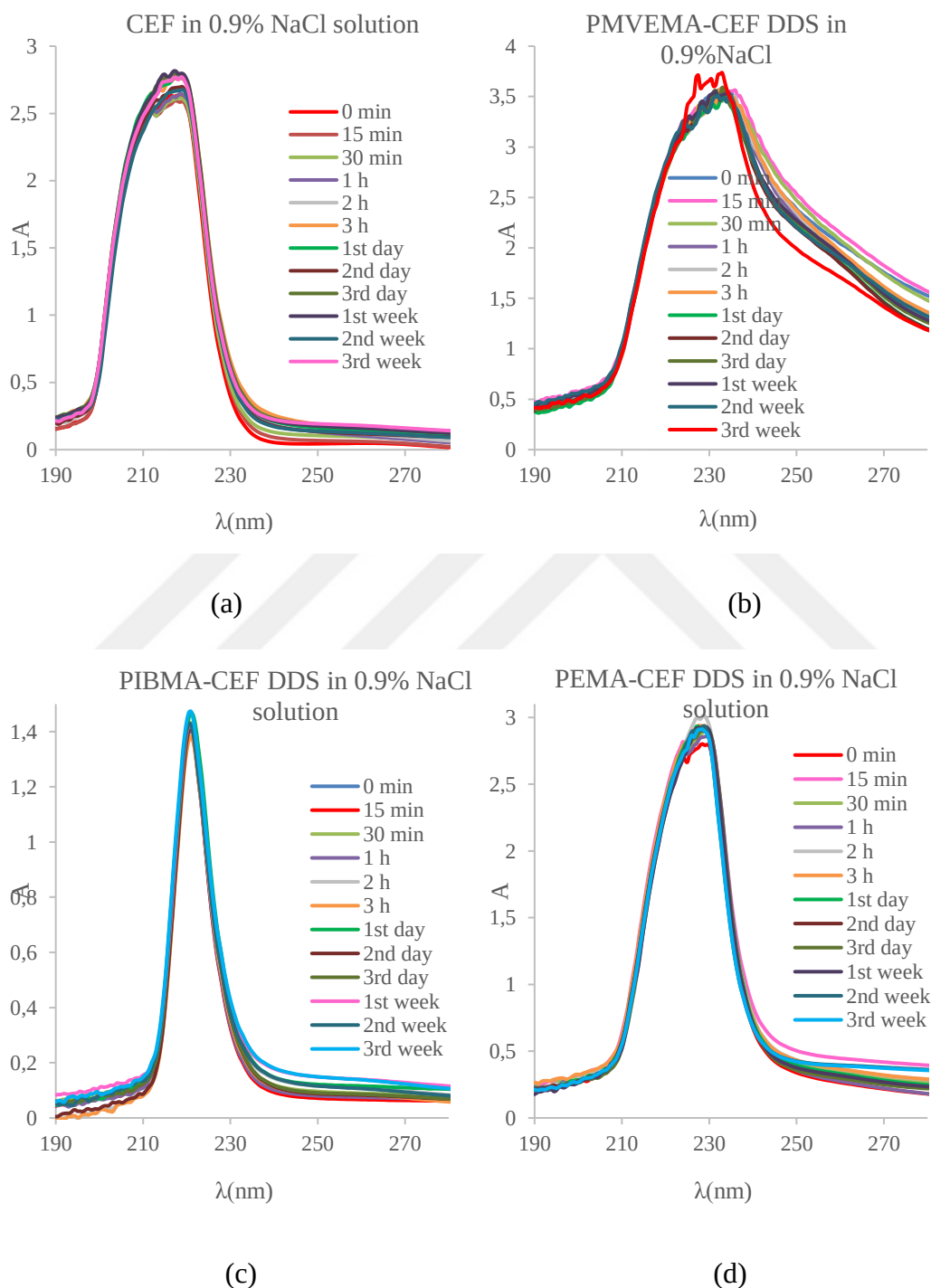


Figure 4.27 Absorption spectra of CEF(a),PMVEMA-CEF DDS (b), PIBMA-CEF DDS (c) and PEMA-CEF DDS (d) at 0.9% NaCl solution

4.18 Evaluation of Activity of Drug Delivery Systems

Maximum and minimum pH and time-dependant activity in simulated body fluids of AMP, CEF, PMVEMA, PIBMA, PEMA, AMP and CEF conjugated DDSs were summarized in Table 4.34.

Table 4.34 pH values and time at which maximum and minimum absorbance values of AMP, CEF, PMVEMA, PIBMA, PEMA, PMVEMA-AMP DDS, PIBMA-AMP DDS, PEMA-AMP DDS, PMVEMA-CEF DDS, PIBMA-CEF DDS and PEMA-CEF DDS

Sample	pH		%5 Dex		PBS		%0.9 NaCl	
	Max	Min	Max	Min	Max	Min	Max	Min
PMVEMA	6	13	0 min	2 nd week	3 rd day	1 h	0 min	3 rd week
PIBMA	7	13	2 nd day	1 st week	2 nd day	30 min	2 nd week	1 st day
PEMA	6	3	0 min	3 rd week	15 min	2 nd week	3 rd day	2 h
AMP	6	13	1 st week	0 min	2 nd week	2 nd day	3 rd week	1 h
PMVEMA-AMP	5	2	3 rd week	3 h	2 nd week	3 rd week	2 nd week	30 min
PIBMA-AMP	2	4	2 nd day	3 rd week	1 st week	3 h	2 nd day	2 h
PEMA-AMP	6	12	2 h	1 st week	3 rd week	15 min	3 rd week	1 h
CEF	6	2	2 nd day	3 rd week	1 st week	2 nd day	1 st week	15 min
PMVEMA-CEF	2	3	0 min	3 rd week	2 nd week	15 min	3 rd week	1 st day
PIBMA-CEF	2	13	2 nd day	3 rd week	2 nd week	30 min	3 rd week	3 h
PEMA-CEF	5	8	1 st week	3 rd week	1 st week	30 min	2 h	0 min

AMP and CEF antibiotics showed maximum activity at acidic media and activity of DDSs were prolonged up to 3 weeks with better circulation time. AMP and CEF antibiotics showed maximum activity mostly at 1st week and after, whereas according to zeta potential values, they already become unstable in a week. In addition to this, since drugs have been eliminated from the body in a couple of hours [24], the activity profile of antibiotics were not in harmony with stability and half-life of the drugs itself. Drugs are more active at pH 6 and unstable in the very acidic gastric medium which is the first place of the GI tract. Before it reaches a higher pH medium, it would be exposed to decomposition in acid.

