



FACULTY OF CHEMISTRY

DEPARTMENT OF PHYSICAL CHEMISTRY AND TECHNOLOGY OF POLYMERS

Aleksy Mazur MSc Eng.

DOCTORAL DISSERTATION

Designing co-delivery drug systems based on graft copolymer carriers prepared from biofunctionalized monomeric ionic liquids

Projektowanie układów współdostarczania leków na bazie nośników o topologii polimerów szczepionych otrzymanych z udziałem biofunkcjonalizowanych monomerycznych cieczy jonowych

A Guide to the Monothematic Publication Cycle

Supervisor: Prof. Dorota Neugebauer, PhD, DSc Eng.

Scientific discipline: Chemical Sciences

Gliwice 2026

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my supervisor, prof. PhD, DSc Eng. **Dorota Neugebauer**, for her invaluable mentorship, continuous support, and guidance throughout my doctoral studies. Our meetings and insightful discussions combined with her broad scientific perspective and invaluable feedback, have been instrumental in shaping my approach to research. Her expertise and encouragement significantly shaped my development as a researcher.

I would like to express my gratitude to the department staff, particularly **Adam, Ania, and Sylwia**, for their daily assistance and cooperation. Furthermore, I want to reserve a special mention for **Kasia**. Thank you for your unwavering support and for creating an environment where I could thrive. Your assistance was invaluable, and finishing it without your help would have been much more difficult.

To my beloved **Magda**; thank you for your infinite patience, love, and for standing by my side throughout this incredibly bumpy road. Thank you for always being there for me when things got stressful, and your unwavering belief in me meant the world. I could not have done this without you.

I am profoundly grateful to my **parents**. Thank you for your unconditional love, endless support, and for providing the foundation that allowed me to reach this point in my life. Everything I have achieved is built upon the opportunities you gave me.

Stepping away from the academic world, I want to express my gratitude to the **Brazilian Jiu-Jitsu (BJJ) community and my teammates from the mats**. BJJ provided the perfect mental reset and a much-needed break from the daily stress. The time spent training kept me grounded, strengthened my resilience, and taught me how to push through difficult moments.

A special shout-out goes to my **friends** and half-brother **Szymon** for our regular get-togethers and countless epic matches of Rebellion. Thank you for helping me clear my mind and for those unique, alternative "brainstorming" sessions. They never failed to spark my creativity and allowed me to look at complex problems from a completely different perspective. Those who know, know.

Finally, I want to take a moment to thank **myself**. Thank you for the perseverance, the countless hours of hard work, and for stubbornly refusing to give up, even when walking away seemed like the easiest option.

STRESZCZENIE ROZPRAWY DOKTORSKIEJ

Innowacyjne jonowe polimery szczepione zostały zaprojektowane jako nośniki dostarczające leki przeciwbakteryjne. Przedstawione układy powstały z udziałem monomerycznych cieczy jonowych (MIL) na bazie choliny, zawierających aniony farmaceutyczne.

Przygotowanie biofunkcjonalizowanych MIL polegało na wprowadzeniu anionów farmaceutycznych w wyniku wymiany anionu w chlorku [2-(metakryloksy)etylo]trimetyloamoniowym (TMAMA). Aniony leków wybrane do badania to: fusydowy (FUS), kloksacyliny (CLX), ampicyliny (AMP), *p*-aminosalicylanu (PAS), piperacyliny (PIP), tazobaktamu (TAZ), ibuprofenu (IBU), diklofenaku (DIC), naproksenu (NAP), nalidyksowe (NAL), walproinowe (VPA), cholowe (CHA) oraz asparaginowe (APT). Pomyślnie biomodyfikowane MIL (TMAMA/FUS, TMAMA/CLX, TMAMA/AMP, TMAMA/PAS, TMAMA/PIP, TMAMA/TAZ, TMAMA/DIC, TMAMA/IBU, TMAMA/VPA) zostały następnie poddane kopolimeryzacji z metakrylanem metylu przy wykorzystaniu kontrolowanej polimeryzacji rodnikowej z przeniesieniem atomu (ATRP) metodą „szczepienia z” wielofunkcyjnego makroinicjatora. Rezultatem były dobrze-zdefiniowane kopolimery szczepione, które zawierały spolimeryzowane cieczы jonowe (PIL) w łańcuchach bocznych, określane jako koniugaty jonowe polimer-lek. Jednakże, polimeryzacje TMAMA modyfikowanej anionami FUS, CLX, AMP, PAS oraz PIP zakończyły się sukcesem. Stopień szczepienia łańcuchów bocznych był zdefiniowany przez makroinicjator, zaś ich długość była regulowana podczas polimeryzacji, aby uzyskać PIL o precyzyjnie dopasowanych parametrach strukturalnych i ściśle określonej zawartości leku.

Oprócz systemów dostarczających jeden lek, w analogiczny sposób zaprojektowano nośniki do terapii skojarzonych. Obejmowało to kopolimeryzację dwóch biomodyfikowanych MIL różniących się anionem farmaceutycznym. Otrzymane koniugaty stanowiły układy podwójne do współdostarczania par anionowych leków FUS-CLX i AMP-CLX. Podczas samoorganizacji amfifilowych koniugatów PAS prowadzących do powstawania struktur micelarnych, izoniazyd był kapsułkowany w celu wytworzenia innego typu układów współdostarczania. Ponadto przeprowadzono wymianę jonową na koniugatach z PIP w celu wprowadzenia jonów tazobaktamu (TAZ). W rezultacie otrzymano kolejny rodzaj koniugatów do współdostarczania anionów PIP-TAZ.

Zachowanie uwalniania leku *in vitro* zazwyczaj wykazywało początkową fazę gwałtownego uwalniania, po której następowała faza przedłużonego uwalniania trwająca do 72 godzin. Na kinetykę uwalniania leku istotny wpływ miały charakter leku i struktura matrycy polimerowej. Podsumowując, zaprojektowane systemy polimerowe na bazie choliny wykazały dużą zdolność do ładowania i kapsułkowania leków, co podkreśla ich potencjał jako zaawansowanych nośników w terapii przeciwbakteryjnej, w tym współuwalniania leków w terapii skojarzonej.

SUMMARY OF DOCTORAL DISSERTATION

The innovative ionic graft polymers were designed as a carriers for antibacterial drug delivery. The presented systems are derived from choline-based monomeric ionic liquids (MILs) containing pharmaceutical anions.

The preparation of biofunctionalized MIL involved the introduction of pharmaceutical anions by exchange of anion in the [2-(methacryloyloxy)ethyl]trimethyl-ammonium chloride (TMAMA). Drug anions selected for this study are: fusidate (FUS), cloxacillin (CLX), ampicillin (AMP), *p*-aminosalicylate (PAS), piperacillin (PIP), tazobactam (TAZ), ibuprofen (IBU), diclofenac (DIC), naproxen (NAP), nalidixic (NAL), valproate (VPA), cholic (CHA), and aspartic (APT). Successfully biofunctionalized MILs (TMAMA/FUS, TMAMA/CLX, TMAMA/AMP, TMAMA/PAS, TMAMA/PIP, TMAMA/TAZ, TMAMA/DIC, TMAMA/IBU, and TMAMA/VPA) were subsequently copolymerized with methyl methacrylate via controlled atom transfer radical polymerization (ATRP) by *grafting from* multifunctional macroinitiator. This generated the well-defined graft copolymers with polymerized ionic liquids (PILs) in side chains defined as ionic graft polymer-drug conjugates. However, the polymerizations of TMAMA modified with FUS, CLX, AMP, PAS and PIP were successful. The grafting degree of side chains was defined by macroinitiator, and the length of side chains was adjusted during polymerization to develop polymer conjugates with precisely tailored structural parameters and drug contents.

Besides single drug delivery systems, the carriers for combination therapies were designed in analogous manner. This involved copolymerization of two biomodified MILs with different pharmaceutical anions. The obtained dual-drug conjugates contained FUS-CLX and AMP-CLX drug anion pairs. During self-assembly of amphiphilic PAS-conjugates into micellar structures, isoniazid (ISO) was encapsulated to produce another type of co-delivery systems. Furthermore, ion exchange was conducted on PIP-conjugates to introduce tazobactam (TAZ) ions. Consequently another type of dual-drug conjugates carrying PIP-TAZ anions were generated.

The drug release behavior evaluated *in vitro* typically demonstrated initial burst release phase, followed by a sustained release lasting up to 72 hours. The kinetic of drug release was influenced by the chemical nature of the drugs and the structure of polymer matrix. Overall, the designed choline-based polymer systems indicated high drug-loading capacities and effective encapsulation, underscoring their potential as advanced carriers for antibacterial therapy, including drug co-delivery in combined therapy.

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GLOSSARY

AMP	Ampicillin
ARD	Percentage amount of the released drug
ATRP	Atom transfer radical polymerization
bpy	2,2'-Bipyridine
dNbpy	4,4'-Dinonyl-2,2'-dipyridyl
BiBB	α -Bromoisobutryl bromide
BIEM	2-(2-bromoisobutyryloxy)ethyl methacrylate
CLX	Cloxacillin
CMC	Critical micelle concentration
CuBr	Copper(I) bromide
CuCl	Copper(I) chloride
CRD	Concentrations of released drug
$\bar{D}$	Dispersity index of polymer
DC	Drug content
DDS	Drug delivery system
DG	Grafting degree
DLC	Drug loading content
DLS	Dynamic light scattering
DMF	<i>N,N</i> -dimethylformamide
DMSO-d6	Deuterated dimethyl sulfoxide
D_H	Hydrodynamic diameter
DP_n	Degree of polymerization of the main chain
DP_{sc}	Degree of polymerization of the side chains
EBiB	Ethyl α -bromoisobutyrate
EPR	Enhanced permeability and retention (effect)
FUS	Fusidate
FT-IR	Fourier-transform infrared (spectroscopy)
F_{TMAMA}	Content of TMAMA fraction
^1H NMR	Proton nuclear magnetic resonance (spectroscopy)
IL	Ionic liquid
ISO	Isoniazid
MMA	Methyl methacrylate
MeOH	Methanol
MI	Macroinitiator
MIL	Monomeric ionic liquid
M_n	Number average molecular weight of polymer
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

n _{sc}	Number of side chains
PAS	<i>Para</i> -aminosalicylate
PBS	Phosphate-buffered saline
PDI	Polydispersity index of polymer particles
PEG	Polyethylene glycol
PIL	Poly(ionic liquid)/polymerized ionic liquid
PIP	Piperacillin
R ²	Correlation coefficient
SEC	Size exclusion chromatography
TAZ	Tazobactam
THF	Tetrahydrofuran
TMAMA	[2-(Methacryloyloxy)ethyl]trimethylammonium chloride
UV-Vis	Ultraviolet-visible (spectrophotometry)
WCA	Water contact angle

LIST OF MONOTHEMATIC PUBLICATIONS, RELATED PATENTS AND AUTHOR CONTRIBUTIONS

Monothematic publications:

This dissertation comprises five monothematic scientific papers, published in Journal Citation Reports (JCR) indexed journals between 2022 and 2025. These papers, detailed below, are cited throughout this thesis using abbreviated identifiers (P). This publication-based dissertation consists of thematically related journal articles. The full texts, author contributions, and the signed co-author declarations are included in the Appendices to the Doctoral Dissertation.

P.1. Pharmaceutical Functionalization of Monomeric Ionic Liquid for the Preparation of Ionic Graft Polymer Conjugates.

Mazur, A., Niesyto, K., and Neugebauer, D.

International Journal of Molecular Sciences, **2022**, 23(23), 14731. (IF₂₀₂₂ = 5.6; MEiN = 140 pkt)

P.2. Characterization of Graft Copolymers Synthesized from *p*-Aminosalicylate Functionalized Monomeric Choline Ionic Liquid.

Mazur, A., Neugebauer, D.

Pharmaceutics, **2023**, 15(11), 2556. (IF₂₀₂₂ = 4.9; MEiN = 140 pkt)

P.3. Ionic liquid-based polymer matrices for single and dual drug delivery: impact of structural topology on characteristics and in vitro delivery efficiency.

Niesyto, K., Keihankhadiv, S., Mazur, A., Mielańczyk, A. and Neugebauer, D.

International Journal of Molecular Sciences, **2024**, 25(2), p.1292. (IF₂₀₂₄ = 4.9; MEiN = 140 pkt, TOP10 wg bazy Scopus)

P.4. Graft Polymers Derived from Pharmaceutically Active Choline-Based Ionic Liquid Monomers: Dual Incorporation of Ampicillin and Cloxacillin.

Mazur, A. and Neugebauer, D.

International Journal of Molecular Sciences, **2025**, 26(19), 9415. (IF₂₀₂₅ = 4.9; MEiN = 140 pkt, TOP10 wg bazy Scopus)

P.5. Sequential Biofunctionalization of a Choline-Based Monomeric Ionic Liquid and Polymerized Ionic Liquid: A Route to Dual Anionic Drug Polymer Conjugates of Piperacillin–Tazobactam

Mazur, A., Neugebauer, D.

ACS Biomaterials Science and Engineering, **2026**, 12(8), 4113–4124. (IF₂₀₂₆ = 6.0; MEiN = 140 pkt)

Patents:

Mazur A., Niesyto K., Neugebauer D. “Method for preparing ionic conjugates and their application”. Polish Patent No. 247411, granted Apr 14, 2025.

Mazur A., Niesyto K., Neugebauer D. “Method for preparing micellar ionic conjugates based on graft polymers for the co-delivery of antibacterial drugs and their application”. Polish Patent No. 249430, granted Feb 2, 2026.

Mazur A., Niesyto K., Keihankhadiv S., Neugebauer D., “Method for preparing ionic conjugates for the co-delivery of drugs with antibacterial activity and their application”. Polish Patent No. 249823, granted Apr 8, 2026.

Mazur A., Niesyto K., Neugebauer D. “Method for preparing micellar ionic conjugates based on graft polymers for the co-delivery of antitubercular drugs and their application”. Polish Patent No. 249375, granted Jan 19, 2026.

Mazur A., Niesyto K., Keihankhadiv S., Neugebauer D., “Method for preparing ionic conjugates for the delivery of drugs with antibacterial activity and their application”. Polish Patent No. 249520, granted Feb 18, 2026.

Summary of the author contribution:

Author contributions to the publications of doctoral candidate **Aleksy Mazur**, including the percentage contribution and CRediT roles.

Participation in the conceptualization and development of the research plan; conducting the synthesis of pharmaceutically modified monomeric ionic liquids, macroinitiator precursors, macroinitiators, as well as ionic graft polymers carrying drugs; preparation of ionic drug conjugates, micelles, as drug carriers containing one or two types of drugs; performing the physicochemical characterization of the obtained carriers; conducting drug release studies; conducting biological studies; data processing, analysis, and interpretation of the results; preparation of the original drafts of publication manuscripts.

P.1

Mazur Aleksy, Niesyto Katarzyna, Neugebauer Dorota; *Pharmaceutical Functionalization of Monomeric Ionic Liquid for the Preparation of Ionic Graft Polymer Conjugates*; International Journal of Molecular Sciences, 2022, 23, 14731–14745.

Percentage contribution of the author: 50%

CRediT roles: Data curation, Formal analysis, Investigation, Writing – original draft.

P.2

Mazur Aleksy, Neugebauer Dorota; *Characterization of Graft Copolymers Synthesized from p-Aminosalicylate Functionalized Monomeric Choline Ionic Liquid*; *Pharmaceutics*, 2023, 15(11), 2556.

Percentage contribution of the author: 60%

CRedit roles: Data curation, Formal analysis, Investigation, Writing – original draft.

P.3

Niesyto Katarzyna, Keihankhadiv Shadi, Mazur Aleksy, Mielańczyk Anna and Neugebauer Dorota; *Ionic Liquid-Based Polymer Matrices for Single and Dual Drug Delivery: Impact of Structural Topology on Characteristics and In Vitro Delivery Efficiency*; *International Journal of Molecular Sciences*, 2024, 25(2), 1292.

