

Development of Pullulan-Based Functional Materials by Graft Copolymerization and Electrospinning

by

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Declaration of AI Use

During the preparation of this thesis, ChatGPT (OpenAI) and Gemini (Google) were used for language editing (grammar, clarity, and readability). The author reviewed and revised the output as needed and takes full responsibility for the final content.

DÉVELOPPEMENT DE MATÉRIAUX FONCTIONNELS À BASE DE PULLULANE PAR COPOLYMÉRISATION GREFFÉE ET ÉLECTROFILAGE

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RÉSUMÉ

Cette thèse examine des stratégies de synthèse et de mise en œuvre afin d'élargir le champ de performance du pullulane, un polysaccharide renouvelable, vers des matériaux fonctionnels modulables et adaptés à diverses applications. L'objectif principal est de comprendre comment la modification du pullulane gouverne la structure et les propriétés du matériau et permet la formation de membranes électrofilées stables en milieu aqueux. Le travail est organisé selon deux axes de recherche complémentaires : (i) une plateforme de polymérisation radicalaire par transfert de chaîne par addition–fragmentation réversible (RAFT) de type « grafting from » basée sur le pullulane, et (ii) un développement préliminaire de membranes électrofilées dérivées du pullulane.

Dans le premier axe, la synthèse repose sur la fonctionnalisation partielle du pullulane par le bromure de bromopropionyle, suivie d'une substitution par un agent de transfert de chaîne de type xanthate préalablement synthétisé, puis de la polymérisation du N-vinylcaprolactame, conduisant à des copolymères pullulane-g-poly(N-vinylcaprolactame) (PULL-g-PNVCL) présentant une croissance contrôlée de chaînes latérales thermo-réactives. Une caractérisation approfondie, incluant la chromatographie par perméation de gel (GPC) et la spectroscopie RMN du proton (^1H RMN), a confirmé la réussite du greffage. La GPC a montré une augmentation de la masse molaire entre le macro-RAFT précurseur et les copolymères greffés, avec des distributions unimodales et des dispersités d'environ 1,74 à 1,78. Les analyses par diffusion dynamique de la lumière (DLS) ont mis en évidence un comportement thermo-réactif, avec une augmentation du diamètre des particules au-delà de la température critique inférieure de solubilité (LCST) du PULL-g-PNVCL. La LCST a par ailleurs été observée comme dépendant de la longueur des chaînes de poly(N-vinylcaprolactame). En outre, la plateforme RAFT a été étendue au greffage médié par xanthate de l'acide acrylique, dans le but de produire des copolymères pullulane-g-poly(acide acrylique) (PULL-g-PAA) sensibles

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au pH. Des analyses structurales détaillées (^1H RMN/DOSY, clivage en conditions basiques catalysées, et GPC) ont montré que la polymérisation de l'acide acrylique à partir du pullulane-xanthate conduit à du PAA greffé ainsi qu'à une fraction substantielle de PAA libre, indiquant que l'homopolymérisation concurrente constitue une réaction secondaire dominante dans les conditions étudiées.

Dans le second axe, des dérivés d'acétate de pullulane présentant différents degrés de substitution ($\text{DS} \approx 4, 7$ et 8) ont été synthétisés puis électrofilés afin de former des membranes fibreuses. Les résultats démontrent que le DS et le système de solvants gouvernent conjointement la solidification du jet et la morphologie des membranes. Des essais d'immersion dans l'eau ont établi une transition, dépendante du DS, de la stabilité en milieu aqueux : la membrane à faible DS a perdu son architecture fibreuse et s'est effondrée en un film, tandis que les membranes à DS plus élevé ont conservé une ossature fibreuse poreuse ; de plus, un traitement à l'eau après électrofilage a permis d'extraire efficacement les solvants résiduels de mise en forme.

Dans l'ensemble, les résultats de cette thèse montrent que l'association d'architecture du pullulane avec des modifications chimiques ciblées constitue une voie cohérente vers des matériaux fonctionnels à base de pullulane présentant des performances pertinentes pour des applications. En intégrant des modifications chimiques durant la conception des polymères, ce travail illustre le développement de matériaux fonctionnels biosourcés en cohérence avec une utilisation durable des ressources.