Comparatively, PDDSs exhibited high activity profiles at very low pH values like in gastric medium. PDDSs were also showing stability in acidic medium, and this

prevents the decomposition of PDDSs. In the simulated body fluids that mimic the gastrointestinal tract, PDDSs were stable at maximum activity conditions.

4.19 Antimicrobial Susceptibility Tests

According to CLSI (Clinical and Laboratory Standards Institute), diameters of inhibition zones for sensitive antimicrobials (S) are accepted as greater or equal to 20 mm, for Intermediate antimicrobials (I) are accepted as between 15-19 mm and for the resistant antimicrobials (R) are accepted as less or equal to 14 mm [112].

In Figure 4.28; DMSO is blank control group for gram positive and gram-negative bacteria. Polymers, drugs and drug delivery systems are tested against gram positive and gram-negative bacteria.

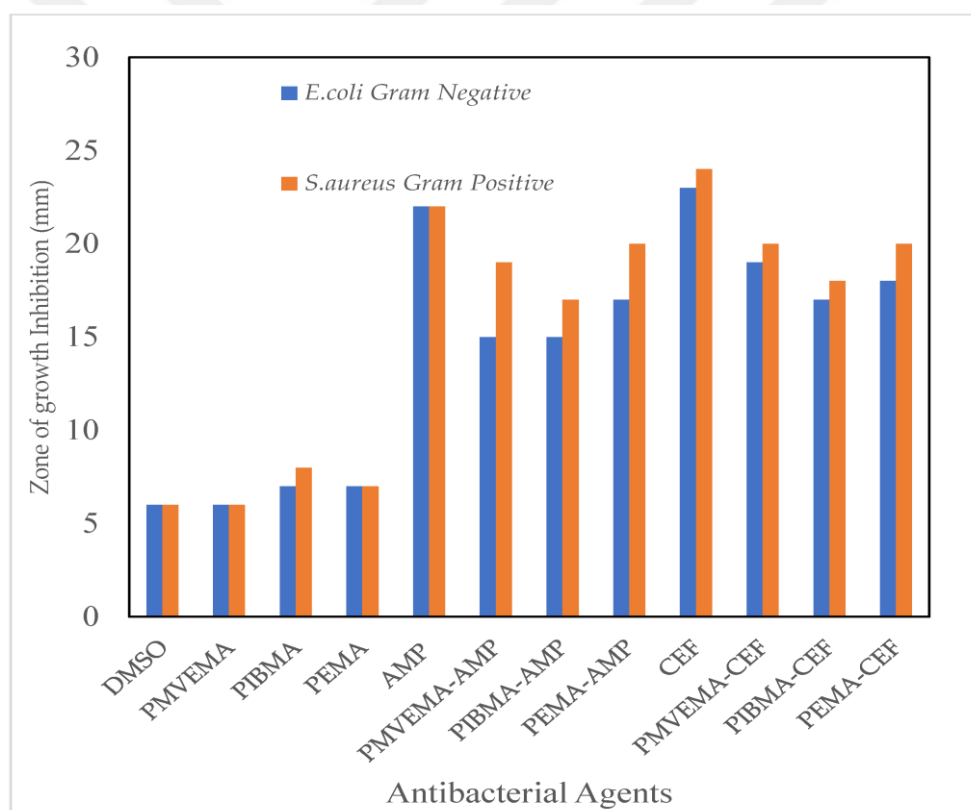


Figure 4.28 Diameters of inhibition zones of samples against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*)

Since DMSO is a blank control group, it shows the radius of the wells (6 mm). Polymers show no antibacterial effect on gram-positive and gram-negative bacteria in figure 4.28. Antibiotic drugs are sensitive to both gram-positive and gram-negative bacteria. All drug delivery systems inhibit gram-positive and gram-

negative bacteria, but they are more effective on gram-positive bacteria than gram-negative bacteria.

Cefalexin monohydrate is hydrophobic compound, so it is retained by gram negative and more effective on gram-positive bacteria whereas ampicillin trihydrate are moderately hydrophilic and less effective on gram-positive bacteria than cephalixin monohydrate. This affects antibacterial activity of DDSs on gram-positive bacteria. Since polymer-drug is not fully (100%) bonded; antibacterial effectivity of DDSs is generally at an intermediate stage.



5 CONCLUSION

To the best of our knowledge, this study was the first drug delivery systems based on these maleic acid derived polymers conjugated with ampicillin trihydrate and cefalexin monohydrate with this method. Chemical characterization of copolymer-antibiotic conjugates was concluded by ^1H NMR and FTIR/ATR spectroscopies. These conjugates at different pH values with simulated body fluids with certain time intervals were analyzed by means of Zeta and UV-VIS spectroscopic measurements. Parameters such as pH values, function of time and composition of solutions affected absorbance of samples.