Percentage contribution of the author: 20%

CRedit roles: Data curation, Formal analysis, Investigation, Writing – original draft.

P.4

Mazur Aleksy, Neugebauer Dorota; *Graft Polymers Derived from Pharmaceutically Active Choline-Based Ionic Liquid Monomers: Dual Incorporation of Ampicillin and Cloxacillin*; *International Journal of Molecular Sciences*, 2025, 26(19), 9415.

Percentage contribution of the author: 65%

CRedit roles: Data curation, Formal analysis, Investigation, Writing – original draft.

P.5

Mazur Aleksy, Neugebauer Dorota; *Sequential Biofunctionalization of a Choline-Based Monomeric Ionic Liquid and Polymerized Ionic Liquid: A Route to Dual Anionic Drug Polymer Conjugates of Piperacillin–Tazobactam*; *ACS Biomaterials Science and Engineering*, 2026, 12(8), 4113–4124.

Percentage contribution of the author: 70%

CRedit roles: Data curation, Formal analysis, Investigation, Writing – original draft.

The declarations of the co-authors detailing their individual contributions can be found in the appendices to this thesis.

1. INTRODUCTION

Essential for human health, drug delivery has experienced rapid growth, benefiting millions of patients and becoming a multi-billion dollar industry. Approximately 40% of approved drugs are hindered by issues such as poor water solubility, quick metabolic degradation, low permeability, and inefficient clearance. Significant progress in treating serious human and animal diseases has been made through the development of drug delivery systems (DDS) to create more effective treatments than conventional oral, nasal, intramuscular, intravenous or inhalation routes. Such systems are designed to transport therapeutic agents directly within the body, ensuring the controlled and more effective treatment. The crucial objectives of DDS include good biocompatibility, efficient and precise drug loading, robust stability, controlled drug release and the ability to overcoming biological barriers. In recent years various nanoparticles have been engineered to overcome these challenges by improving drug bioavailability, solubility and targeting, as well as by enhancing tissue permeation and prolonging retention within the body. Moreover, DDS address issues such as premature drug degradation and toxic drug concentrations and the emergence of drug resistance [1–3].

Current advancements in DDS research are increasingly directed towards utilizing polymeric biomaterials [4]. Natural polymers, e.g. albumin [5], alginate [6], chitosan [7–8], hyaluronic acid [9], collagen [10], ethyl cellulose [11], gelatin [12], or dextran [13], exhibit biocompatibility, biodegradability and a lack of immunogenicity. Several synthetic polymers, such as polyethylene glycol (PEG) [14], poly(ϵ -caprolactone) (PCL) [15], poly(lactide-co-glycolide) acid (PLGA) [16], polymethacrylates [17] including poly(2-hydroxyethyl methacrylate) [18], polyacrylates [19], or poly(vinyl alcohol) [20], offer various chemical, mechanical and degradation properties, therefore they have gained Food and Drug Administration approval and are actively used in the clinical practice.

1.1. CLASSES OF DRUG DELIVERY SYSTEMS

DDS technologies exploit nanoparticles including capsules, micelles, liposomes and also nanosuspensions or hydrogels [12, 21]. Nanosuspensions are stable, polymer colloidal dispersions of particles less than 1000 nm in size, stabilized by polymers or surfactants. They are applied in oral, pulmonary and ocular DDS [22]. Liposomes including lipid–polymer hybrid systems are spherical lipid structures, typically less than 200 nm in size, composed of phospholipid bilayers encapsulating an aqueous core. They are utilized for fungal infections, leukemia, ovarian and breast cancer [23]. Nanocapsules are composed of polymer shell and liquid core with diameter less than 1 micrometer. They have found applications in dermal, anticancer, anti-inflammatory therapies [24]. Hydrogels comprise hydrophilic polymers that form a crosslinked network in an aqueous solution, possessing the ability to absorb and retain significant amounts of water. This enables the delivery of hydrophilic drugs and cells. Their high water content and soft consistency effectively mimic extracellular matrix, making them valuable for wound healing and tissue engineering. Furthermore, their porosity, combined with mechanisms such as network degradation, swelling, and mechanical deformation, facilitates the efficient transport and controlled release of bioactive molecules, thereby enhancing their utility as moisturizing agents [25–27]. In addition it should be mentioned that other technologies have been currently utilized as nanocarriers such as carbon–based nanomaterials (nanotubes [28], quantum dots [29], graphene oxide [30]) as well as nanorobots [31]. Nanoparticle–based DDS can be synthesized through various chemical methods (such as controlled polymerizations, microemulsions, co–precipitation, chemical or electrochemical deposition) as well as physical methods (e.g. self–assembly, vapor deposition). Consequently, they offer high functionality with precise control over size, composition, and drug loading. These systems can encapsulate both lipophilic and hydrophilic drugs, providing sufficient stability and enhanced permeability through biological barriers. However, challenges remain, including issues with reproducibility costly scalability, and poor drug loading. Furthermore, there are

potential risks regarding toxicity, immunogenicity, and limited aggregation on targeted cells [32–34].

DDS can be categorized into active and passive. Former one, also called site-targeting DDS, aim for precise, site-specific therapeutic delivery, maximizing efficacy by minimizing off-target effects [35]. A strategy involves stimuli-responsive "smart" polymers that alter drug release based on exogenous or endogenous triggers. Exogenous stimuli comprise temperature (once a critical temperature is reached, it causes phase separation and conformational changes in amphiphilic macromolecules near 37°C) [36], ultrasound (inducing cavitation and heat, leading to nanostructure destabilization) [37], light/radiation (metallic nanoparticles generating localized heat), and magnetic fields (alternating fields allow imaging and modulated distribution) [38]. Endogenous stimuli include pH gradients (altering polymer ionization, leads to conformational changes in chains, and in consequence disassembly or swelling) [39], enzymatic activity (carriers contain degrading peptide links that are cleaved by specific enzymes at the target site) [40], and redox potential (e.g. disulfide bonds in polymers cleavage at high glutathione concentrations in tumors) [41]. However, such systems often face significant disadvantages. These include complex design and manufacturing processes, as well as issues like rapid clearance or deposition of the drug at specific site. Furthermore, the efficacy of some stimuli-responsive systems is hindered when the affected area does not significantly differ from normal tissues in terms of temperature or pH. While an alternative approach involves directly binding targeting moieties to drug molecules, 1:1 ratio between the targeting moiety and the therapeutic agent is not efficient [34]. In contrast, passive targeting occurs under physiological conditions and is significantly less specific. It relies on the properties of the drug carriers and the driving force gradient between the carrier and its environment. Drug release in passive systems primarily depends on diffusion, dispersion or erosion and does not require external intervention. For this type of targeting to be effective, DDS must be long-circulating in the bloodstream, maintaining a sufficient concentration of the drug carrier over extended periods after a single administration. This ensures

adequate accumulation at the target site, and the longevity of these DDS can often be improved by incorporating hydrophilic polymers [34, 42].

Furthermore, long-acting drug delivery formulations are crucial passive DDS as well as for treating chronic diseases that require extended therapy and for patients with limited healthcare access [2, 43]. Biodegradable injectable formulations, frequently exhibit a high initial burst release, especially for hydrophilic drugs. This can be problematic for drugs with a narrow therapeutic window and often reduces the formulation's retention. So far, biocompatible polymeric carriers enabled sustainable drug release over extended periods in human immunodeficiency virus, cancer, ocular, emphysema treatment, opioid withdrawal, hormone therapies [44]. Release rate of drug depends on diffusion, nanoparticle matrix biodegradation and solubility. Furthermore, drug release kinetics can be predicted and controlled by many factors: diffusion, glass transition temperature of amorphous polymers, environment in case of stimuli-responsive polymers (pH, temperature, solvent, redox, light), polymer's properties (hydrophobic/hydrophilic balance, composition, topology, chain length, molecular weight), drug size and initial loading, polymer-drug interactions, drug and polymer molecular weight [45–48]. This dissertation, focuses on passive DDS which primarily involve chemical (ionic) drug-polymer interactions. In addition, some formulations involving drug incorporation by physical interactions are also presented. Synthesized polymers are based on ionic liquid units and several systems are designed for the co-delivery of a pair of drugs. Therefore, the subsequent sections are discussing these specific concepts.

1.2. POLYMERIC CONJUGATES

A common strategy to produce DDS involves linking polymers and drug molecules by chemical interactions. Specifically, polymer-drug conjugates are typically defined as the covalent attachment of a therapeutic agent to a polymeric chain; however, novel ionic conjugation methods will be explored later in this work (section 3.2.). Polymeric conjugates, due to their sufficiently strong covalent linkage, generally offer satisfying stability, bioavailability, and solubility of the therapeutic agent. However, polymer-drug conjugates may have a lower drug

content, and in case of relatively strong polymer–drug bond can itself limit drug release. Furthermore, high molecular weight polymer–drug conjugates can leverage the enhanced permeability and retention (EPR) effect and achieve longer circulation times. This, however, simultaneously increases the potential for immunogenic long-term side effects and can impede their clearance. Additionally, drug release from polymeric conjugates is often not purely diffusion–dependent and can become less specific or retarded. Polymer–drug conjugates are capable of active drug discharge, targeting or imaging moieties can also be conjugated. Drugs are usually attached via biodegradable or stimuli–responsive linkers, which require that the polymers possess suitable functional groups. In addition, steric effects can reduce conjugation efficiency and limit drug content [49,50, 51].

To optimize polymer–drug conjugate systems, diverse polymer architectures have been examined and applied, including linear [52], graft [53], star [54], and dendritic [55]. Block copolymers are also frequently utilized [56]. Most approved DDS formulations consist of drugs conjugated to PEG [57–58], with alternatives including copolymers of *N*-(2–hydroxypropyl)methacrylamide [59], poly(2–oxazoline) [60], polysaccharides [61–64], poly(lactic acid) [65], and PLGA [66]. In addition, polymer–drug conjugates can be composed of hydrophilic or amphiphilic carriers. The latter are relevant as they are able to self–assemble into nanoparticles. However, simultaneously achieving good carrier degradability, solubility, and a high number of conjugation sites remains a significant challenge [67].

Polymeric conjugates are typically manufactured in two ways: (1) by incorporating a drug into a pre–formed polymer, or (2) by incorporating a drug into a monomer prior to polymerization. The latter approach offers higher control over drug content, which is dependent on the conversion of drug functionalized monomer and the polymerization degree [67]. Several advanced polymerization reactions have been employed for polymer–drug conjugates production, including atom transfer radical polymerization (ATRP), reversible addition–fragmentation transfer polymerization, ring–opening polymerization, and ring–opening metathesis polymerization [50].

1.3. POLYMERIC MICELLES

Beyond nanocarriers that chemically conjugate biomolecules, drug molecules can also be physically entrapped by polymers. Among the various types of nanoparticles, micellar structures have been extensively researched for drug delivery purposes. Polymeric micelles are self-assembled particles formed from amphiphilic polymers, often block copolymers, containing distinct segments with opposite affinities toward a solvent. In aqueous solutions, these polymers self-organize above a critical concentration into a core-shell structure. This consists of an inner hydrophobic core, which is protected and stabilized by an outer hydrophilic shell [68–69].

Polymeric micelles offer significant advantages over other nanocarriers, including ease of formulation, biocompatibility, and favorable pharmacokinetics. Their unique structure allows them to encapsulate water-insoluble therapeutic agents within the hydrophobic core. This shielding effect enhances drug solubility, prolongs blood circulation, and facilitates the EPR effect [70–71]. Drug encapsulation typically occurs during micelle formation using methods such as mixing, dialysis, direct dissolution, lyophilization or solvent evaporation [72–73]. However, a key challenge is stability; polymeric micelles can disassemble upon dilution in the bloodstream following administration. Therefore, copolymers with low critical micelle concentration (CMC) values are highly desired to maintain integrity. Stability can be further improved through the use of polymers with nonlinear topologies, including grafted structures, or cross-linking strategies [74]. Additionally, recent developments have focused on (multi)stimuli-sensitive micelles to increase drug delivery efficiency [75].

Regarding morphology, polymeric micelles generally range in size up to 100 nm, although drug encapsulation, fusion or aggregation can significantly increase their dimensions. The size and shape of micelles are critical, as they influence cellular accumulation, circulation time, pharmacokinetics, and biodistribution. Polymeric micelles exhibit different shapes, including spheres, rigid rods, flexible worm-like structures or vesicles. These morphological properties are dictated by the polymer's composition, architecture, chain length and the surrounding

environment or drug type and drug content [68–69,76]. Furthermore, electrostatic or steric repulsions can induce morphological transitions, such as the fusion of spherical micelles into larger cylindrical superstructures [77].

1.4. POLYMERIZED CHOLINIUM–BASED IONIC LIQUIDS

Ionic liquids (ILs) are fluids, comprised entirely of organic and/or inorganic ions. In general they possess relatively low melting temperatures (below 100°C). However, highly viscous ILs never form crystalline phase. Most common biocompatible cations are phosphonium, imidazolium, cholinium, alkylammonium, pyridinium, pyrrolidinium [78]. ILs possess unique and tunable properties such as negligible vapor pressure, high thermal and chemical stability excellent solvating properties and capability of ion exchange therefore they found numerous applications [79].

Certain ILs show significant promise in drug delivery, primarily due to their remarkable biocompatibility, negligible toxicity, and ability to solubilize or conjugate various therapeutic agents [80]. Phosphonate cations are able to improve solubility of anti-inflammatory agents [81]. Furthermore imidazole cations enhanced the solubility of psychostimulants [82], immunosuppressants [83], antibiotics [84], anti-inflammatory agents [85], antiviral agent [86], or antidepressant [87]. The reports for cholinium cation have described conjugation with various biologically active agents such as antioxidants [88], anticancer [89], antifungal [90], aminoacids [91], polyphenols [92], antivirals [93]. Moreover, choline-based ILs, including [2-(methacryloyloxy)ethyl]trimethylammonium chloride (TMAMA), demonstrate antibacterial, anti-inflammatory, antioxidant and antimicrobial activities and effective biological barrier penetration themselves [94].

However, performance of ILs can be limited due to high viscosity or low conductivity caused by the aggregation of ions [95]. To enhance their potential as DDS, ILs containing (meth)acrylic groups so called monomeric ionic liquids (MILs) can undergo primarily controlled radical polymerizations to form polymerized ILs (PILs) [96]. Imidazolium [97], cholinium [98], pyridinium [99], guanidinium [100] based MILs have been used in polymerization reactions in order to produce solid or gel materials characterized by improved processability, mechanical properties and

durability. Furthermore PILs with controlled content of the ILs species are qualified as excellent innovative materials for biomedical applications [101].

PIL-based nanoparticles were designed to transport encapsulated anticancer doxorubicin [102–103], polyphenol curcumin [104], anti-inflammatory sulfasalazine [105], antioxidant, anti-inflammatory, and vasoprotective rutin [106], immunosuppressant quercetin, anti-inflammatory indomethacin and antibiotic erythromycin [107], antibiotic rifampicin [108], or tazobactam [109]. Moreover, since PILs are susceptible to ion exchange, the pharmaceutical anions have been introduced to produce innovative ionic conjugates. Limited number of ionic conjugates have been developed e.g. systems for delivery of anti-inflammatory mefenamic acid [110] or naproxen [111], tryptophan and proline amino acids [112], anti-inflammatory, and antimicrobial salicylates [113–114], antibiotic fusidates [108], piperacillin [109], ampicillin [115–116] and cloxacillin anions [116]. Nevertheless, the examples discussed above involve ionic polymer–drug conjugates based on linear architectures [112, 114–116] or utilize DDS where drugs are incorporated into the formed nonlinear polymers via post-polymerization modification [108–110, 112–113]. Crucially, even though the same medications were investigated, this dissertation employs innovative synthetic strategy using pre-polymerization biofunctionalization, to achieve novel DDS.