Pharmacological properties of drugs such as stability and activity were prolonged by copolymer conjugation, where antibacterial effect of drugs was still preserved. Stability of synthesized copolymer-drug delivery systems were arranged as PMVEMA-AMP DDS > PEMA-AMP DDS > PIBMA-AMP DDS and PMVEMA-CEF DDS > PEMA-CEF DDS > PIBMA-CEF DDS. Hydrophilicity of polymer made the drug delivery systems more stable. Size of the particles of drug delivery systems were in sub-micro scale, whereas; particles of drugs became micro-sized in temporal and pH dependent changes due to the formation of aggregation of clusters among the particles. By the copolymer conjugation on antibiotics, activity of drug was extended in longer period of time. DDSs were stable in an extended pH range compared to the pure drug. In general, drugs and DDSs were more stable at pH 2-6 band. Among the tested body fluids, 5% Dextrose solution and 0.9% isotonic NaCl Solution led to more stable results for DDSs compared to PBS solution due to basicity of this solution. All particles are in sub-micro scale less than 1000 nm. The size of the particles between 1-1000 nm, have special advantages in oral administration of drugs [113].

Antibacterial tests proved that there was a bactericidal effect of the drug delivery systems. The antimicrobial effect on these bacteria could be simply improved by the increase in the ratio of drug to polymer binding. According to antibacterial studies, cefalexin monohydrate-based drug delivery systems exhibited more antibacterial effect on bacteria. All DDSs were more efficient on gram positive

bacteria than gram-negative bacteria. As far as stability, activity, in vitro antibacterial efficiency concerned, the most prominent drug delivery system was PMVEMA-CEF drug delivery system.

The results revealed that the method of conjugation of the drug via an amide bond resulted in a more stable macromolecular conjugate form. The resulting DDS allowed improved the pharmacokinetics of a pharmacologically active substance. The results are promising for the application of the obtained macromolecular conjugates as implants in pharmaceutical technology.



REFERENCES

- [1] R. S. Vardanyan and V. J. Hruby, "Antiviral Drugs," in *Synthesis of Essential Drugs*, Elsevier, 2006, pp. 549–557. doi: 10.1016/B978-044452166-8/50036-4.
- [2] T. Bergan, "Pharmacokinetics of beta-lactam antibiotics.," *Scand J Infect Dis Suppl*, vol. 42, pp. 83–98, 1984.
- [3] N. D. Stebbins, M. A. Ouimet, and K. E. Uhrich, "Antibiotic-containing polymers for localized, sustained drug delivery," Nov. 30, 2014, *Elsevier B.V.* doi: 10.1016/j.addr.2014.04.006.
- [4] I. K. Paterson, A. Hoyle, G. Ochoa, C. Baker-Austin, and N. G. H. Taylor, "Optimising antibiotic usage to treat bacterial infections," *Sci Rep*, vol. 6, Nov. 2016, doi: 10.1038/srep37853.
- [5] M. A. Ouimet *et al.*, "Biodegradable ferulic acid-containing poly(anhydride-ester): Degradation products with controlled release and sustained antioxidant activity," *Biomacromolecules*, vol. 14, no. 3, pp. 854–861, Mar. 2013, doi: 10.1021/bm3018998.
- [6] Y. K. Sung and S. W. Kim, "Recent advances in polymeric drug delivery systems," Jun. 06, 2020, *BioMed Central Ltd.* doi: 10.1186/s40824-020-00190-7.
- [7] A. Srivastava, T. Yadav, S. Sharma, A. Nayak, A. Akanksha Kumari, and N. Mishra, "Polymers in Drug Delivery," *J Biosci Med (Irvine)*, vol. 04, no. 01, pp. 69–84, 2016, doi: 10.4236/jbm.2016.41009.
- [8] K. Harrison, "Introduction to polymeric drug delivery systems," in *Biomedical Polymers*, Elsevier Inc., 2007, pp. 33–56. doi: 10.1533/9781845693640.33.
- [9] R. Duncan, "Drug-polymer conjugates: potential for improved chemotherapy," *Anticancer Drugs*, vol. 3, no. 3, 1992, [Online]. Available: https://journals.lww.com/anti-cancerdrugs/fulltext/1992/06000/drug_polymer_conjugates__potential_for_improved.1.aspx.15.07.2024.
- [10] D. C. Bibby, N. M. Davies, and I. G. Tucker, "Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems," 2000. [Online]. Available: www.elsevier.com/locate/ijpharm.12.05.2023.
- [11] I. S. Jerbic, "Biodegradable synthetic polymers and their application in advanced drug delivery systems (DDS)," *Journal of Chemical Engineering & Process Technology*, vol. 09, 2018, doi: 10.4172/2157-7048-c1-011.
- [12] N. Kamaly, B. Yameen, J. Wu, and O. C. Farokhzad, "Degradable controlled-release polymers and polymeric nanoparticles: Mechanisms of