1.5. NON-LINEAR POLYMER ARCHITECTURES FOR DDS – GRAFT COPOLYMERS

As mentioned in the previous sections, DDS can utilize linear or nonlinear polymer topologies, the latter including grafted architectures. Linear polymers are easily eliminated by renal filtration because they easily pass through the pores of the renal filtration membranes and thus branched architectures can be used for increasing the drugs body circulation. Moreover, branched polymers exhibit greater stability compared to carriers generated by linear polymers thus they prevent the premature and quick release of drug. In addition such nanocarriers often are characterized by lower sizes and better solubility [117–118].

Graft copolymers or copolymer brushes are composed of a linear backbone of one composition within distributed branches (side chains) of a different

composition. Well-defined graft copolymers are usually synthesized by combining controlled radical polymerizations with: *grafting from*, *grafting through* or *grafting to methods*. Controlled polymerization processes enable precise control over copolymer composition, functionality, dispersity, backbone and branch length, as well as branch spacing (grafting degree (DG)). Therefore physicochemical properties of these graft copolymers, such as their hydrophobic/hydrophilic balance, can be finely tuned [119–126]. *Grafting from* uses pre-formed macromolecules containing initiating sites (multifunctional macroinitiators (MIs)) which start subsequent polymerization of monomers [127–131]. For instance, this method allowed to obtain amphiphilic graft copolymers which were further used for loading of anti-inflammatory indomethacin [132–133], quercetin [133], or curcumin [134], antibacterial fusidic acid, rifampicin or *p*-aminosalicylic acid [108–109, 113]. *Grafting through* procedure utilizes polymerizable macromonomers, which can be copolymerized with low molecular weight monomers to regulate degree of grafting [131,135–139]. For example, copolymers composed of polyether-*b*-polymethacrylate side chains obtained via this method served as carriers for delivery indomethacin [140,141] or anticancer doxorubicin [142–143]. In case of *grafting to* various "click" chemistries are currently employed, for example alkyne-azide reaction [144–147]. Graft copolymers developed using this method demonstrated potential for the delivery of various antioxidants, such as covalently conjugated lipoic and ferulic acids [148] or encapsulated arbutin, vitamins C and E [149–150] as well as anticancer doxorubicin [151–152]. The polymers presented in this work were synthesized via *grafting from* technique combined with controlled ATRP which uses a dynamic equilibrium between active and dormant species [153]. This allowed to obtain well-defined copolymers with grafted topology, uniform distribution of polymeric chain lengths and precisely regulated compositions.

1.6. COMBINATION DRUG THERAPY

Combination therapy is a well-established strategy that enhances treatment efficiency by simultaneously delivering multiple therapeutic agents with different mechanisms of action [154]. This approach is widely utilized for managing conditions such as pain [155], hypertension [156], arrhythmia [157], cancer [158],

diabetes mellitus [159], and bipolar disorders [160]. Furthermore, the escalating global health concern of drug resistance, coupled with the decreasing development of new antibiotics, makes previously treatable infections increasingly difficult to manage. To counter this, combination therapy is frequently employed to combat antimicrobial resistance, leading to improved therapeutic outcomes in various infections, including antibiotic-resistant diseases. Nevertheless, a profound understanding of drug–drug interaction mechanisms is crucial to avoid adverse side effects, moreover these interactions can significantly alter drug bioavailability, pharmacokinetics, and pharmacodynamics [161]. Antibiotic co–delivery therapy, has been successfully applied to treat infections caused by resistant Gram–positive bacteria (e.g., *Staphylococcus aureus* [162]), resistant Gram–negative bacteria (e.g., *Pseudomonas aeruginosa* [163], *Acinetobacter baumannii* [164]), *Mycobacterium tuberculosis* [165], *Helicobacter pylori* [166], or *Escherichia coli* [167].

Commonly, most dual–drug delivery systems use non–ionic polymer carriers co–loading of two drugs by encapsulation and/or conjugation [168], a blend of two single–drug covalent conjugates or the encapsulation of drug molecules during the self–assembly of single drug covalent conjugates [169–170]. While literature reports limited examples of systems based on the ionic polymers, the development of dual–DDS utilizing PILs based on choline and ionic conjugation is a major focus of the research group directed by Professor Neugebauer. Such co–delivery platforms can be produced by:

- co–encapsulation of two drugs, e.g. chitosan–g–PEG composed of methoxide anions was used for the loading of methotrexate and doxorubicin [171] or anionic poly(glutamic acid)–PEG served as a carrier for paclitaxel and doxorubicin delivery [172],
- covalent conjugation of one drug combined with an encapsulation of another drug e.g. doxorubicin–polyethyleneimine conjugate was successful in the loading of DNA [173], whereas the cationic micelles composed of PEG–b–PCL–b–poly(2-aminoethyl ethylene phosphate)

copolymers were used for the conjugation of siRNA and the encapsulation of paclitaxel [174],

- covalent conjugation of two drugs e.g. PEGylated chitosan nanoparticles were used for the conjugation of anticancer methotrexate and mitomycin [175],
- ionic conjugation of one drug combined with the entrapment of a second drug, e.g. salicylate anions with indomethacin or erythromycin [107], fusidate anions with rifampicin [108], or piperacillin ions with tazobactam [109],
- ionic conjugation of two drugs, e.g. linear cholinium-based ionic polymer conjugates for co-delivery of ampicillin and cloxacillin ions [116].

As evidenced from the discussion above, significant advancements can still be made in the development of PIL-based ionic conjugates. Current approaches focus mostly on post-modification of PILs, or direct syntheses of drug conjugates limited to linear polymers. The state of knowledge in this area underscores the need to explore and optimize alternative synthetic methods for ionic conjugate carriers in DDS. This includes investigating non-linear PILs, which provides an opportunity to introduce larger content of drug anions within the branches, and developing their dual-drug ionic systems.

2. AIM AND METHODOLOGY OF THE RESEARCH

The primary objective of this research was to investigate and optimize novel synthetic strategies for the development of DDS based on the ionic polymer–drug conjugates of PILs with grafted architectures. The synthesis approach relied on the application of monomeric cholinium ionic liquids (MILs) functionalized with pharmaceutical anions suitable for controlled ATRP. In this case, the potential of [2–(methacryloyloxy)ethyl]–trimethylammonium chloride (TMAMA) was explored for the introduction of various pharmaceutical anions. The chloride counterions in a TMAMA underwent anion exchange with the sodium salts of selected drugs. This reaction introduced pharmaceutical anions such as cloxacillin (CLX), fusidate (FUS), *para*–aminosalicylate (PAS), ampicillin (AMP), piperacillin (PIP) into the monomer structure. The successfully prepared MILs were then polymerized by grafting from multifunctional initiator (MI) to produce grafted ionic polymer–drug conjugates in a direct manner. These DDS were designed to facilitate both single and dual–drug delivery. DDS for the combined delivery involved either completely ionic conjugate or the encapsulation of a drug within the matrix of an ionic drug–polymer conjugate.

The following main synthetic strategies were employed:

1. Direct synthesis of ionic polymer–drug conjugates:
 - a) Synthesis of macroinitiator precursors, followed by esterification to introduce initiating groups.
 - b) Copolymerization of pharmaceutically modified TMAMA and methyl methacrylate (MMA) utilizing the "grafting–from" technique from a MIs to produce well–defined graft copolymers serving as single–drug systems. The study yielded single drug ionic polymer conjugates carrying FUS, CLX, PAS, AMP, or PIP anions.
 - c) Terpolymerization of two MILs biofunctionalized with different pharmaceutical anions and MMA using MI to synthesize dual–drug systems. They were developed to carry the FUS–CLX and AMP–CLX drug anion pairs.

The ionic content was controlled by adjusting the initial comonomer feed ratios, monomer to macroinitiator ratios, number of initiating sites in MI, and monomer conversion.

2. Encapsulation of drug in the matrix of polymer–drug conjugate: amphiphilic nature of TMAMA based graft copolymers was exploited to induce self-assembly into micelles. This allowed for the physical encapsulation of selected drug. By combining ionically conjugated PAS with physically encapsulated isoniazid (ISO), advanced dual-drug micellar conjugate systems were developed for combination therapy.
3. Modification of polymer–drug conjugate: ionic PIP-based polymer conjugates were modified via ion exchange to introduce tazobactam (TAZ) anions and generate co-delivery systems.

Physicochemical characterization of TMAMA monomers and the obtained graft copolymers included proton nuclear magnetic resonance (^1H NMR) spectroscopy, Fourier transform infrared spectroscopy (FT-IR), size exclusion chromatography (SEC), dynamic light scattering (DLS), and goniometry (water contact angle (WCA) and critical micelle concentration (CMC)). Comprehensive *in vitro* studies were performed in phosphate-buffered saline (PBS) using Ultraviolet Visible (UV-Vis) spectrophotometry to analyze drug content and kinetic drug release profiles to show correlation of structural parameters with delivery performance, thereby identifying the most efficient polymer carriers. An assessment of the cytotoxicity of selected DDS was performed.

3. RESULTS AND DISCUSSION

3.1. SYNTHESIS OF CHOLINE BASED MILS WITH PHARMACEUTICAL ANIONS (P.1; P.2; P.4; P.5)

The TMAMA composed of a polymerizable methacrylic group, a quaternary ammonium cation, and a chloride counterion prone to ionic exchange, was biofunctionalized with various selected pharmaceutical anions (Figure 1, Table 1). Successful ionic conjugation to TMAMA was achieved with the sodium salts of CLX, FUS, PAS, AMP, PIP, TAZ, ibuprofen, diclofenac, and valproate in the molar ratio 1:1 using methanol (MeOH) as a solvent. However, the ion exchange reactions with anti-inflammatory ibuprofenate and diclofenac were slow and required prolonged reaction times to achieve satisfying yields. Furthermore, other biologically active agents, including nalidixic, cholic, aspartic, valproic, and naproxen were examined, but their ion exchange reactions with TMAMA proved inefficient. Specifically, the efficiency of ion exchange was hindered for drug anions containing pyridyl or dicarboxylic groups or phenyl ring with methoxy substituent. In these cases, when reaction times exceeded 4h, the process was dominated by the competitive transesterification of the TMAMA with MeOH. Consequently, the efficiently biofunctionalized MILs with incorporated CLX, FUS, PAS, AMP, PIP, TAZ, ibuprofen, diclofenac or valproate anions were selected for further steps of this study.

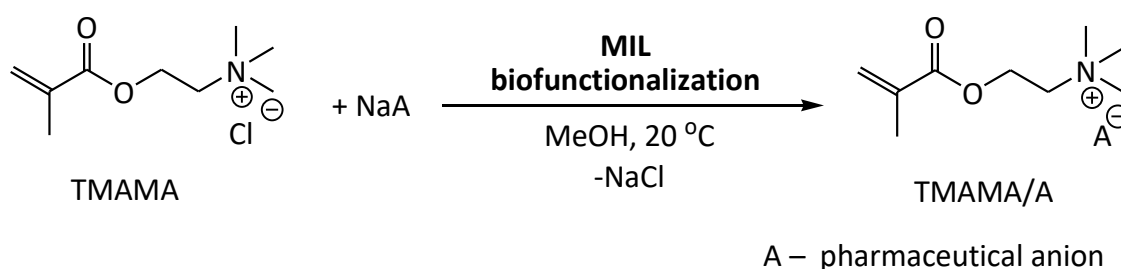


Figure 1. Reaction scheme for the biomodification of TMAMA.

The ion exchange reaction conditions and procedures for the reactions performed are detailed in publications. The structures of the obtained compounds, that is TMAMA/A as the MILs, were confirmed using ^1H NMR spectroscopy (P.1 Fig.2, Fig. S3; P.2 Fig.1; P.5 Fig.2). Efficiencies of ion exchanges were evaluated by comparison of the integrals of signals at 3.0–3.2 ppm assigned to the protons in

trimethylammonium groups ionically bonded with chloride and pharmaceutical anions.

Table 1. Modification of TMAMA MIL by ionic exchange with various pharmaceutical anions.

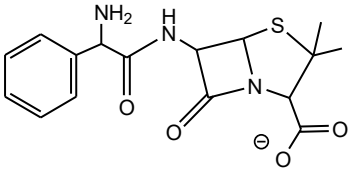
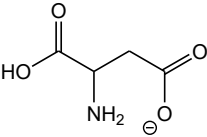
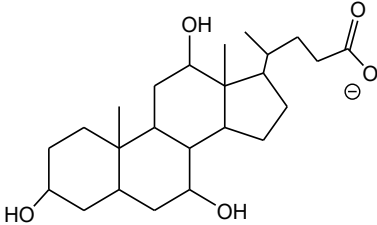
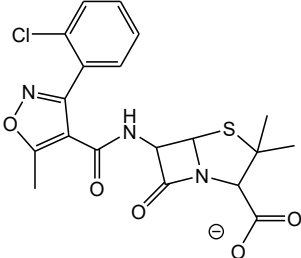
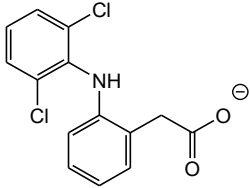
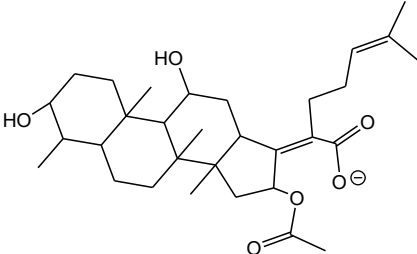
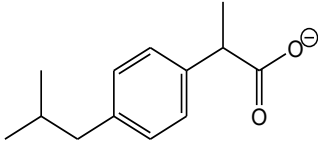
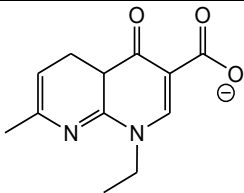
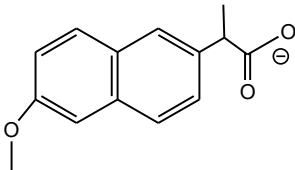
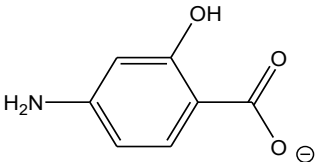
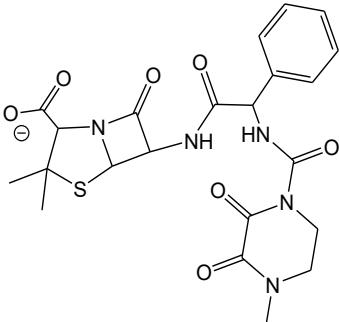
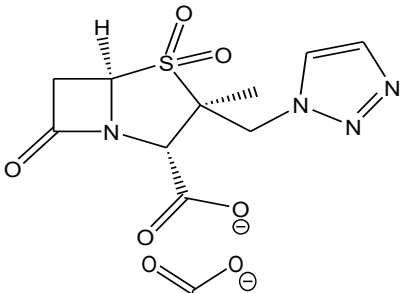
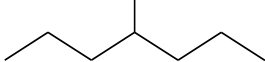
Pharmaceutical anion (A)	Structure	Pharmaco-logical property	Ion exchange [%]	Comment
Ampicillin (AMP)		Broad-spectrum penicillin	89	transesterification hindered by THF addition, [P.4]
Aspartic		Neurotransmitter, metabolic intermediate, protein building block	13	maximum efficiency of exchange after 3 days, [P.5]
Cholic		Drug absorption enhancer, digestive aid	14	v. long reaction (7 days), transesterification, [P.5]
Cloxacillin (CLX)		β -lactam antibiotic	84	4h reaction, [P.1, P.4]
Diclofenac		Non-steroidal anti-inflammatory drug	46	long reaction (3 days), transesterification, [P.5]
Fusidate (FUS)		Protein synthesis inhibitor	75	2h reaction, [P.1]
Ibuprofenate		Non-steroidal anti-inflammatory drug	54	v. long reaction (7 day), [P.5]

Table 1. Modification of TMAMA MIL by ionic exchange with various pharmaceutical anions (continued).