- controlling drug release,” Feb. 24, 2016, *American Chemical Society*. doi: 10.1021/acs.chemrev.5b00346.
- [13] C. J. Yarce, J. D. Echeverri, M. A. Palacio, C. A. Rivera, and C. H. Salamanca, “Relationship between surface properties and in vitro drug release from compressed matrix containing polymeric materials with different hydrophobicity degrees,” *Pharmaceuticals*, vol. 10, no. 1, Mar. 2017, doi: 10.3390/ph10010015.
 - [14] S. D. Sütökin, A. B. Atıcı, O. Güven, and A. S. Hoffman, “Controlling of free radical copolymerization of styrene and maleic anhydride via RAFT process for the preparation of acetaminophen drug conjugates,” *Radiation Physics and Chemistry*, vol. 148, pp. 5–12, Jul. 2018, doi: 10.1016/j.radphyschem.2018.02.012.
 - [15] Ghosh S., “Recent research and development in synthetic polymer-based drug delivery systems,” *J Chem Res*, pp. 241–246, 2004.
 - [16] R. De Souza, P. Zahedi, C. J. Allen, and M. Piquette-Miller, “Polymeric drug delivery systems for localized cancer chemotherapy,” 2010, *Informa Healthcare*. doi: 10.3109/10717541003762854.
 - [17] H. Kamada *et al.*, “Design of a pH-Sensitive Polymeric Carrier for Drug Release and Its Application in Cancer Therapy,” 2004, *Clin Cancer Res*.doi: 10.1158/1078-0432.ccr-03-0544.
 - [18] K. Tsukigawa *et al.*, “Synthesis and therapeutic effect of styrene-maleic acid copolymer-conjugated pirarubicin,” *Cancer Sci*, vol. 106, no. 3, pp. 270–278, Mar. 2015, doi: 10.1111/cas.12592.
 - [19] E. Kaplan, T. Ince, E. Yorulmaz, F. Yener, E. Harputlu, and N. T. Laçın, “Controlled delivery of ampicillin and gentamycin from cellulose hydrogels and their antibacterial efficiency,” *J Biomater Tissue Eng*, vol. 4, no. 7, pp. 543–549, 2014, doi: 10.1166/jbt.2014.1198.
 - [20] A. Zafar *et al.*, “Formulation of Self-Nanoemulsifying Drug Delivery System of Cephalexin: Physiochemical Characterization and Antibacterial Evaluation,” *Polymers (Basel)*, vol. 14, no. 5, Mar. 2022, doi: 10.3390/polym14051055.
 - [21] D. K. Ho, B. L. B. Nichols, K. J. Edgar, X. Murgia, B. Loretz, and C. M. Lehr, “Challenges and strategies in drug delivery systems for treatment of pulmonary infections,” Nov. 01, 2019, *Elsevier B.V.* doi: 10.1016/j.ejpb.2019.09.002.
 - [22] P. Bhattacharya, S. Dutta, K. Chandra, and A. Basak, “The Never-Ending Story of β -Lactams: Use as Molecular Scaffolds and Building Blocks,” in *Beta-Lactams: Novel Synthetic Pathways and Applications*, B. K. Banik, Ed., Cham: Springer International Publishing, 2017, pp. 373–419. doi: 10.1007/978-3-319-55621-5_12.
 - [23] “WHO EML 23rd List (2023),” 2023. [Online]. Available: <http://apps.who.int/bookorders.03.06.2024>.

- [24] P. W. Smith *et al.*, “Pharmacokinetics of β -Lactam Antibiotics: Clues from the Past To Help Discover Long-Acting Oral Drugs in the Future,” *ACS Infect Dis*, vol. 4, no. 10, pp. 1439–1447, Oct. 2018, doi: 10.1021/acsinfecdis.8b00160.
- [25] D. Lednicer, “Beta Lactam Antibiotics,” in *Strategies for Organic Drug Synthesis and Design*, 2008, pp. 545–575. doi: <https://doi.org/10.1002/9780470399613.ch14>.
- [26] M. E. Fox, F. C. Szoka, and J. M. J. Fréchet, “Soluble Polymer Carriers for the Treatment of Cancer: The Importance of Molecular Architecture,” *Acc Chem Res*, vol. 42, no. 8, pp. 1141–1151, Aug. 2009, doi: 10.1021/ar900035f.
- [27] E. Bacu, G. C. Chitanu, A. Couture, P. Grandclaudon, Gh. Singurel, and A. Carpov, “Potential drug delivery systems from maleic anhydride copolymers and phenothiazine derivatives,” *Eur Polym J*, vol. 38, no. 8, pp. 1509–1513, 2002, doi: [https://doi.org/10.1016/S0014-3057\(02\)00040-X](https://doi.org/10.1016/S0014-3057(02)00040-X).
- [28] I. Orienti, G. Gentilomi, F. Bigucci, B. Luppi, and V. Zecchi, “Substituted Poly(Methyl Vinyl Ether-alt-Maleic Anhydride) for the Release Control and Targeting of Methotrexate.”
- [29] P. Liras and J. F. Martín, “ β -Lactam Antibiotics,” in *Encyclopedia of Microbiology*, Elsevier, 2009, pp. 274–289. doi: 10.1016/B978-012373944-5.00039-0.
- [30] L. M. Lima, B. N. M. da Silva, G. Barbosa, and E. J. Barreiro, “ β -lactam antibiotics: An overview from a medicinal chemistry perspective,” Dec. 15, 2020, *Elsevier Masson s.r.l.* doi: 10.1016/j.ejmech.2020.112829.
- [31] J.-M. Ghuysen, “Serine β -Lactamases And Penicillin-Binding Proteins,” *Annu Rev Microbiol*, vol. 45, no. 1, pp. 37–67, Oct. 1991, doi: 10.1146/annurev.mi.45.100191.000345.
- [32] Bush K., “ β -Lactam antibiotics. Antibiotic and Chemotherapy ch14,” Ninth Edition., vol. Ch.14, G. D. , W. R. J. , and N. S. R. Finch R.G., Ed., Saunders, 2010, pp. 200–225.
- [33] B. D. Raynor, “Penicillin and ampicillin,” *Prim Care Update Ob Gyns*, vol. 4, no. 4, pp. 147–152, 1997, doi: [https://doi.org/10.1016/S1068-607X\(97\)00012-7](https://doi.org/10.1016/S1068-607X(97)00012-7).
- [34] H. Bundgaard, “Spectrophotometric determination of ampicillin sodium in the presence of its degradation and polymerization products,” *Journal of Pharmacy and Pharmacology*, 26: 385-392 1974.
- [35] S. Suwanrumpha and R. B. Freast, “Identification of Metabolites of Ampicillin Using Liquid Chromatography/Thermospray Mass Spectrometry and Fast Atom Bombardment Tandem Mass Spectrometry,” *Biomed Environ Mass Spectrom*. 1989. doi: 10.1002/bms.1200181106
- [36] J. H. C. Nayler, “Structure-Activity Relationships in Semi-Synthetic Penicillins,” *Proceedings of the Royal Society of London*, 1971.
- [37] R. E. W. Hancock and A. Bell, “• Antibiotic Uptake into Gram-Negative Bacteria,” *Eur J Clin Microbiol Infect Dis*. 1988. doi: 10.1007/BF01975036