Pharmaceutical anion (A)	Structure	Pharmacological property	Ion exchange [%]	Comment
Nalidixic		Quinolone antibiotic	33	primarily transesterification occurred after 4h, [P.5]
Naproxen		Non-steroidal anti-inflammatory drug	19	4h of exchange, transesterification, [P.5]
<i>P</i> -amino salicylate (PAS)		Antitubercular agent	92	4h reaction, [P.2]
Piperacillin (PIP)		Extended-spectrum penicillin	66	4h reaction, [P.5]
Tazobactam (TAZ)		β -lactamase inhibitor	85	4h reaction, [P.5]
Valproic		Mood stabilizer, anticonvulsant	76	24h of exchange, transesterification, [P.5]

3.2. POLYMERIZATION OF MILs – ROUTE TO GRAFTED PILs AS SINGLE DDS (P.1, P.2, P.4, P.5)

Designing of graft PILs, denoted also as grafted ionic drug–polymer conjugates, required in the first step preparation of MIs, which were obtained via ATRP using copper bromide/4,4′-dinonyl–2,2′-bipyridyl (CuBr/dNbpy) as catalyst system in anisole. Initially, methyl methacrylate (MMA) was copolymerized with 2-hydroxyethyl methacrylate (HEMA), which in the resulting copolymer was modified to 2-(2-bromoisobutyryloxy)ethyl methacrylate (BIEM) with initiating units, subsequently serving as a multifunctional MI (P(MMA-co-BIEM)) (Table 2, Figure 2a). Specific synthetic details for this stage can be found in P.4, procedure S1. Subsequently, the prepared MILs with pharmaceutical anions, that is TMAMA/A, was copolymerized with MMA by *grafting from* these MIs to obtain copolymers with PIL side chains, denoted as P(MMA-co-(BIEM-graft-P(TMAMA/A-co-MMA))) (Figure 2b).

Table 2. Characteristics of MI.

MI	[HEMA] ₀ : [MMA] ₀	DP _n	DP _{BIEM}	M _n ·10 ⁻³ [g/mol]	Đ
I	25:75	165	48	20.9	1.3
II	25:75	365	65	41.2	1.5
III	50:50	207	99	42.0	1.6
IV	25:75	229	63	43.5	1.3
V	50:50	214	103	36.4	1.2

Conditions: ATRP [HEMA+MMA]₀:[EBiB]₀: [CuBr]₀: [dNbpy]₀=600:1:1:2, anisole, 70°C, reaction time of 2 h; esterification [HEMA]:[BiBB]=1:3, pyridine, room temperature, reaction time of 24 h; DP_n – degree of polymerization, DP_{BIEM} number of BIEM initiating units in a polymer chain, M_n number-average molecular weight and Đ dispersity index determined by the SEC analysis. Initiators: EBiB ethyl α-bromoisobutyrate, BiBB α-bromoisobutyryl bromide.

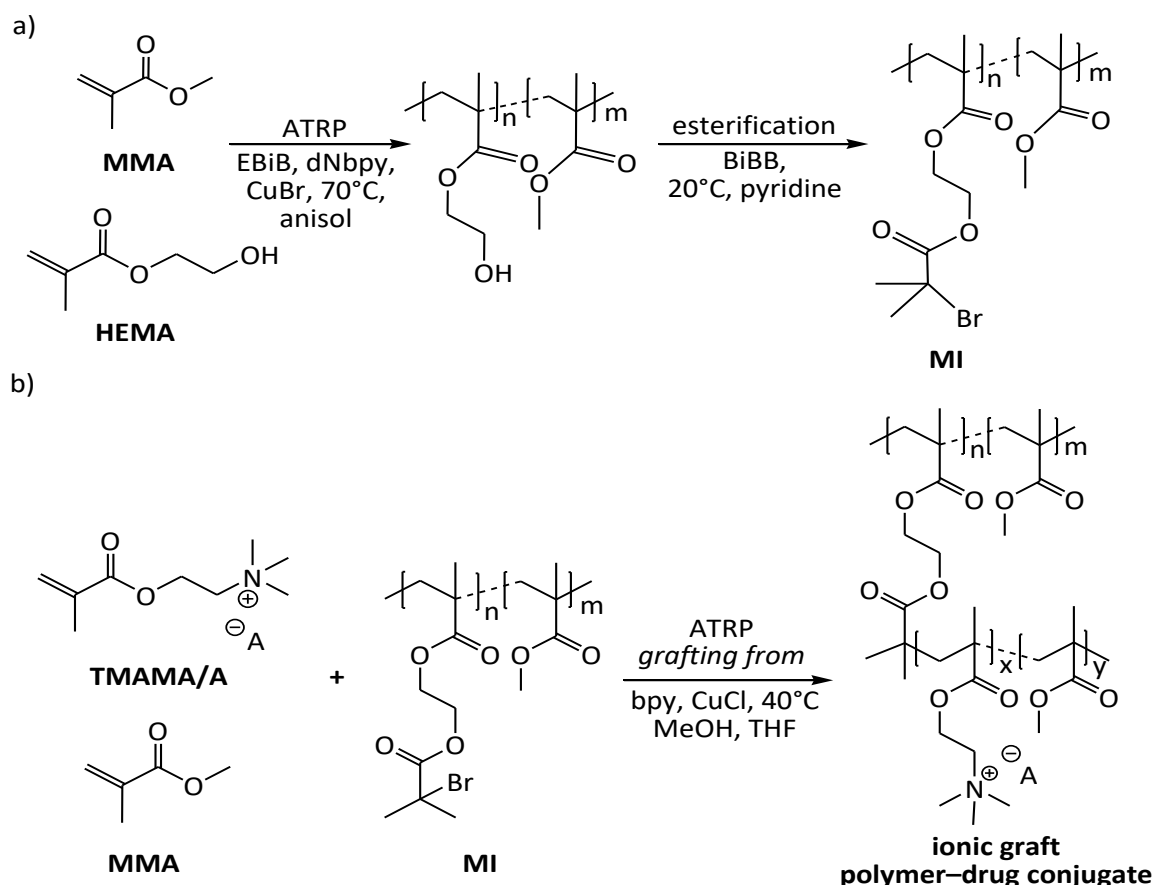


Figure 2. Reaction scheme for synthesis of MI (a) and grafted ionic polymer–drug conjugates (b), where A is pharmaceutical anion.

To design a comprehensive series of DDS, TMAMA/A and MMA were copolymerized using two initial molar ratios (25:75, 50:50) and catalyst system of copper chloride/bipyridyl (CuCl/bpy). The incorporation of MMA units allows for precise tuning of the hydrophobic–hydrophilic balance within the graft copolymer. Furthermore, MMA contributes to the biocompatibility and stability of the resulting polymeric matrix. Furthermore, an TMAMA:MMA molar ratio of 75:25 was avoided, as the high steric hindrance of pharmaceutical anions, solubility issues, and occurrence of extensive transesterification of TMAMA hindered the reaction. Various monomer:MI ratios (50:1, 100:1, 200:1) were also examined to optimize the degree of polymerization of the side chains (DP_{sc}) and the ionic fraction content (F_{TMAMA}). Furthermore, the solvent mixture ratio as methanol/tetrahydrofuran (MeOH:THF v:v) and the proportion of TMAMA/A:MeOH have been carefully optimized to ensure proper dissolution of monomers, suppress transesterification of TMAMA/A with MeOH and prevent precipitation or cross-linking. In addition, preparation of most carriers required an increased solvent volume thus reaction

time had to be extended up to 20 h to achieve sufficient monomer conversions. As a result, polymerization of TMAMA modified with CLX, FUS, AMP, PAS or PIP yielded a series of ionic conjugates (Figure 2b). However, the direct polymerization of the biomodified MILs containing TAZ, ibuprofen, diclofenac or valproate anions failed because these drug anions preferentially formed copper–complexes, thus reacting with the ATRP catalyst instead of propagating.

The characteristics of the drugs contained in the graft polymer conjugates, which were introduced in via biofunctionalized TMAMA are as follows:

- CLX is beta–lactamase–resistant penicillin antibiotic which targets staphylococcal, pneumococcal, and β –hemolytic streptococcal infections. It is commonly used for conditions such as septic arthritis, pneumonia, endocarditis, impetigo, and cellulitis but it is not effective against methicillin–resistant *Staphylococcus aureus* [176].
- FUS is an antibiotic primarily effective against Gram–positive bacteria, including staphylococci, β –hemolytic streptococci. It is applied in treating bacterial infections of the blood, lungs, skin, and bone [177]. However, FUS exhibits significantly lower activity against *S. aureus* compared to CLX. FUS and CLX are often co–delivered, particularly for serious staphylococcal infections [178].
- AMP is a broad–spectrum antibiotic, active against various Gram–positive and Gram–negative bacteria. It is widely used clinically for infections of the throat, respiratory tract, nose, ear, skin, urinary tract and gastrointestinal system [179]. AMP–CLX combination is commercially available due to their synergistic antibacterial activity [180].
- PAS is primarily used in the treatment of *Mycobacterium tuberculosis* infections, especially those affecting the lungs. Its efficacy is due to synergistic interactions with other antitubercular medications. PAS also plays a role in enhancing the plasma concentration of other antituberculosis drugs [181].
- PIP is a penicillin β –lactam antibiotic used for bacterial infections, including *Streptococcus pneumoniae* or *Pseudomonas aeruginosa*. Its effectiveness is compromised by bacteria producing β –lactamase enzymes [182].

The length of the main chain of the PIL brushes was defined as total degree of polymerization (DP_n) of the MI. The number of P(TMAMA/A-co-MMA) side chains (n_{sc}) and the grafting degree (DG, which is defined as a ratio of the n_{sc} to the DP_n) were precisely tuned by controlling the amount of initiating units present in the MI. Furthermore, DP_{sc} was controlled by monomer conversion. These factors, alongside the reactivities of the pharmaceutically functionalized MIL and comonomer, determined the F_{TMAMA} within the side chains, the overall number-average molecular weight (M_n), and the dispersity ($\mathcal{D}$) of the copolymers (Table 3). Structural parameters, including length of polymer chains and composition, were calculated on the base of ^1H NMR spectra. SEC analysis confirmed the excellent control of the polymerization reactions regardless of the presence of pharmaceutical anions. The obtained grafted PILs exhibited narrow molecular weight distributions with $\mathcal{D}$ ranging from 1.19 to 1.63 (P.4 Fig. S3; P.5 Fig. S1).

The detailed procedures and conditions for the performed reactions are detailed in publications P.1, P.2, P.4 and P.5. The structures of the obtained polymers were confirmed using ^1H NMR and FT-IR spectroscopy (P.1 Fig. 3, Fig. S2; P.2 Fig. 3; P.4 Fig. 2, Fig. S1, Fig. S4, Fig. S5; P.5 Fig. 3).

Table 3. Characteristics of selected graft copolymers based on MILs with drug anions.

Drug (A)	DDS	MI	DG [%]	[T] ₀ :[M] ₀	DP _{sc}	F _{TMAMA} [mol%]	M _n ·10 ⁻³ [g/mol]	Đ	DC [%]
FUS	F1	I	29	25:75	26	50	517.1	1.50	53
PAS	S1	II	18	25:75	35	57	431.3	1.36	35
	S2	III	48	25:75	34	24	289.2	1.36	23
	S3	II	18	50:50	75	73	405.0	1.46	39
	S4	III	48	50:50	89	40	841.9	1.40	43
AMP	A1	IV	28	25:75	44	24	284.1	1.60	52
	A2	V	48	25:75	30	22	316.0	1.56	48
	A3	IV	28	50:50	57	44	483.1	1.63	62
	A4	V	48	50:50	52	52	820.2	1.52	70
CLX	C1	IV	28	25:75	56	11	277.0	1.25	27
	C2	V	48	25:75	60	14	600.0	1.28	31
	C3	IV	28	50:50	21	30	172.8	1.22	65
	C4	V	48	50:50	29	23	322.0	1.19	62
PIP	P1	IV	28	25:75	36	33	283.4	1.21	74
	P2	V	48	25:75	32	25	362.1	1.18	78
	P3	IV	28	50:50	24	42	225.4	1.25	92
	P4	V	48	50:50	21	26	259.4	1.22	91

DG was calculated as the ratio of the n_{sc} to the DP_n , $[T]_0:[M]_0 = [TMAMA/A]_0:[MMA]_0$, $[TMAMA/A+MMA]_0:[MI]_0 = 200:1$, except F1 where $[TMAMA/A+MMA]_0:[MI]_0 = 100:1$ and S4 where $[TMAMA/A+MMA]_0:[MI]_0 = 50:1$; $[MI]_0:[CuCl]_0:[bpy]_0 = 1:1:2$. All reactions were carried out for 20 h. The MeOH:TMAMA (v/wt) and MeOH:THF (v/v) ratios were applied as follows: 3:1 and 3:2.25 for series C1-C4 and P1-P4; 4:1 and 4:3 for series A1-A4 and S4; 2:1 and 1:1 for F1; 8:1 and 4:3 for S1; 7:1 and 3:2.25 for S2; and 6:1 and 3:2.25 for S3, respectively. MI data in Table 2; DP_{sc} , F_{TMAMA} , M_n were determined by ¹H NMR; Đ was determined by the SEC analysis; DC refers to drug content determined by UV-vis.

Grafted copolymers exhibited a wide range of DP_{sc} values, including polymers with short PIL side chains ($DP_{sc} = 21-44$) or long PIL grafts ($DP_{sc} = 52-89$). Since different MIs were employed, it is important to emphasize the structural variations among the synthesized nonlinear copolymers:

- Densely grafted PILs (DG = 48 %): carrying PAS, AMP, CLX, and PIP anions, generally characterized by similar n_{sc} , DP_n , and DG.
- Loosely grafted PILs (DG = 18–28 %; PAS vs. AMP/CLX/PIP): PILs conjugated with PAS exhibit a n_{sc} similar to those carrying AMP, CLX, and PIP. However, they are characterized by a longer backbone and a lower DG.
- Loosely grafted PIL with the DG = 29 % containing FUS or CLX/FUS: PILs conjugated with FUS or a CLX/FUS combination show lower n_{sc} and shorter main chains. Despite these differences, their DG remains similar to that of PILs carrying AMP/CLX/PIP.

Analysis of the correlation between the initial comonomer mixture ($[T]_0:[M]_0$) and the final copolymer composition (ionic fraction content), confirmed the F_{TMAMA} of the synthesized graft polymers was strongly affected by the number of initiating sites in MI and reactivity of used MIL including steric hindrance implied by particular drug anion (P.2 Fig. 4; P.4 Fig. 3). Synthesis of loosely grafted FUS–polymer conjugate (F1) indicated a higher reactivity of MIL than MMA. This was evidenced by the notably higher content of ionic units incorporated into the resulting copolymer relative to the initial content of MIL in the reaction feed, thereby suggesting higher reactivity ratio for the ionic monomer. Most of AMP and PIP based polymer conjugates (A1–A4 and P1–P3) appeared to be a statistical copolymers showing a similar reactivity of MIL and MMA, resulting in side chains with compositions close to the initial feed of comonomers. In contrast, the presence of CLX primarily caused a depletion of ionic fraction in the copolymer relative to the feed yielding predominance of MMA units. For the PAS series F_{TMAMA} was strongly influenced by the number of active sites in MI. Similar MIL and MMA reactivities were noticed during syntheses of densely grafted copolymers (S2 and S4), whereas during synthesis of loosely grafted S1 and S3, TMAMA/PAS was more rapidly incorporated into the polymer chain than MMA, suggesting the formation of gradient copolymers.

The percentage amount of drug contained in the polymer matrix defined as the drug content (DC), was assessed by UV–Vis spectra. Satisfactory DC was achieved; PIP–conjugates indicated the highest DC (74–92%), then AMP–conjugates (48–70%) and FUS–conjugate (53%), whereas PAS and CLX based conjugates indicated moderate DC (23–43% and 27–65%, respectively). The correlations between DC of selected PILs with respect to the content of ionic fraction as well as distribution and length of the branches are shown in the Figure 3.

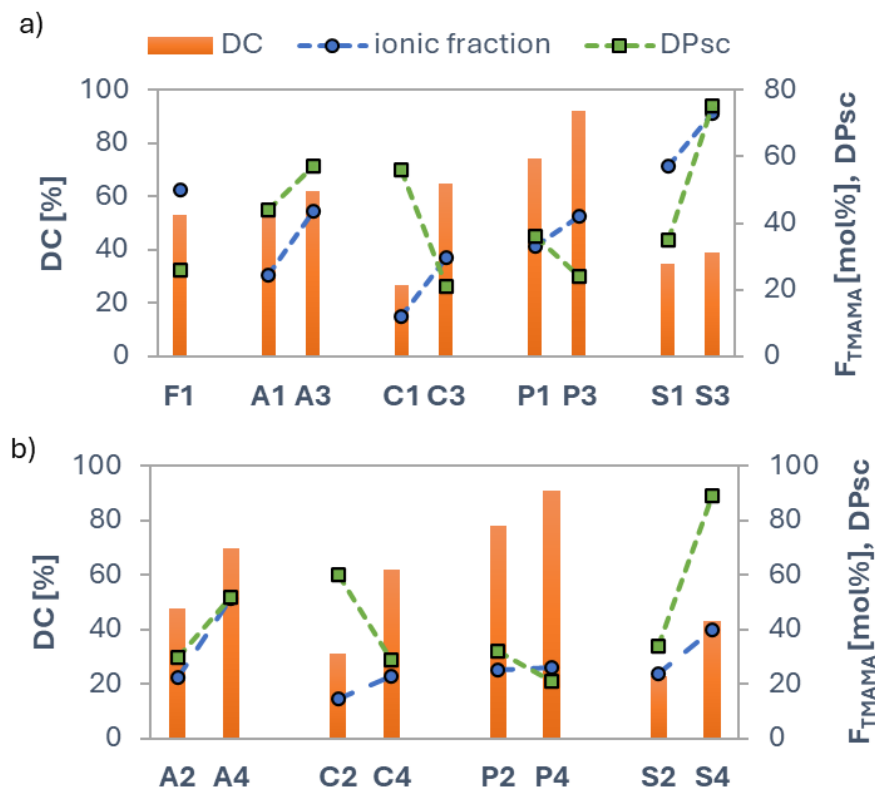


Figure 3. Relationship of ionic fraction and drug content with side-chain length in loosely (a) and densely grafted PILs (b).

For most of both loosely and densely grafted PILs, the DC increased with the F_{TMAMA} . Furthermore, specific trends included:

- A1–A4: higher AMP content was associated with longer side chains,
- C1–C4 and P1–P4: an inverse trend comparing to AMP-conjugates was observed, likely due to CLX and PIP steric hindrance,
- S1–S4: Loosely grafted PILs S1 and S3 exhibited similar PAS contents. Conversely, substantial reduction in DC was observed in dense graft copolymers despite of increasing F_{TMAMA} . Moreover, due to low spatial bulk of PAS, even distinct differences in the lengths of the side chains did not influence the DC.

3.3. PREPARATION OF DUAL-DDSs BASED ON GRAFTED PILs (P.1, P.3, P.4, P.5)

The DDSs for combination delivery were obtained in three ways:

- Terpolymerization of MMA with two MILs containing different pharmaceutical anions (P.1, P.4): to directly obtain series of grafted ionic polymer conjugates containing anion drug pairs composed of CLX–FUS and AMP–CLX (Figure 4a).

- Encapsulation of non-ionic drug in nanoparticles formed by amphiphilic grafted ionic polymer conjugates containing drug anions (P.3): this strategy was utilized to prepare PAS-ISO dual-drug systems (Figure 4b).
- Post-modification of grafted ionic polymer conjugates containing drug anions (P.5): TAZ anions were introduced to PIP-based graft PILs via ion exchange (Figure 4c).

The characteristics of the second drugs introduced into the matrices of the graft polymer conjugates containing anionic drugs are as follows:

- ISO is a primary antituberculosis drug, frequently used in combination with PAS. Combined administration of PAS and ISO is usually employed because PAS is known to prolong the half-life of ISO and increase its plasma concentration [183].
- TAZ is a bacterial β -lactamase inhibitor, commonly administrated in combination with PIP to restore its activity against resistant bacteria. This PIP-TAZ combination provides an expanded spectrum of antibacterial action and is a standard, broad-spectrum formulation in clinical practice [184].

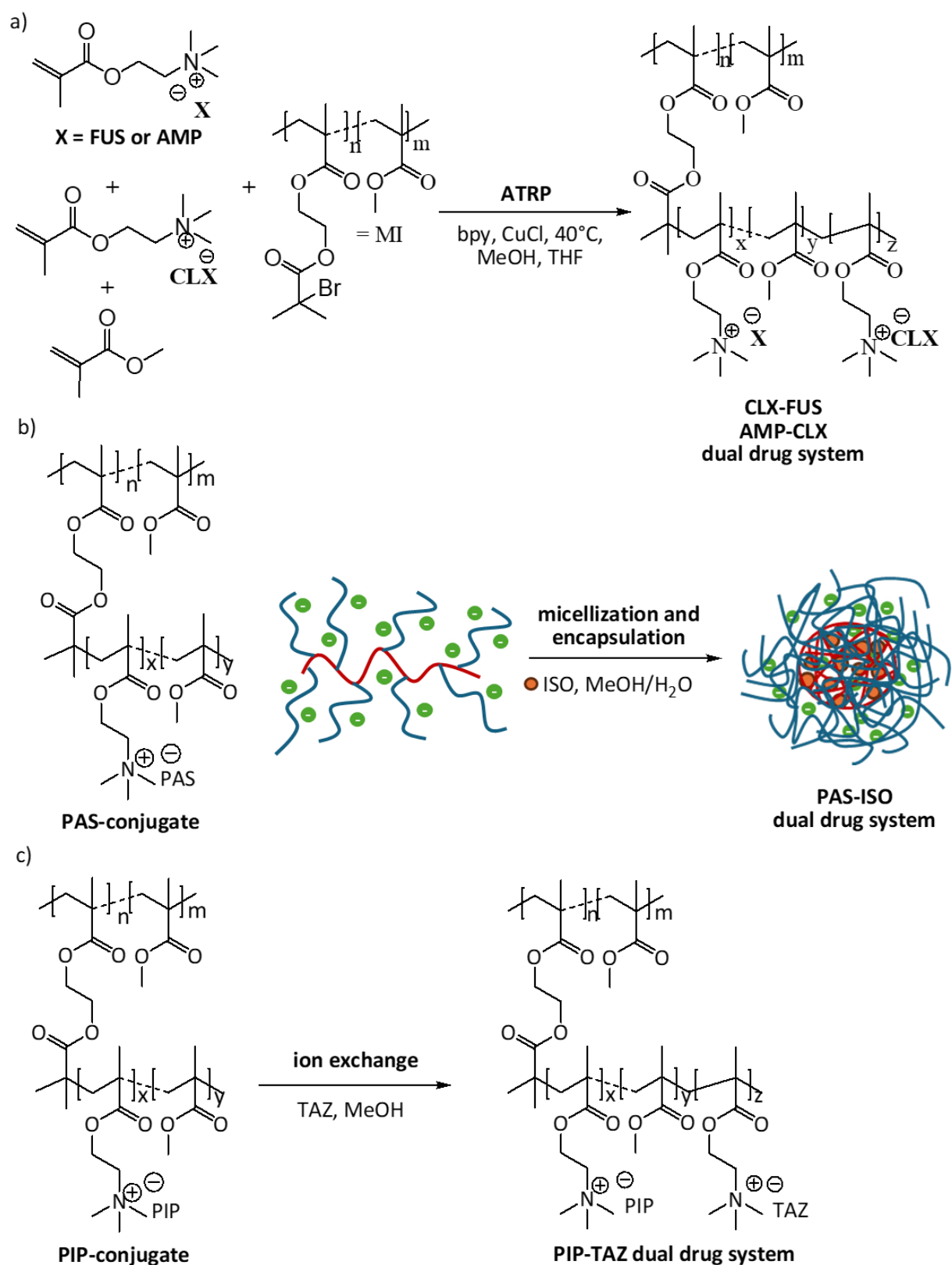


Figure 4. Pathways for dual-drug systems: direct synthesis of dual-drug ionic graft polymer conjugates (a), ionic graft polymer-drug conjugates with encapsulated drug (b), introduction of drug anion by modification of ionic graft polymer-drug conjugates (c).

The main goal of this part of work was to synthesize dual-drug systems via polymerization (strategy #1). However, since PAS is clinically administered in combination with non-ionic drugs such as ISO, an alternative strategy #2 was employed. Specifically, the ability of the synthesized amphiphilic graft PILs containing drug anion to self-organize into micellar structures in aqueous solutions was exploited to encapsulate non-ionic ISO molecules. Furthermore, dual PIP-TAZ systems were synthesized by introducing TAZ into PIP-conjugates (strategy #3). This stepwise approach was employed because the direct polymerization of TMAMA/TAZ MIL failed, likely due to TAZ coordinating with the copper catalyst ions. Detailed procedures for the preparation of these dual-drug systems are reported in publication P.1, P.3, P.4, and P.5. Reaction conditions were optimized analogously to those described in the previous section. The same analytical techniques were utilized to confirm the structures of the PIL conjugates and to estimate their parameters, which are presented in Table 4 and Table 5 (P.1 Fig.3c, Fig.S2, Fig.S4; P.3 Fig.3; P.4 Fig.2, Fig.S6; P.5 Fig.4).

Table 4. Characteristics of dual-drug systems based on graft copolymers composed of MILs with drug anions designed by the strategy #1.

Drugs	DDS	MI	DG [%]	[T] ₀ : [M] ₀	DP _{sc}	F _{TMAMA} [mol%]	M _n · 10 ⁻³ [g/mol]	Đ	DC [%]
CLX & FUS	CF1	I	29	12.5:12.5:75	DP _{total}	21	283.5	1.90	CLX 41
					DP _{TMAMA/CLX}	3			FUS 33
					DP _{TMAMA/FUS}	3			
AMP & CLX	AC1	IV	28	12.5:12.5:75	DP _{total}	27	180.3	1.76	47
					DP _{TMAMA/AMP}	4			
					DP _{TMAMA/CLX}	2			
	AC2	V	48	12.5:12.5:75	DP _{total}	39	397.0	1.76	61
					DP _{TMAMA/AMP}	5			
					DP _{TMAMA/CLX}	3			
	AC3	IV	28	25:25:50	DP _{total}	44	395.2	1.75	74
					DP _{TMAMA/AMP}	13			
					DP _{TMAMA/CLX}	7			
	AC4	V	48	25:25:50	DP _{total}	37	627.6	1.43	79
					DP _{TMAMA/AMP}	13			
					DP _{TMAMA/CLX}	7			

CF1 [T]₀: [M]₀ = [TMAMA/CLX]₀: [TMAMA/FUS]₀: [MMA]₀, AC1-AC4 [T]₀: [M]₀ = [TMAMA/AMP]₀: [TMAMA/CLX]₀: [MMA]₀; MeOH:TMAMA (v/wt) and MeOH:THF (v/v) ratios were applied as follows: 3.75:1 and 3.75:2.8 for series AC1-AC2; 4:1 and 4:3 for series AC3-AC4; and 2:1 and 1:1 for FC1, respectively, time of reactions 20h; DP_{sc}, F_{TMAMA}, M_n determined by ¹H NMR (M_n, NMR); Đ was determined by the SEC analysis; DC refers to drug content determined by UV-vis.

Table 5. Characteristics of dual-drug systems based on graft copolymers composed of PILs with drug anions designed by the strategy #2 and #3.

Drugs	Modified conjugate	DDS	DG [%]	n_{sc}	DP_{sc}	F_{TMAMA} [mol%]	DC/DLC [%]
PAS&ISO	S4	SI1	48	99	89	40	PAS 43 ISO 65
	S1	SI2	18	65	35	57	PAS 35 ISO 68
	S3	SI3	18	65	75	73	PAS 39 ISO 54
	P1	PT1	28	63	36	11	PIP 48 TAZ 34
	P2	PT2	48	103	32	14	PIP 58 TAZ 41
	P3	PT3	28	63	24	30	PIP 68 TAZ 31
PIP&TAZ	P4	PT4	48	103	21	23	PIP 62 TAZ 36

Characteristics of the modified conjugates forming the dual-drug systems are detailed in Table 3. DG was calculated as the ratio of the n_{sc} to the DP_n , DLC refers to the percentage content of ISO loaded into the micelles, DC corresponds to percentage content of conjugated PAS, PIP or TAZ anions.

Terpolymerization enabled the direct synthesis of dual-drug ionic polymer conjugates, allowing for the fine-tuning of both the F_{TMAMA} and DC. However, these parameters were notably influenced by drug-drug interactions. Structurally, FUS-CLX and AMP-CLX conjugates were characterized by shorter side chains compared to their analogous single-drug CLX, FUS, or AMP conjugates. Furthermore, this synthetic strategy yielded statistical copolymers ($[TMAMA]_0 \sim F_{TMAMA}$) for similar relative reactivities of the TMAMA/CLX and TMAMA/FUS ($DP_{TMAMA/CLX}:DP_{TMAMA/FUS}=1:1$), whereas TMAMA/AMP appeared to be more reactive than TMAMA/CLX ($DP_{TMAMA/AMP}:DP_{TMAMA/CLX} \sim 2:1$). Overall, satisfactory control over polymerization was maintained when employing two MILs with different pharmaceutical anions. However, dispersity indices of these copolymers were generally higher ($\bar{D} > 1.5$), likely due to combined steric hindrance of the connected anions in both types of TMAMA and the arising occurrence of side reactions.

A summary correlating DC with polymer parameters, emphasizing differences in DG, is presented in Figure 5. When comparing DC in the FC1 system to analogous single-drug systems, the CLX content remained similar (~40%), whereas the FUS content was notably lower (33 vs. 53%, Figure 5a). The total drug content (AMP+CLX) varied from 40% to 75% (Figure 5b); however, most systems only

slightly exceeded the individual DC values observed in the majority of analogous single-drug DDS. In all AC1–AC4 PIL conjugates, DC significantly increased with F_{TMAMA} regardless of the DG. Additionally, for loosely grafted copolymers, DC was influenced by the length of the side chains. Unlike the direct polymerization of MILs, the efficiencies of polymer modification and encapsulation processes are strongly dependent on the polymer's composition and structural parameters in conjunction with steric effects generated by both the conjugated drug anions and the incoming pharmaceuticals. Specifically, the loading efficiency of ISO (DLC ~ 55–70%, Figure 5c) remained similar across both densely and loosely grafted PIL conjugates (SI1–SI3), despite substantial differences in their structure parameters. This was attributed to the low steric hindrance presented by both PAS and ISO molecules. Moreover, the encapsulation process did not hinder the PAS content. While DLC values were generally high, they did not exceed 70%, a limitation likely resulted from the PILs' high ionic fraction. Regarding the ion exchange process conducted on PIP–PIL conjugates, a sufficient TAZ content (~30–40%) was achieved (Figure 5d). It should be noted that the biomodification of the MILs usually does not reach 100% conversion. Since the TMAMA/A and remaining unreacted TMAMA cannot be effectively separated using solvent-based method, they were polymerized without separation. The incorporation of the chloride TMAMA units into the polymer chains does not generate a biological concern, as the analogous graft copolymers containing solely chloride counterions have demonstrated low cytotoxicity [185]. Nevertheless, the presence of chloride anions in the PILs had to be considered during the subsequent introduction of TAZ. The modification efficiency was consistent across all this series regardless of variations in polymer characteristics. However, due to the high PIP content in the PIL and the significant steric hindrance induced by these anions, TAZ did not solely exchange with chloride anions. Instead, it displaced both Cl and PIP anions, resulting in a decrease in PIP content by approximately 20% yielding finally DC~50–70%.