- [38] E. Quaresima, M. O. Tinti, P. Foresta, P. De Witt, And M. T. Ramacci, "Semisynthetic .BETA.-lactam antibiotics. II. Cephalosporin derivatives in the naphthalene series Chemical and microbiological properties.," *J Antibiot (Tokyo)*, vol. 32, no. 12, pp. 1311–1318, 1979, doi: 10.7164/antibiotics.32.1311.
- [39] J. M. T. Hamilton-Miller, "Comparative activity of ampicillin and seven cephalosporins against group D streptococci," 1974. [Online]. Available: <http://jcp.bmj.com/>. 10.05.2022.
- [40] C. M. Kunin, "Oral Cephalexin and Ampicillin: Antimicrobial Activity, Recovery in Urine, and Persistence in Blood of Uremic Patients," *Ann Intern Med*, vol. 72, no. 3, p. 349, Mar. 1970, doi: 10.7326/0003-4819-72-3-349.
- [41] Q. Z. Zhai and X. D. Li, "Immobilization and sustained release of cefalexin on MCF nano-mesoporous material," *J Dispers Sci Technol*, vol. 40, no. 11, pp. 1675–1685, Nov. 2019, doi: 10.1080/01932691.2019.1615936.
- [42] G. Tiwari *et al.*, "Drug delivery systems: An updated review," *Int J Pharm Investig*, vol. 2, no. 1, p. 2, 2012, doi: 10.4103/2230-973x.96920.
- [43] L. Y. Qiu and Y. H. Bae, "Polymer architecture and drug delivery," *Pharm Res*. Jan. 2006. doi: 10.1007/s11095-005-9046-2.
- [44] S. Ghosh, "Recent research and development in synthetic polymer-based drug delivery systems," *J Chem Res*, vol. 2004, no. 4, pp. 241–246, Apr. 2004, doi: 10.3184/0308234041209158.
- [45] A. Khazaei *et al.*, "Grafting amino drugs to poly(styrene-alt-maleic anhydride) as a potential method for drug release," *J Braz Chem Soc*, vol. 24, no. 7, pp. 1109–1115, 2013, doi: 10.5935/0103-5053.20130145.
- [46] Y. Song, Y. Cong, B. Wang, and N. Zhang, "Applications of Fourier transform infrared spectroscopy to pharmaceutical preparations," *Expert Opin Drug Deliv*, vol. 17, no. 4, pp. 551–571, Apr. 2020, doi: 10.1080/17425247.2020.1737671.
- [47] I. Popescu, D. Suflet, I. Pelin, and G. C. Chițanu, "Biomedical applications of maleic anhydride copolymers," *Revue Roumaine de Chimie*, vol. 56, pp. 173–188, Mar. 2011.
- [48] N. Kamaly, B. Yameen, J. Wu, and O. C. Farokhzad, "Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release.," *Chem Rev*, vol. 116, no. 4, pp. 2602–63, Feb. 2016, doi: 10.1021/acs.chemrev.5b00346.
- [49] Q. Feng and R. Tong, "Anticancer nanoparticulate polymer-drug conjugate," *Bioeng Transl Med*, vol. 1, no. 3, pp. 277–296, Sep. 2016, doi: <https://doi.org/10.1002/btm2.10033>.
- [50] D. Hood and O. Musa, "Application of Maleic Anhydride-Based Materials," 2016. doi: 10.1007/978-3-319-29454-4_10.
- [51] T. Pompe *et al.*, "Maleic Anhydride CopolymersA Versatile Platform for Molecular Biosurface Engineering," *Biomacromolecules*, vol. 4, no. 4, pp. 1072–1079, Jul. 2003, doi: 10.1021/bm034071c.

- [52] M. D. Franko, J. E. Gates, and R. M. Ottenbrite, "Synthesis and in vitro Antimicrobial Activity of Chloramphenicol- Conjugated Copolymers," *J Bioact Compat Polym*, vol. 5, no. 3, pp. 283–292, Jul. 1990, doi: 10.1177/088391159000500304.
- [53] S. M. Mortada and N. A. Boraie, "The possible application of polyvinyl methyl ether/maleic anhydride," *Pharmazie*, vol. 43, pp. 627–629, 1988.
- [54] C. L. Burnett *et al.*, "Final Report of the Amended Safety Assessment of PVM/MA Copolymer and Its Related Salts and Esters as Used in Cosmetics," *Int J Toxicol*, vol. 30, no. 5_suppl, pp. 128S–144S, Oct. 2011, doi: 10.1177/1091581811407934.
- [55] De Souza Ligia Elene, "Poly(vinyl methyl ether-alt-maleic anhydride) based nanoparticles and nanocapsules - formulation and characterization," 2017. [Online]. Available: <http://dx.doi.org/10.25673/2185>. 12.04.2022
- [56] U. Schmidt, S. Zschoche, and C. Werner, "Modification of poly(octadecene-alt-maleic anhydride) films by reaction with functional amines," *J Appl Polym Sci*, vol. 87, no. 8, pp. 1255–1266, Feb. 2003, doi: <https://doi.org/10.1002/app.11527>.
- [57] Z. Jin *et al.*, "Modification of Poly(maleic anhydride)-Based Polymers with H₂N–R Nucleophiles: Addition or Substitution Reaction?," *Bioconjug Chem*, vol. 30, no. 3, pp. 871–880, Mar. 2019, doi: 10.1021/acs.bioconjchem.9b00008.
- [58] P. McCarron, A. Woolfson, R. Donnelly, G. Andrews, A. Zawislak, and J. Price, "Influence of Plasticiser type and storage conditions on the properties of poly(methyl vinyl ether-co-maleic anhydride) bioadhesive films," *J Appl Polym Sci*, vol. 91, pp. 1576–1589, Feb. 2004, doi: 10.1002/app.13228.
- [59] L. Goetz, M. Foston, A. P. Mathew, K. Oksman, and A. J. Ragauskas, "Poly(methyl vinyl ether-co-maleic acid)–Polyethylene Glycol Nanocomposites Cross-Linked In Situ with Cellulose Nanowhiskers," *Biomacromolecules*, vol. 11, no. 10, pp. 2660–2666, Oct. 2010, doi: 10.1021/bm1006695.
- [60] E. Elizondo, A. Córdoba, S. Sala, N. Ventosa, and J. Veciana, "Preparation of biodegradable poly (methyl vinyl ether-co-maleic anhydride) nanostructured microparticles by precipitation with a compressed antisolvent," *J Supercrit Fluids*, vol. 53, no. 1–3, pp. 108–114, Jun. 2010, doi: 10.1016/j.supflu.2010.02.018.
- [61] F. Bettahar *et al.*, "Tough Hydrogels Based on Maleic Anhydride, Bulk Properties Study and Microfiber Formation by Electrospinning," *Polymers (Basel)*, vol. 13, no. 6, p. 972, Mar. 2021, doi: 10.3390/polym13060972.
- [62] G. Lawson, J. Ogwu, and S. Tanna, "Quantitative screening of the pharmaceutical ingredient for the rapid identification of substandard and falsified medicines using reflectance infrared spectroscopy," *PLoS One*, vol. 13, no. 8, pp. e0202059-, Aug. 2018, [Online]. Available: <https://doi.org/10.1371/journal.pone.0202059>. 10.05.2022

- [63] A. V. Ewing and S. G. Kazarian, "Recent advances in the applications of vibrational spectroscopic imaging and mapping to pharmaceutical formulations," *Spectrochim Acta A Mol Biomol Spectrosc*, vol. 197, pp. 10–29, May 2018, doi: 10.1016/j.saa.2017.12.055.
- [64] J. Shi, L. Liu, X. Sun, S. Cao, and J. F. Mano, "Biom mineralized Polysaccharide Beads for Dual-Stimuli-Responsive Drug Delivery," *Macromol Biosci*, vol. 8, no. 3, pp. 260–267, Mar. 2008, doi: 10.1002/mabi.200700177.
- [65] N. Alotaibi *et al.*, "Toward Point-of-Care Drug Quality Assurance in Developing Countries: Comparison of Liquid Chromatography and Infrared Spectroscopy Quantitation of a Small-Scale Random Sample of Amoxicillin," *Am J Trop Med Hyg*, vol. 99, no. 2, pp. 477–481, Aug. 2018, doi: 10.4269/ajtmh.17-0779.
- [66] C. A. Coutts-Lendon, N. A. Wright, E. V Mieso, and J. L. Koenig, "The use of FT-IR imaging as an analytical tool for the characterization of drug delivery systems," *Journal of Controlled Release*, vol. 93, no. 3, pp. 223–248, 2003, doi: <https://doi.org/10.1016/j.jconrel.2003.06.003>.
- [67] V. Balan *et al.*, "Vibrational Spectroscopy Fingerprinting in Medicine: from Molecular to Clinical Practice," *Materials*, vol. 12, no. 18, p. 2884, Sep. 2019, doi: 10.3390/ma12182884.
- [68] M. J. Walsh *et al.*, "FTIR Microspectroscopy Coupled with Two-Class Discrimination Segregates Markers Responsible for Inter- and Intra-Category Variance in Exfoliative Cervical Cytology," *Biomark Insights*, vol. 3, p. BMI.S592, Jan. 2008, doi: 10.4137/BMI.S592.
- [69] S.-L. Chau, H.-W. Tang, and K.-M. Ng, "Gold nanoparticles bridging infrared spectroscopy and laser desorption/ionization mass spectrometry for direct analysis of over-the-counter drug and botanical medicines," *Anal Chim Acta*, vol. 919, pp. 62–69, May 2016, doi: 10.1016/j.aca.2016.03.023.
- [70] Y. Tozuka, A. Wongmekiat, K. Kimura, K. Moribe, S. Yamamura, and K. Yamamoto, "Effect of Pore Size of FSM-16 on the Entrapment of Flurbiprofen in Mesoporous Structures," *Chem Pharm Bull (Tokyo)*, vol. 53, no. 8, pp. 974–977, 2005, doi: 10.1248/cpb.53.974.
- [71] H. Zhang *et al.*, "A systematic study of captopril-loaded polyester fiber mats prepared by electrospinning," *Int J Pharm*, vol. 439, no. 1–2, pp. 100–108, Dec. 2012, doi: 10.1016/j.ijpharm.2012.09.055.
- [72] J. Lu, S. Obara, F. Liu, W. Fu, W. Zhang, and S. Kikuchi, "Melt Extrusion for a High Melting Point Compound with Improved Solubility and Sustained Release," *AAPS PharmSciTech*, vol. 19, no. 1, pp. 358–370, Jan. 2018, doi: 10.1208/s12249-017-0846-6.
- [73] N. Ramazanzadeh and N. O. Mahmoodi, "Drug delivery system for controlled release of empagliflozin from alginate-chitosannanocarrier system," *Turk J Chem*, vol. 46, no. 3, pp. 805–813, Jan. 2022, doi: 10.55730/1300-0527.3370.