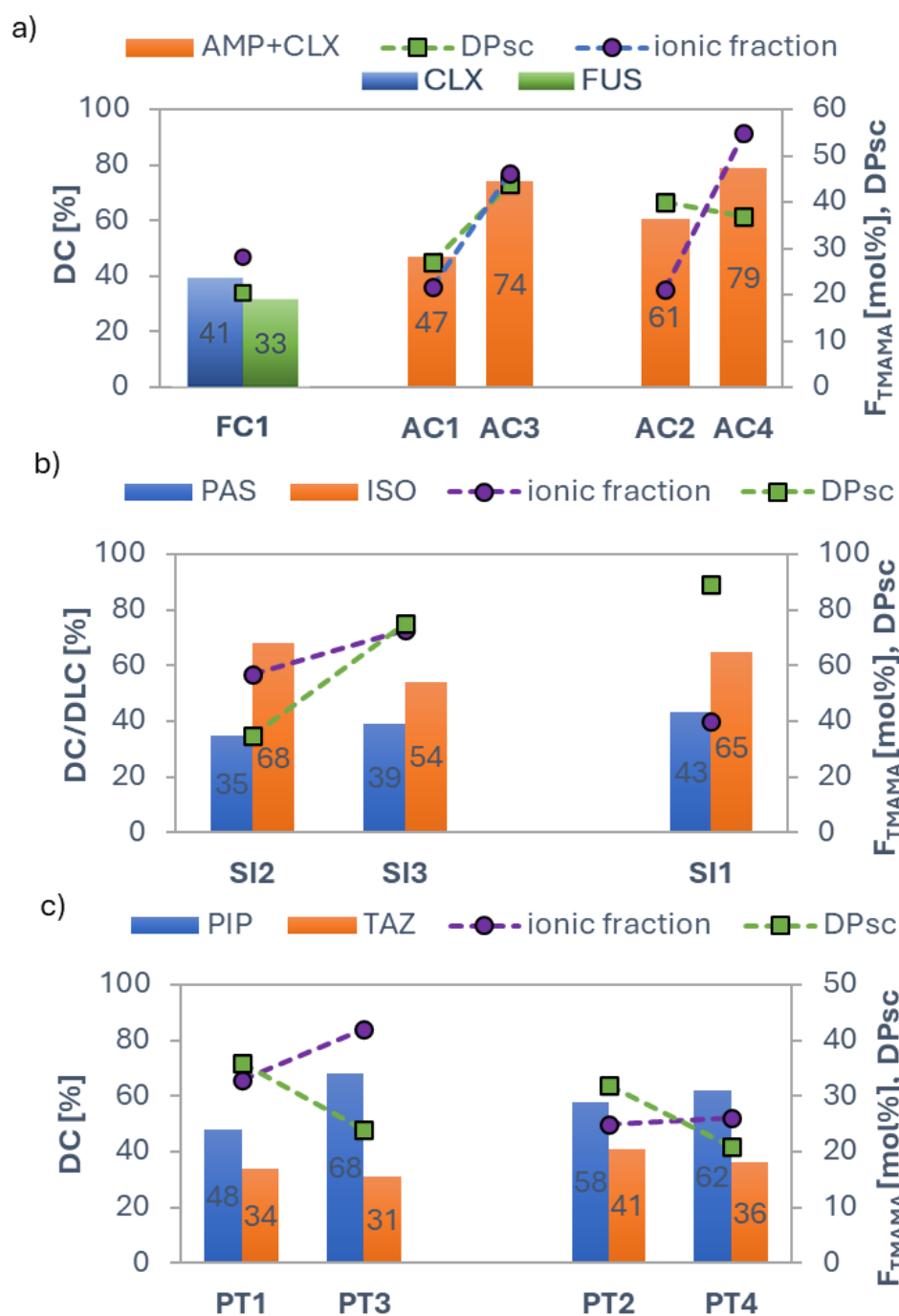


Figure 5. Relationship of DC with F_{TMAMA} and DP_{sc} including differences in DG (loosely grafted PILs are presented on the left, whereas densely grafted PILs on the right side) in CLX–FUS and AMP–CLX (a), PAS–ISO (b) and PIP–TAZ (c) co-delivery systems.

3.4. BEHAVIOR OF DRUG CONJUGATED GRAFT PIL IN AQUEOUS SOLUTION (P.1- P.5)

The ability of ionic graft polymer–drug conjugates to form the self-assembling micellar superstructures was confirmed by determining the CMC of PAS–conjugates derived from interfacial tension (IFT) measurements of aqueous solutions of copolymer at varying concentrations (P.3 Fig.2d, Fig. S2c) using the

pendant drop method via goniometry. Despite of SI3 high ionic content is displayed the lowest CMC (0.02 mg/mL) since the particles were stabilized by the long side chains. Conversely, SI2 characterized by significantly shorter grafts, exhibited the highest CMC (0.06 mg/mL), while SI1 with high DP_{sc} and the lowest ionic fraction, formed nanostructures at an intermediate CMC (0.04 mg/mL). Differences in DG did not varied CMC values due to low steric bulk of PAS.

DLS measurements revealed that the ionic conjugates formed homogeneous self-assemblies in aqueous solutions (P.3 Fig. 5b, Fig. S3; P.4 Fig.5, Tab.S1; P.5 Tab.2, Fig.S2). Single-drug systems showcased one prevailing fractions of particles with hydrodynamic sizes (D_H) falls within ~190–230 nm for AMP, ~180–360 nm for CLX, and ~260–330 nm for PIP based graft polymer conjugates (Figure 6). Particles carrying AMP were generally smaller than those containing CLX due to higher DC, what resulted in more compact packing. In case of dual-drug systems often more densely packed structures, with reduced D_H were formed. Prevailing fractions of the generated nanoparticles were smaller for most of PIP–TAZ and PAS–ISO systems (~180 and ~70–240 nm, respectively). Other dual-drug systems containing AMP–CLX had a similar size range to micelles carrying one drug but with more pronounced bimodal distributions yielding particle fractions in the range of ~90–130 and ~180–300 nm. Most single-drug systems (AMP, half of the CLX, most PIP) and PAS–ISO dual systems exhibited particles with narrow polydispersity index ($PDI = 0.01–0.17$), indicating uniform self-assembly. However, most dual-drug systems, tend to have broader PDI values ($0.25–0.50$) due to the increased complexity of drug interactions and a higher tendency for the formation of aggregates. Polymer structural parameters influenced D_H . Higher ionic fraction generally enhanced electrostatic repulsion between polymer chains leading to larger particle sizes. However combination of both high F_{TMAMA} and distribution of the side chains often promoted the formation of aggregates. While longer side chains often led to more dense packing and additional stability of nanoparticles.

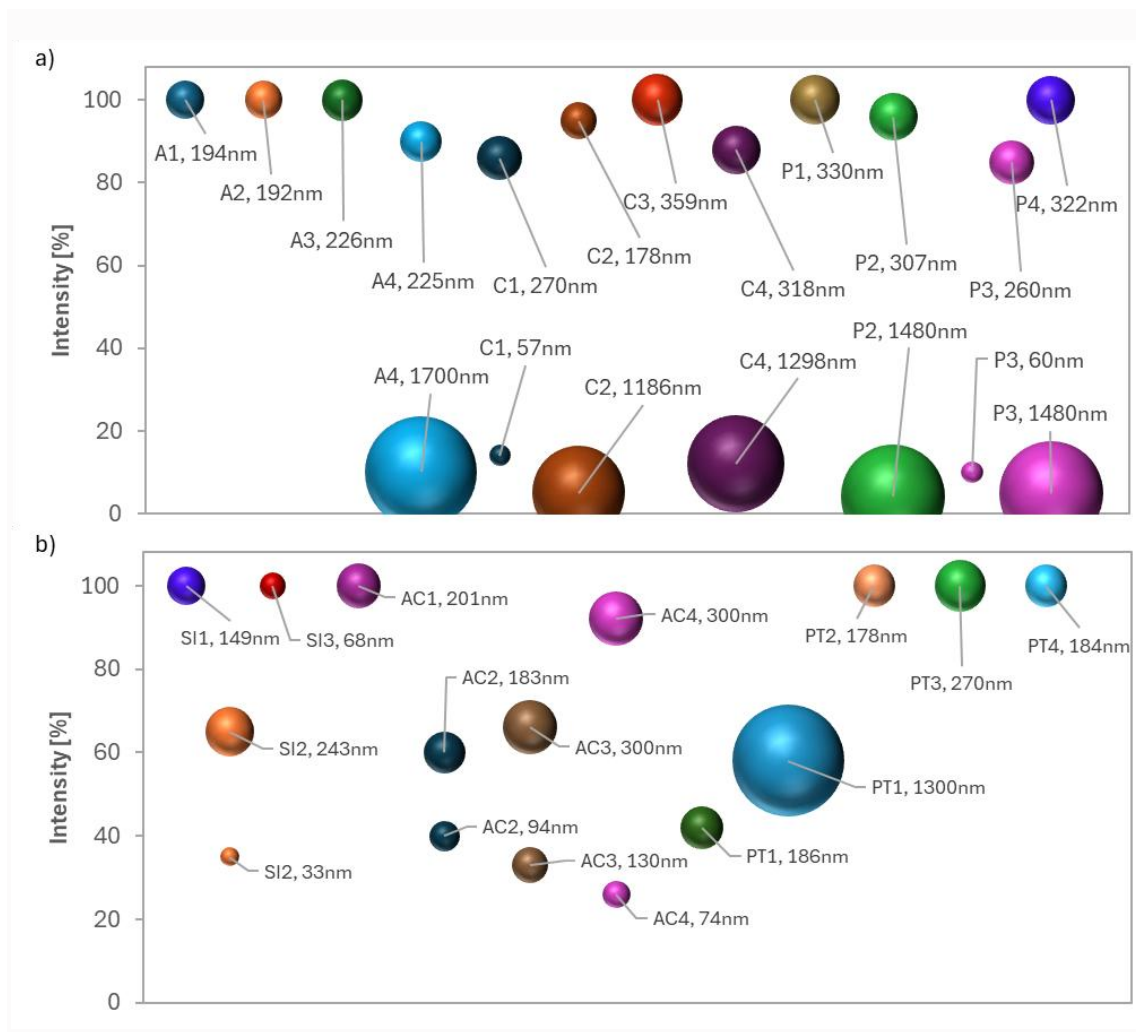


Figure 6. Hydrodynamic diameters of micelles formed by single-drug (a) and dual-drug (b) systems.

WCA measurements, conducted using a goniometer via the sessile drop method, were employed to evaluate hydrophilic/hydrophobic balance of the polymer film surface as the hydrophilicity degree of polymer. These surface properties were affected by both characteristics of the polymer and conjugated ionic drug (P.3 Fig.2b, P.4 Fig.6, P.5 Fig. 6). For almost all series of graft PIL conjugates WCA decreased with increasing F_{TMAMA} , indicating enhanced hydrophilicity regardless of DG or DP_{sc} (Figure 7). Moreover, influence of DG was drug-specific. PAS conjugates with a lower degree of grafting and longer side chains exhibited the higher wettability. Densely grafted single AMP- or CLX-based polymer conjugates, containing high ionic contents, demonstrated the greatest wettability. In contrast, for PIP conjugates, higher DG led to a less hydrophilic surfaces. Dual-drug systems containing AMP and CLX indicated comparable WCA to their single-drug

counterparts, whereas analogical PIP-TAZ systems exhibited an increase in overall hydrophilicity.

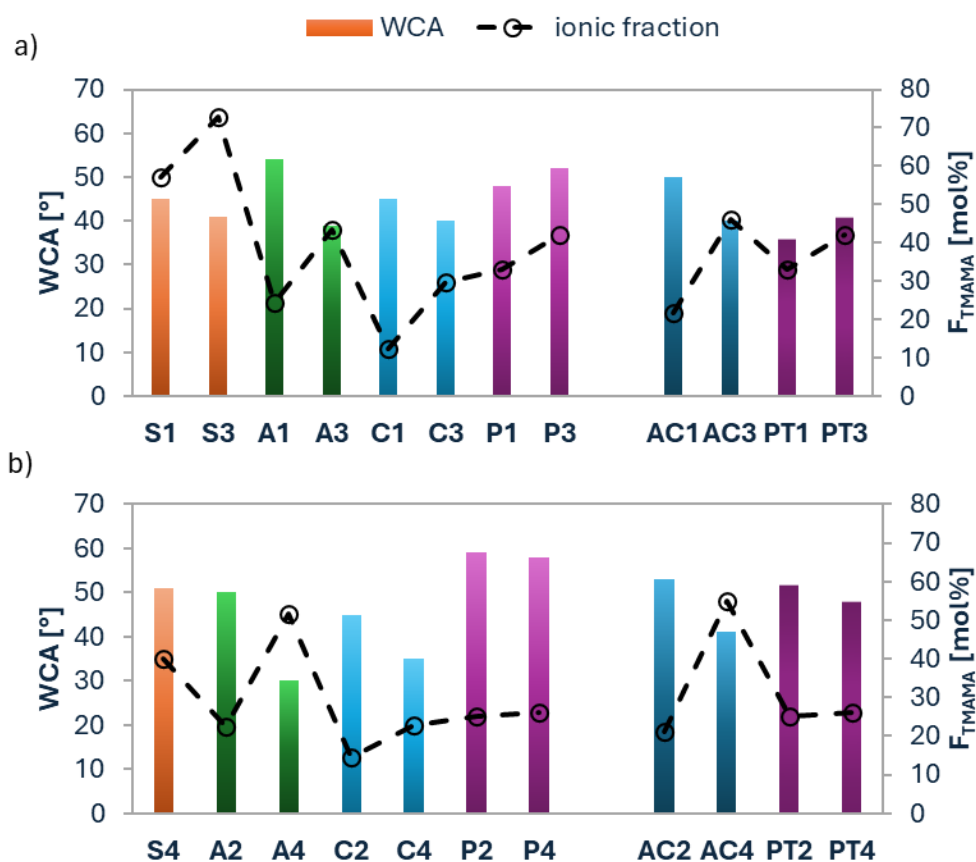


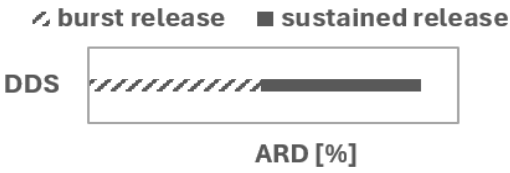
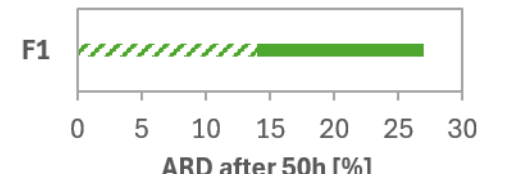
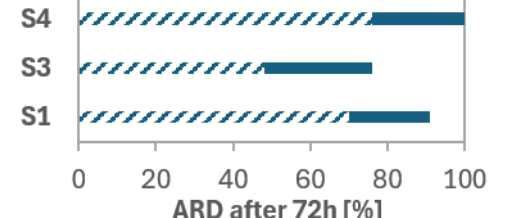
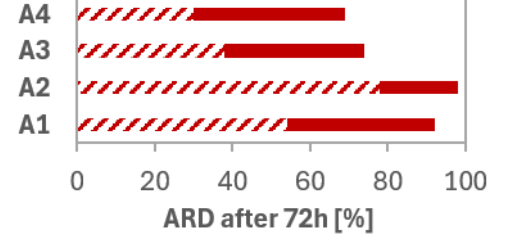
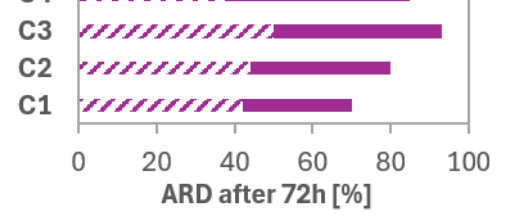
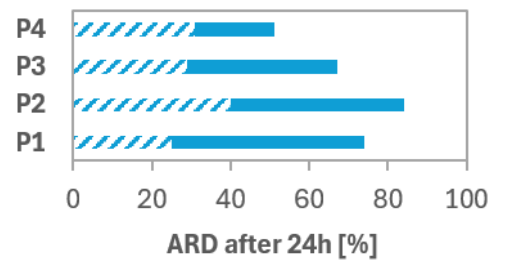
Figure 7. Contact angles for PIL conjugates with a lower (a) and a higher (b) degree of grafting in correlation with ionic fraction content.

3.5. *IN-VITRO* RELEASE OF DRUGS FROM SINGLE- AND DUAL- DDSs (P.1, P.2, P.3, P.4, P.5)

The therapeutic potential of the ionic graft conjugates presented in this doctoral dissertation was assessed by monitoring their *in vitro* performance. Accordingly, drug release studies were accomplished using the dialysis method in PBS, in conditions selected to simulate human physiological fluids (pH 7.4 and 37°C). UV-Vis spectroscopy was employed to estimate amount of released drug over time until plateau was reached (FUS-CLX: P.1 Fig.4, PAS: P.2, Fig.7, PAS-ISO: P.3 Fig.6e, AMP-CLX: P.4 Fig.7, PIP-TAZ: P.5 Fig.7). The summary of generalized results attained from drug release studies are presented in Table 6 and Table 7. The release was triggered in the presence of phosphate anions which exchanged the conjugated pharmaceutical anions, and then the drug diffused through the micellar

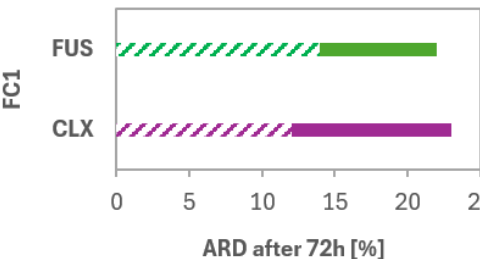
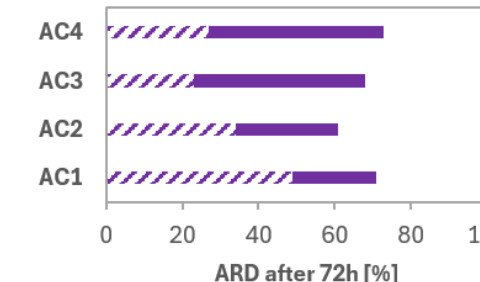
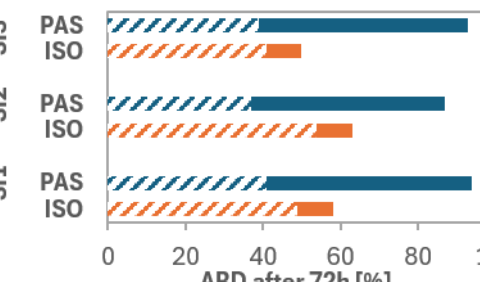
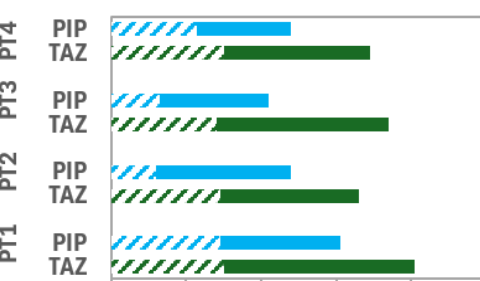
superstructure. In the case of ISO the release was driven only by the diffusion from polymer matrix.

Table 6. Summary of approximated data for *in vitro* released drugs, kinetic phases, and best-fit kinetic models for the investigated single-DDS.

Drug released		CRD [μg/mL]	Best-fit kinetic model
FUS		4	Higuchi ($R^2 = 0.40-0.75$)
PAS		8-10	Korsmeyer-Peppas ($R^2 = 0.85-0.99$ $n=0.17-0.35$)
AMP		12-13	First order / Higuchi ($R^2 = 0.60-0.99$) Korsmeyer-Peppas ($R^2 = 0.85-0.99$ $n=0.16-0.48$)
CLX		5-16	
PIP		11-16	

ARD is percentage amount of released drug, CRD is concentration of released drug.

Table 7. Summary of approximated data for *in vitro* released drugs, kinetic phases, and best-fit kinetic models for the investigated dual-DDS.

Drugs released		 DDS  ARD [%]	CRD [µg/mL]	Best-fit kinetic model
FUS + CLX	FUS		2	Higuchi ($R^2 = 0.60-0.70$) Korsmeyer-Peppas ($R^2 = 0.90-0.94$, $n=0.16-0.20$)
	CLX		2	
AMP + CLX	AMP + CLX		9-15	First order / Higuchi ($R^2 = 0.60-0.94$) Korsmeyer-Peppas ($R^2 = 0.90-0.98$, $n=0.20-0.40$)
PAS + ISO	PAS		9-10	First order / Higuchi ($R^2 = 0.76-0.80$) Korsmeyer-Peppas ($R^2 = 0.85-0.95$, $n=0.23-0.38$)
	ISO		5-8	First order ($R^2 = 0.45-0.55$) Korsmeyer-Peppas ($R^2 = 0.73-0.94$, $n=0.13-0.16$)
PIP + TAZ	PIP		7	First order / Higuchi ($R^2 = 0.69-0.99$) Korsmeyer-Peppas ($R^2 = 0.70-0.94$, $n=0.22-0.43$)
	TAZ		6-7	

In general, all systems followed concentration-dependent (First-order model) and diffusion-driven kinetics (Higuchi model). The diffusion process, characterized by the Korsmeyer–Peppas model, was typically quasi-Fickian. For all investigated systems, release proceeded in two stages: an initial burst release phase (usually lasting 0.5–1 hour), followed by a sustained release phase until a plateau was reached (usually after 50–72h). The drug release profiles depended heavily on the polymer matrix structure, the nature of the drug, drug content, and drug–drug interactions. Particularly:

- FUS and CLX: The effective diffusion of FUS and FUS–CLX from an micellar polymer matrix of loosely distributed side chains was limited by steric hindrance of the drug moieties, reaching completion within two days. Notably, interactions between FUS and CLX did not mutually limit release compared to single DDS (~25 vs. 20%).
- AMP and CLX: The burst release phase could be controlled by adjusting the ionic content. AMP–conjugates with higher ionic content (A3, A4) displayed a slower initial release compared to A2, where effective release lasted only four hours. However, systems combining both high F_{TMAMA} and DP_{sc} formed more densely packed structures regardless of DG, thereby overall release was restricted. Release from CLX–conjugates within the first hour was faster than that from most AMP–conjugates due to the higher steric hindrance induced by CLX. Release was facilitated by increasing the ionic fraction in copolymers with both low DP_{sc} and DG, whereas it remained similar for densely grafted PILs. The cumulative release of the drugs from dual–drug systems was predetermined by drug–drug interactions, which limited total release to ~60–70% regardless of polymer matrix structure. However, extending the effective drug release duration was possible by tuning ionic content similarly as in case of AMP–conjugates (AC3–AC4 vs. AC1).
- PAS and ISO: The low steric hindrance of PAS allowed for efficient release over 3 days (~70–100%); however, a significant fraction (~40–60%) was released within the first hour. Copolymers with loosely distributed side chains and high ionic content generally displayed a higher ARD, though release was slower for

those with longer side chains (S1 vs. S3). Conversely, almost the total amount of conjugated PAS was released from copolymers with high DG (S2), due to stronger repulsive interactions. In dual-drug systems, PAS release was not limited by ISO and remained similar to analogous single DDS. ISO release was influenced by micelle stability. For instance, the lowest release was observed for SI3, which formed the most stable nanoparticles (lowest CMC). However, most of the ISO was still released during the burst phase, with effective release ending after 4 hours.

- PIP and TAZ: The release from conjugates with higher DC was more hindered (P1, P2 vs. P3, P4). Additionally, high DG in conjunction with low F_{TMAMA} (P2) promoted more effective release comparing to other carriers (P1, P4). While, PIP release from all single carriers was relatively fast compared to abovementioned single DDS and was completed within 24h, its behavior changed in co-delivery systems. Interactions between PIP-TAZ extended the effective release of PIP, up to 3 days. Nevertheless, total amount of PIP released from most systems decreased by about 15–25%. Furthermore, since TAZ anions being smaller than PIP species; their diffusion rate from the nanoparticles was enhanced (70–80% of TAZ vs. 40–60% of PIP was released).

3.5.1. COMPARISON OF THE OBTAINED IONIC POLYMER–DRUG CONJUGATES TO THEIR LITERATURE ANALOGS

Designed ionic graft conjugates carrying particular drugs can be compared to previously reported: (a) linear analogs synthesized by the same pre-polymerization method (linear conjugates (LC)), or (b) polymer–drug conjugates obtained via post-polymerization modification of grafted PILs, where chloride anions in the copolymer were exchanged for the pharmaceutical ones (modified graft conjugates (MGC)).

- Current graft systems exhibited effective and sustained release lasting up to 72 hours, whereas LC generally achieved complete drug release within 4 hours, making them more advantageous for potential long-term therapeutic applications. However, higher stability of presented in this work, graft systems sometimes was accompanied by a lower ARD [P.2, P.3, P.4 vs ref. 114,115,186].

- The directly synthesized PAS-based graft PIL conjugates demonstrated superior release efficiency (~90%) compared to MGC (~30–45%) [P.2 vs ref. 113]. However, when considering dual PAS–ISO systems, the ARD values for PAS and ISO were comparable to MGC, whereas in comparison to LC, the ISO release was similar, but PAS release was higher (~90 vs. ~50–80 %). [P.3].
- Comparing graft copolymers carrying FUS, the F1 carrier achieved a similar DC to MGC with lower DP_{sc} and higher F_{TMAMA} , but its ARD was halved [P.1 vs ref. 108].
- The single PIP conjugates proved advantageous, yielding significantly higher CRD values (~7 vs. 3 $\mu\text{g/mL}$) compared to MGC. Moreover, the presented study enhanced the drug release efficiency for PIP–TAZ co-delivery over previous methods where TAZ was physically encapsulated in MGC. The use of TAZ anions led to much less limited PIP release and higher CRD for PIP (7 vs. 3 $\mu\text{g/mL}$), while maintaining a similar CRD for TAZ (~7 $\mu\text{g/mL}$) [P.5 vs ref. 109].
- Presented graft topology enhanced both the rate and amount of CLX release compared to LC (ARD: ~70–90% vs. ~60–70%, CRD: 14–16 $\mu\text{g/mL}$ vs. 10–13 $\mu\text{g/mL}$). Conversely, for AMP–conjugates, the LC provided higher CRD values (15–25 vs. 12 $\mu\text{g/mg}$) compared to the current graft systems. Moreover, LC allowed for a higher total AMP+CLX release [P.4 vs ref. 115].

3.5.2. CYTOTOXIC EVALUATION (P.1, P.3)

The designed drug carriers were evaluated by biological studies, the cytotoxicity of the selected single and dual DDS was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay against human bronchial epithelial cells (BEAS-2B) or normal human dermal fibroblasts (NHDF). Figure 8 presents the cell viability results of MTT assay for drug-based graft PIL conjugates solutions with two extremely different concentrations (FUS–CLX: P.1 Fig. 5; PAS–ISO: P.3. Fig. 8., PIP–TAZ: [109]).

Studies showcased a slight cytotoxic impact for the drug carriers, which intensified with increasing concentration. This trend was observed in contrast to the PIP-based PIL conjugate, which did not display any cytotoxicity. The cytotoxic effect was most pronounced at 100 $\mu\text{g/mL}$ following treatment with both single and dual

systems carrying CLX and FUS. Co-delivery systems incorporating PAS and ISO presented a similar effect on cell survival as those containing conjugated PIP and encapsulated TAZ, reducing cell proliferation to approximately 60%. At lower concentrations (3 $\mu\text{g/mL}$), only a slight reduction in metabolic activity ($\sim 5\text{--}15\%$) was perceived. It is important to note that the cytotoxicity of the both PIP and PIP-TAZ conjugates presented in this work was not re-evaluated here. These systems used the same polymer matrices which were previously investigated by myself and dr. Niesyto but using alternative synthetic strategies [109].

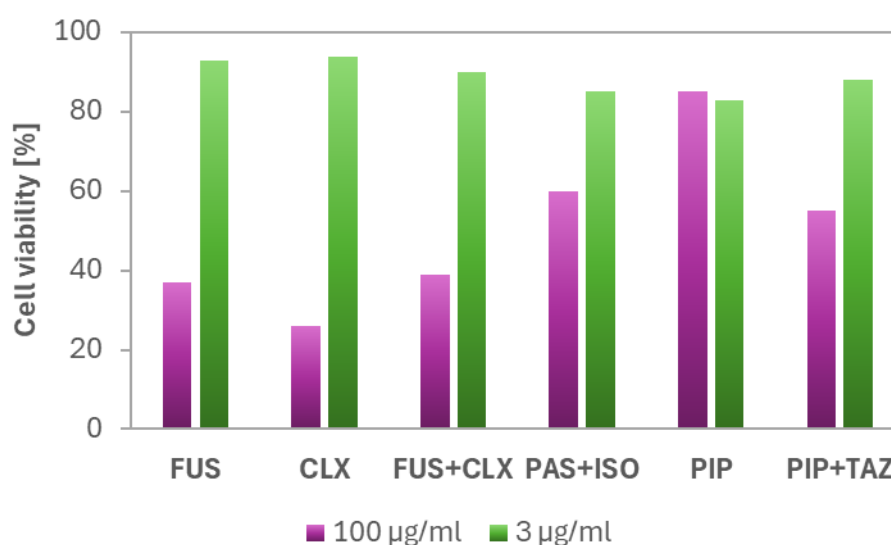


Figure 8. Cell viability of BEAS-2B cells for ionic conjugates (F1, GCLX⁻ [P.1], FC1, SI1, GP3_PIP⁻ and GP3_PIP⁻/TAZ⁻ [109], respectively) assessed at the highest (100 $\mu\text{g/mL}$) and lowest (3 $\mu\text{g/mL}$) concentrations after 72 hours of incubation. Before treatment, cell viability was assumed to be 100%.

4. SUMMARY AND CONCLUSIONS

- Modification of choline-based TMAMA with various pharmaceutical anions was successful providing MILs with FUS, CLX, PAS, AMP, PIP, TAZ, DIC, IBU and VPA anions, yielding high ion exchange degrees (75–90%). However, attempts to incorporate APT, NAP, CHA, and NAL anions were unsuccessful (efficiencies of biofunctionalization were 13–33%).
- Graft copolymers, synthesized by ATRP using multifunctional MI and biomodified TMAMA MILs, yielded well-defined structures with precisely controlled ionic contents, side chain distributions and lengths of PIL side chains.