- [74] F. N. Kasim Archana Somasekharan; Lalitha Balachandran Aswathy; Sooraj Moorikoval Parambil; Nair Anoop Somasekharan, "Preparation, Characterization and Evaluation of a Novel Drug Carrier for the Controlled Release of Curcumin," *Drug Res*, vol. 73, no. 04, pp. 224–231, 2023, doi: 10.1055/a-1995-5303.
- [75] S. G. Kazarian and K. L. A. Chan, "Applications of ATR-FTIR spectroscopic imaging to biomedical samples," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1758, no. 7, pp. 858–867, 2006, doi: <https://doi.org/10.1016/j.bbamem.2006.02.011>.
- [76] S. E. Glassford, B. Byrne, and S. G. Kazarian, "Recent applications of ATR FTIR spectroscopy and imaging to proteins," *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1834, no. 12, pp. 2849–2858, 2013, doi: <https://doi.org/10.1016/j.bbapap.2013.07.015>.
- [77] V. Pincioli, V. Rizzo, F. Angelucci, M. Tatò, and A. Vigevani, "Characterization of Methacrylamide 1H NMR Polymer Conjugates with the Anti-Cancer Drug Doxorubicin," *Magn. Reson. Chem.* 1997.
- [78] J. U. Izunobi and C. L. Higginbotham, "Polymer Molecular Weight Analysis by 1H NMR Spectroscopy," *J Chem Educ*, vol. 88, no. 8, pp. 1098–1104, Aug. 2011, doi: 10.1021/ed100461v.
- [79] K. Saalwächter, "Chapter 1: Applications of NMR in Polymer Characterization-An Introduction," in *New Developments in NMR*, vol. 2019-January, no. 20, Royal Society of Chemistry, 2019, pp. 3–22. doi: 10.1039/9781788016483-00001.
- [80] F. Podczeck, C. L. Mitchell, J. M. Newton, D. Evans, and M. B. Short, "The gastric emptying of food as measured by gamma-scintigraphy and electrical impedance tomography (EIT) and its influence on the gastric emptying of tablets of different dimensions," *Journal of Pharmacy and Pharmacology*, vol. 59, no. 11, pp. 1527–1536, Feb. 2010, doi: 10.1211/jpp.59.11.0010.
- [81] Söderlind E. and Dressman J.B., "Physiological Factors Affecting Drug Release and Absorption," in *Oral Drug Absorption*, Second Edition., Söderlind E. and Dressman J.B., Eds., 2010.
- [82] Nugent S.G., Kumar D., Rampton D.S., and Evans D.F., "Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs," *Gut*. vol. 48, no. 4, pp. 571–577, Apr. 2001.
- [83] M. R. C. Marques, R. Loebenberg, and M. Almukainzi, "Simulated biological fluids with possible application in dissolution testing," 2011, *Dissolution Technologies Inc.* doi: 10.14227/DT180311P15.
- [84] S. S. Castle, "Ampicillin," *xPharm: The Comprehensive Pharmacology Reference*, pp. 1–6, Jan. 2007, doi: 10.1016/B978-008055232-3.61227-9.
- [85] H. Lode, P. Janisch, G. Kiipper, and H. Weuta, "Comparative Clinical Pharmacology of Three Ampicillins and Amoxicillin Administered Orally Downloaded from," 2015. [Online]. Available: <http://jid.oxfordjournals.org/>. 05.06.2022

- [86] J. Jiang, G. Oberdörster, and P. Biswas, "Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies," *Journal of Nanoparticle Research*, vol. 11, no. 1, pp. 77–89, Jan. 2009, doi: 10.1007/s11051-008-9446-4.
- [87] P. R. Lockman, J. M. Koziara, R. J. Mumper, and D. D. Allen, "Nanoparticle Surface Charges Alter Blood–Brain Barrier Integrity and Permeability," *J Drug Target*, vol. 12, no. 9–10, pp. 635–641, Dec. 2004, doi: 10.1080/10611860400015936.
- [88] C. Stillhart *et al.*, "Impact of gastrointestinal physiology on drug absorption in special populations—An UNGAP review," *European Journal of Pharmaceutical Sciences*, vol. 147, p. 105280, 2020, doi: <https://doi.org/10.1016/j.ejps.2020.105280>.
- [89] M. Koziolok *et al.*, "Investigation of pH and Temperature Profiles in the GI Tract of Fasted Human Subjects Using the Intellicap® System," *J Pharm Sci*, vol. 104, no. 9, pp. 2855–2863, 2015, doi: <https://doi.org/10.1002/jps.24274>.
- [90] K. Khalid, R. Ishak, and Z. Z. Chowdhury, "Chapter 15 - UV–Vis spectroscopy in non-destructive testing," in *Non-Destructive Material Characterization Methods*, A. Otsuki, S. Jose, M. Mohan, and S. Thomas, Eds., Elsevier, 2024, pp. 391–416. doi: <https://doi.org/10.1016/B978-0-323-91150-4.00021-5>.
- [91] A. Asan and N. Seddiq, "A Simple Spectrophotometric Determination of Amoxicillin in Drug Samples," *Journal of the Turkish Chemical Society Section A: Chemistry*, vol. 9, pp. 483–492, Mar. 2022, doi: 10.18596/jotcsa.978686.
- [92] W. A., T. B., S. Gholve, Dr. O. Bhusnure, T. S.S., and V. Thakare, "UV Spectrophotometric Stability Indicating Method Development and Validation for the Determination of Tenoxicamin Bulk and Dosage Form," *Int. J. Pharm. Sci. Rev. and Res.*, vol. 45, pp. 74–77, Jul. 2017.
- [93] P. Polyák, D. M. Tilinger, and B. Pukánszky, "A simple spectroscopic method for the determination of the release kinetics of drugs from PHB," *Polym Test*, vol. 81, p. 106269, 2020, doi: <https://doi.org/10.1016/j.polymertesting.2019.106269>.
- [94] K. Cakiral and D. Sakar, "Modification of poly(methyl vinyl ether – alt – maleic anhydride) with pregabalin drug active substance via ring opening polymerization of anhydride ring in/noncatalyst media," *Polymer Bulletin*, Jul. 2022, doi: 10.1007/s00289-022-04410-z.
- [95] G. Sandri, M. C. Bonferoni, F. Ferrari, S. Rossi, and C. M. Caramella, "The Role of Particle Size in Drug Release and Absorption," 2014, pp. 323–341. doi: 10.1007/978-3-319-00714-4_11.
- [96] A. A. Barba, S. Bochicchio, A. Dalmoro, D. Caccavo, S. Cascone, and G. Lamberti, "Polymeric and lipid-based systems for controlled drug release: An engineering point of view," in *Nanomaterials for Drug Delivery and Therapy*, Elsevier, 2019, pp. 267–304. doi: 10.1016/B978-0-12-816505-8.00013-8.

- [97] E. Joseph and G. Singhvi, "Multifunctional nanocrystals for cancer therapy: A potential nanocarrier," in *Nanomaterials for Drug Delivery and Therapy*, Elsevier, 2019, pp. 91–116. doi: 10.1016/B978-0-12-816505-8.00007-2.
- [98] K. H. Buschow, R. W. , Cahn, M. C. Flemings, B. Ilschner, E. J. Kramer, and S. Mahajan, "Encyclopedia of Materials: Science and Technology," *MRS Bull*, vol. 29, no. 7, pp. 512–513, Jul. 2004, doi: 10.1557/mrs2004.147.
- [99] R. J. Hunter, *Zeta Potential in Colloid Science*. Elsevier, 1981. doi: 10.1016/C2013-0-07389-6.
- [100] C. Freitas and R. H. Müller, "Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLNTM) dispersions," *Int J Pharm*, vol. 168, no. 2, pp. 221–229, 1998, doi: [https://doi.org/10.1016/S0378-5173\(98\)00092-1](https://doi.org/10.1016/S0378-5173(98)00092-1).
- [101] M. Mehta, J. Shah, T. Khakhkhar, R. Shah, and K. G. Hemavathi, "Anticonvulsant hypersensitivity syndrome associated with carbamazepine administration: Case series," *J Pharmacol Pharmacother*, vol. 5, no. 1, pp. 59–62, Mar. 2014, doi: 10.4103/0976-500X.124428.
- [102] M. Danaei *et al.*, "Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems," *Pharmaceutics*, vol. 10, no. 2, p. 57, May 2018, doi: 10.3390/pharmaceutics10020057.
- [103] M. Instruments, "Dynamic Light Scattering Common Terms Defined," 2011. [Online]. Available: www.malvern.com/contact. 15.04.2021.
- [104] M. Instruments, "Tech Note: Zeta potential - An introduction in 30 minutes."
- [105] S. N. M. Zainon and W. H. Azmi, "Recent Progress on Stability and Thermo-Physical Properties," 12, vol. 2, p. 176, 2021, doi: <https://doi.org/10.3390/mi12020176>.
- [106] J. Goscińska, A. Ejsmont, A. Stasiłowicz-Krzemień, S. Sip, and J. Cielecka-Piontek, "Enhancing antimicrobial activity of β -lactam antibiotic via functionalized mesoporous carbon-based delivery platforms," *Microporous and Mesoporous Materials*, vol. 343, p. 112160, 2022, doi: <https://doi.org/10.1016/j.micromeso.2022.112160>.
- [107] A. V. Delgado, F. González-Caballero, R. J. Hunter, L. K. Koopal, and J. Lyklema, "Measurement and Interpretation of Electrokinetic Phenomena (IUPAC Technical Report)," *Pure and Applied Chemistry*, vol. 77, no. 10, pp. 1753–1805, Jan. 2005, doi: 10.1351/pac200577101753.
- [108] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," Apr. 01, 2016, *Xi'an Jiaotong University*. doi: 10.1016/j.jpha.2015.11.005.
- [109] S. Aydemir, Z. Gulay, U. Hasdemir, and Z. C. Karahan, "Antibiyotik Duyarlılık Testleri, EUCAST: Uygulama, Yorum ve Uzman Kurallar." [Online]. Available: <https://www.researchgate.net/publication/309732190>. 22.10.2021.
- [110] C. Bisson-Boutelliez, S. Fontanay, C. Finance, and F. Kedzierewicz, "Preparation and Physicochemical Characterization of Amoxicillin β -cyclodextrin Complexes," *AAPS PharmSciTech*, vol. 11, no. 2, pp. 574–581, 2010, doi: 10.1208/s12249-010-9412-1.

- [111] A. Zielinska *et al.*, “Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology,” Aug. 01, 2020, MDPI AG. doi: 10.3390/molecules25163731.
- [112] J. A. Hindler and Clinical and Laboratory Standards Institute., *Analysis and presentation of cumulative antimicrobial susceptibility test data : approved guideline*.
- [113] M. Guo *et al.*, “Impacts of particle shapes on the oral delivery of drug nanocrystals: Mucus permeation, transepithelial transport and bioavailability,” *Journal of Controlled Release*, vol. 307, pp. 64–75, 2019, doi: <https://doi.org/10.1016/j.jconrel.2019.06.015>.



PUBLICATIONS FROM THE THESIS

Conference Papers

1. Aysegul Kahraman and Dolunay Şakar, “Synthesis And Characterization of Poly(ethylene-alt-maleic anhydride)-Ampicillin Trihydrate Polymer-Drug Delivery System In a Sub-Micro Scale” in *2nd International Graduate Studies Congress (IGSCONG’22)*, 2022, pp. 738–739.

Papers

1. A. Kahraman, D. Sakar, and M. Altikatoglu Yapaoz, “Maleic Anhydride-Derived Copolymers Conjugated with Beta-Lactam Antibiotics: Synthesis, Characterization, In Vitro Activity/Stability Tests with Antibacterial Studies,” *Applied Sciences*, vol. 14, no. 14, p. 6112, Jul. 2024, doi: 10.3390/app14146112.