- Synthesized single drug delivery systems carried conjugated FUS, CLX, PAS, AMP and PIP anions. Ionic conjugates contained satisfactory drug contents ($DC_{FUS} \sim 50\%$, $DC_{CLX} \sim 30\text{--}65\%$, $DC_{PAS} \sim 40\%$, $DC_{AMP} \sim 50\text{--}70\%$, $DC_{PIP} \sim 70\text{--}90\%$). However, attempts to synthesize other ionic conjugates carrying ibuprofenate, diclofenac, valproate or TAZ anions were unsuccessful because these drug anions preferentially reacted with the ATRP catalyst.
- Successful copolymerization of two distinct pharmaceutically functionalized MILs, specifically TMAMA/CLX and TMAMA/FUS, TMAMA/AMP and TMAMA/CLX yielded ionic graft PIL conjugates carrying two types of drug anions. Contents of dual-drugs in polymer structures were precisely assessed ($DC_{FUS+CLX} \sim 40\%$, $DC_{AMP+CLX} \sim 50\text{--}80\%$).
- Graft polymer–drug conjugates demonstrated the ability to self-organize into micellar structures, as confirmed by CMC evaluation (0.02–0.06 mg/mL) and DLS analysis. Amphiphilic nature of PAS–conjugates ($DC \sim 35\text{--}45\%$), enabled the effective encapsulation of ISO ($DLC \sim 55\text{--}70\%$) during micellization, which in turn led to PAS–ISO co–delivery systems.
- Systems for combined delivery of PIP and TAZ were prepared by incorporating TAZ anions through ion exchange onto PIP–conjugates ($DC_{PIP \text{ in } PIPTAZ} \sim 70\text{--}90\%$, $DC_{TAZ \text{ in } PIPTAZ} \sim 30\text{--}40\%$).
- The sizes of nanoparticles ranged from 190–360nm and 70–300nm for single and dual-drug systems, respectively. The hydrophilic nature of the ionic conjugates was confirmed by the WCA for their film surface, which ranged from 20° to 50°.
- *In vitro* drug release experiments, demonstrated the effective (co–)release from the designed graft PIL carriers. Drug release exhibited a biphasic pattern: burst phase (up to 1 h, followed by sustained phase. This sustained release typically lasted for most systems for 48 or 72 hours, though for some, release reached plateau within 4 or 24 hours. It was elaborated in detail how drug release profiles were influenced by the polymer matrix structure, the nature of the drug and its content as well as drug–drug interactions in case of co–delivery systems.

- The overall release efficiencies of the ionic graft PIL conjugates varied significantly depending on the ionic content, drug, or structural parameters such as DG or DP_{sc}. The systems can be ranked from the lowest to the highest average release efficiencies: FUS and dual FUS/CLX (20–30%) > ISO from PAS/ISO, PIP from single and dual systems, dual AMP/CLX (40–70%) > PAS, AMP, CLX, and TAZ from single DDS, and PAS from dual systems (70–100%).
- The drug release performance depends on the ionic interactions (ionic content) which regulate the ARD and release rates of AMP and CLX or FUS and CLX, whereas micelle stability heavily dictates the release of ISO from PAS-conjugates. Steric hindrance affects the diffusion process, notably reducing the release of bulkier molecules (FUS, CLX, and PIP) compared to smaller ones (PAS, TAZ). In addition, the incorporation of TAZ can be utilized to regulate and prolong the effective release of PIP anions.
- Cytotoxic evaluation showcased good biocompatibility with human bronchial epithelial cells treated by graft PIL carriers containing: FUS-CLX, PAS-ISO and PIP-TAZ formulations.

In summary, during this doctoral dissertation ionic graft polymer-drug conjugates were successfully designed using innovative methodology via drug-containing monomers. Single and dual-drug delivery systems utilizing grafted PIL architectures with conjugated pharmaceutical anions or encapsulated drugs, demonstrate significant potential to serve as drug carriers based on their favorable physicochemical properties, drug delivery performance and cytotoxicity profiles. The integration of biocompatible choline based biofunctionalized MIL units with antibacterial drugs within the polymer matrix, positions these systems for potential application in treating diverse bacterial diseases. These DDS prove valuable for combination therapies, as they enable the effective co-delivery of synergistic drug pairs. In conclusion, promising properties of the designed DDS display the strong clinical potential, justifying further *in vivo* biological studies to confirm their efficacy and safety.

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FIGURES AND TABLES

Figure 1. Reaction scheme for the biomodification of TMAMA.

Table 1. Modification of TMAMA MIL by ionic exchange with various pharmaceutical anions.

Table 2. Characteristics of MI.

Figure 2. Reaction scheme for synthesis of MI (a) and grafted ionic polymer–drug conjugates (b).

Table 3. Characteristics of selected graft copolymers based on MILs with drug anions.

Figure 3. Relationship of ionic fraction and drug content with side–chain length in loosely (a) and densely grafted PILs (b).

Figure 4. Pathways for dual–drug systems: direct synthesis of dual–drug ionic graft polymer conjugates (a), ionic graft polymer–drug conjugates with encapsulated drug (b), introduction of drug anion by modification of ionic graft polymer–drug conjugates (c).

Table 4. Characteristics of dual–drug systems based on graft copolymers composed of MILs with drug anions designed by the strategy #1.

Table 5. Characteristics of dual–drug systems based on graft copolymers composed of MILs with drug anions designed by the strategy #2 and #3.

Figure 5. Relationship of DC with F_{TMAMA} and DP_{sc} including differences in DG (loosely grafted PILs are presented on the left, whereas densely grafted PILs on the right side) in CLX–FUS (a), AMP–CLX (b), PAS–ISO (c) and PIP–TAZ (d) co–delivery systems.

Figure 6. Hydrodynamic diameters of micelles formed by single–drug (a) and dual–drug (b) ionic graft conjugates.

Figure 7. Contact angles for PIL conjugates with a lower (a) and a higher (b) degree of grafting in correlation with ionic fraction content.

Table 6. Summary of approximated data for *in vitro* released drugs, kinetic phases, and best–fit kinetic models for the investigated single–drug delivery systems.

Table 7. Summary of approximated data for *in vitro* released drugs, kinetic phases, and best–fit kinetic models for the investigated dual–drug delivery systems.

Figure 8. Cell viability of BEAS–2B cells for polymer–drug conjugates, assessed at the highest (100 $\mu\text{g/mL}$) and lowest (3 $\mu\text{g/mL}$) concentrations after 72 hours of incubation. Before treatment, cell viability was assumed to be 100%.

SCIENTIFIC ACHIEVEMENTS

SCIENTIFIC PAPERS

I. Scientific papers published in a journal included in the ministerial list of scientific journals and peer-reviewed international conference proceedings in the year of their final publication.

1. **Mazur Aleksy**, Niesyto Katarzyna, Neugebauer Dorota; Pharmaceutical Functionalization of Monomeric Ionic Liquid for the Preparation of Ionic Graft Polymer Conjugates; International Journal of Molecular Sciences; 2022, 23, 14731-14745. MEiN = 140 pkt; IF₂₀₂₂ = 5.6.
2. **Mazur Aleksy**, Neugebauer, Dorota; Characterization of Graft Copolymers Synthesized from *p*-Aminosalicylate Functionalized Monomeric Choline Ionic Liquid; Pharmaceutics, 2023, 15(11), 2556. MEiN = 140 pkt; IF₂₀₂₂ = 4.9.
3. Niesyto Katarzyna, **Mazur Aleksy**, Neugebauer Dorota; Piperacillin/Tazobactam co-delivery by Micellar Ionic Conjugate Systems Carrying Pharmaceutical Anions and Encapsulated Drug; Pharmaceutics; 2024, 16, 198-211; MEiN = 100 pkt; IF₂₀₂₂ = 5.4.
4. Niesyto Katarzyna, Keihankhadiv Shadi, **Mazur Aleksy**, Mielańczyk Anna and Neugebauer Dorota; Ionic Liquid-based Polymer Matrices for Single and Dual Drug Delivery: Impact of Structural Topology on Characteristics and in vitro Delivery Efficiency. International Journal of Molecular Sciences, 2024, 25(2), 1292. MEiN = 140 pkt, IF₂₀₂₄ = 4.9; TOP10 wg bazy Scopus.
5. Konieczkowska Jolanta, Neugebauer Dorota, Kozanecka-Szmigiel Anna, **Mazur Aleksy**, Kotowicz Sonia, and Schab-Balcerzak Ewa; Photoresponse of New Azo Pyridine Functionalized Poly(2-hydroxyethyl methacrylate-co-methyl methacrylate). Scientific Reports, 2024, 14(1), 9078. MEiN = 140 pkt, IF₂₀₂₄ = 3.9; TOP10 wg bazy Scopus.
6. **Mazur Aleksy**, Neugebauer Dorota; Graft Polymers Derived from Pharmaceutically Active Choline-Based Ionic Liquid Monomers: Dual Incorporation of Ampicillin and Cloxacillin. International Journal of Molecular Sciences, 2025, 26(19), 9415. MEiN = 140 pkt, IF₂₀₂₅ = 4.9; TOP10 wg bazy Scopus.
7. **Mazur Aleksy**, Neugebauer Dorota; Sequential Biofunctionalization of a Choline-Based Monomeric Ionic Liquid and Polymerized Ionic Liquid: A Route to Dual Anionic Drug Polymer Conjugates of Piperacillin-Tazobactam.

ACS Biomaterials Science and Engineering, 2026, 12(8), 4113–4124. MEiN = 140 pkt, IF₂₀₂₆ = 6.0.

II. Chapters in scientific monographs which, in the year of publication in their final form, were included in the ministerial list of publishing houses

Niesyto Katarzyna, Keihankhadiv Shadi, **Mazur Aleksy**, Neugebauer Dorota; Design of innovative polymer nanocarriers based on choline ionic liquids for the co-delivery of bioactive compounds. In: *Materiały przyszłości*, Eds. Czichy Małgorzata, Kazek-Kęsik Alicja. Silesian University of Technology Press, 2025, 287. ISBN: 978-83-68390-42-1.

NATIONAL PATENTS

1. **Mazur A.**, Niesyto K., Neugebauer D. “Method for preparing ionic conjugates and their application”. Polish Patent No. 247411, granted Apr 14, 2025.
2. **Mazur A.**, Niesyto K., Neugebauer D. “Method for preparing micellar ionic conjugates based on graft polymers for the co-delivery of antibacterial drugs and their application”. Polish Patent No. 249109, granted Dec 3, 2025.
3. **Mazur A.**, Niesyto K., Neugebauer D. “Method for preparing micellar ionic conjugates based on graft polymers for the co-delivery of antibacterial drugs and their application”. Polish Patent No. 249430, granted Feb 2, 2026.
4. **Mazur A.**, Niesyto K., Keihankhadiv S., Neugebauer D. “Method for preparing ionic conjugates for the co-delivery of drugs with antibacterial activity and their application”. Polish Patent No. 249823, granted Apr 8, 2026.
5. **Mazur A.**, Niesyto K., Neugebauer D. “Method for preparing micellar ionic conjugates based on graft polymers for the co-delivery of antitubercular drugs and their application”. Polish Patent No. 249375, granted Jan 19, 2026.
6. **Mazur A.**, Niesyto K., Keihankhadiv S., Neugebauer D. “Method for preparing ionic conjugates for the delivery of drugs with antibacterial activity and their application”. Polish Patent No. 249520, granted Feb 18, 2026.

CONFERENCE PRESENTATIONS

2022

Mazur Aleksy, Niesyto Katarzyna, Neugebauer Dorota; Functionalization of monomeric ionic liquid with pharmaceutical anions for the preparation of ionic graft conjugates serving as drug delivery systems; XXVI Gliwice Scientific Meetings; Gliwice, 18-19.11.2022

2023

Mazur Aleksy, Neugebauer Dorota; Ionic nanocarriers for the delivery of pharmaceutical anions in antituberculosis therapies;
7th International Conference InterNanoPoland 2023; Katowice,
11- 12.10.2023

Mazur Aleksy, Neugebauer Dorota; Ionic graft conjugates for delivering pharmaceutical anions in tuberculosis treatments;
XXVII Gliwice Scientific Meetings; Gliwice, 16-17.11.2023

2024

Mazur Aleksy, Neugebauer Dorota; Ionic graft conjugates for dual delivery of anti-tuberculosis drugs;
EuroScience Open Forum 2024; Katowice, 12-15.06.2024

Mazur Aleksy, Neugebauer Dorota; Drug release studies and biological in vitro evaluation of choline based dual drug polymer systems;
XXVIII Gliwice Scientific Meetings; Gliwice, 21-22.11.2024

2025

Mazur Aleksy, Neugebauer Dorota; Synthesis of ionic graft copolymers carrying ampicillin anions;
The Silesian Meetings on Polymer Materials 2025; 2025

Mazur Aleksy, Neugebauer Dorota; Development of graft polymeric conjugates bearing ampicillin and cloxacillin ions derived from pharmaceutically active choline- ionic liquid monomers;
PolymerTalent - International Workshop on Polymer Structures for Medical Applications; Zabrze, 2025

Mazur Aleksy, Neugebauer Dorota; Solvent Responsive Ionic Graft Conjugates for Dual Delivery of Antituberculosis Drugs;
International Conference on Polymers for Sustainable Development - 2025; Bengaluru (online), 07.02.2025

RESEARCH PROJECTS

Researcher, CUPOLA Project (2026): Participation in the international research project "Carbon-neutral pathways of recycling marine plastic waste" (CUPOLA). The project is funded by the European Union under the Marie Skłodowska-Curie Actions (MSCA) Staff Exchanges (Horizon Europe), Grant Agreement No. 101086071. The research is implemented in cooperation with the Silesian University of Technology.

STATUTORY RESEARCH PROJECTS OF THE SILESIAN UNIVERSITY OF TECHNOLOGY

1. "Preparation of 3 or more delivery and co-delivery systems for single and dual drugs, based on para-aminosalicylate and/or nicotinic acid or another pharmaceutical anion, using graft-topology polymers obtained from monomeric ionic liquids modified with a drug anion."

04/040/BKM23/0263

2. "Synthesis and characterization of graft copolymers using monomeric ionic liquids modified with pharmaceutical anions."

04/040/BKM24/0291

INTERNSHIPS AND RESEARCH COLLABORATIONS

2024: Research collaboration with the **Centre of Polymer and Carbon Materials of the Polish Academy of Sciences (CMPW PAN) in Zabrze**, resulting in the joint publication: J. Konieczkowska, D. Neugebauer, A. Kozanecka-Szmigiel, A. Mazur, S. Kotowicz, and E. Schab-Balcerzak, "Photoresponse of new azo pyridine functionalized poly(2-hydroxyethyl methacrylate-co-methyl methacrylate)", Scientific Reports, 2024, 14, 1–9.

31.07.2026 – 01.09.2026: One-month scientific **internship at Xi'an Jiaotong University (China) at the country's best faculty of energy and power engineering**. The research focused on the catalytic pyrolysis of polymers, including polyethylene terephthalate (PET), glycol-modified polyethylene terephthalate (PETG), polypropylene (PP), polylactic acid (PLA), and high-density polyethylene (HDPE). The work also involved the collection of gaseous and liquid products and the analysis of their composition using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

WORKSHOPS AND TRAININGS

06.2023 – Participation in the scientific workshop titled "Progress in controlled radical polymerization," conducted by Krzysztof Matyjaszewski at the Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences in Łódź.

05–06.2023 – Training course on research project management: "Preparation for the role of a research project manager," organized at the Silesian University of Technology.

SCIENCE OUTREACH

Scientific Supervisor within the Student Scientific Society of Chemists,
Silesian University of Technology

Student: Kinga Kluczevska

Project title: Synthesis and characterization of drug carriers based on
copolymers containing ionic liquid units.

SCHOLARSHIPS AND AWARDS

2022/2023 – Rector's Scholarship for the Best Doctoral Students

02.2022 – 3rd Prize for the Best Diploma Thesis, SITPChem Competition,
Silesian University of Technology.