Mots-clés : Pullulane, polymérisation RAFT/MADIX, greffage, poly(N-vinylcaprolactame), acide acrylique, électrofilage

DEVELOPMENT OF PULLULAN-BASED FUNCTIONAL MATERIALS BY GRAFT COPOLYMERIZATION AND ELECTROSPINNING

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ABSTRACT

This thesis investigates synthesis and processing strategies to expand the performance envelope of pullulan, a renewable polysaccharide, toward tunable, application-relevant functional materials. The main objective is to understand how pullulan modification governs material structure and properties and enables the formation of water-resilient electrospun membranes. The work is organized into two complementary research paths: a reversible addition-fragmentation chain transfer (RAFT) radical polymerization “grafting from” platform based on pullulan, and a preliminary development of pullulan-derived electrospun membranes.

In the first path, the synthesis involved partial functionalization of pullulan with bromopropionyl bromide, followed by substitution with a pre-synthesized xanthate-based chain transfer agent, and subsequent polymerization of N-vinyl caprolactam, yielding pullulan-g-poly(N-vinyl caprolactam) (PULL-g-PNVCL) with controlled growth of temperature-responsive side chains. Comprehensive characterization, including Gel Permeation Chromatography (GPC) and Proton Nuclear Magnetic Resonance spectroscopy (^1H NMR), confirmed successful graft copolymerization. GPC showed molar mass increases from the macro-RAFT precursor to the graft copolymers, with unimodal distributions and dispersities of $\sim 1.74 - 1.78$. Dynamic light scattering (DLS) analyses demonstrated a temperature-responsive behavior, with particle diameter increasing upon surpassing the lower critical solution temperature (LCST) of PULL-g-PNVCL. The LCST was observed to vary as a function of the poly(N-vinyl caprolactam) chain length. Furthermore, the RAFT platform was extended to xanthate-mediated grafting of acrylic acid, aiming to produce pullulan-g-poly(acrylic acid) (PULL-g-PAA). Detailed structural analyses (^1H NMR/DOSY, base-catalyzed cleavage, and GPC) showed that acrylic acid polymerization from pullulan-xanthate

produces grafted PAA together with a substantial fraction of free PAA, indicating that competing homopolymerization is a dominant side reaction under the investigated conditions.

In the second research path, pullulan acetate derivatives with different degrees of substitution ($DS \approx 4, 7$, and 8) were synthesized and electrospun to form fibrous membranes. The results demonstrated that DS and the solvent system jointly govern jet solidification and membrane morphology. Water-immersion testing established a DS-dependent transition in aqueous stability: the lower-DS membrane lost its fibrous architecture and collapsed into a film, whereas higher-DS membranes retained a porous fibrous scaffold; importantly, post-spinning water treatment effectively extracted residual spinning solvents.

Together, the results of this thesis demonstrate that coupling pullulan architectures with targeted chemical modification provides a coherent route to pullulan-based functional materials with application-relevant performance. Moreover, integrating chemical modifications with polymer design, this research showcases the development of bio-based functional materials aligned with sustainability-driven resource utilization.

Keywords: Pullulan, RAFT/MADIX polymerization, Grafting, Poly(N-vinyl caprolactam), Acrylic acid, Electrospinning

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	Acrylic acid
AIBN	2,2'-Azobisisobutyronitrile
ATR-FTIR	Attenuated total reflectance Fourier transform infrared spectroscopy
ATRP	Atom transfer radical polymerization
BPB	2-bromopropionyl bromide
CDCl ₃	Deuterated chloroform
CMP	Carboxymethyl pullulan
COSY	Correlation spectroscopy (¹ H– ¹ H)
CRP	Controlled radical polymerization
CTA	Chain transfer agent
CTAs	Chain transfer agents
D ₂ O	Deuterium oxide
DLS	Dynamic light scattering
DMF	N, N-dimethylformamide
DMF/LiBr	DMF containing lithium bromide (GPC eluent)
DMSO	Dimethyl sulfoxide
DMSO-d ₆	Deuterated dimethyl sulfoxide
DOSY	Diffusion-ordered spectroscopy
DS	Degree of substitution
DSC	Differential scanning calorimetry
GPC	Gel permeation chromatography

GRAS	Generally recognized as safe
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol
KEX	Potassium ethyl xanthogenate
LCST	Lower critical solution temperature
MADIX	Macromolecular design via interchange of xanthates
MgSO ₄	Magnesium sulfate
NaIO ₄	Sodium periodate
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
NVCL	N-vinylcaprolactam
OSA	Octenyl succinic anhydride
PAA	Poly(acrylic acid)
PNVCL	Poly(N-vinylcaprolactam)
PdI	Polydispersity index (Particle size distribution)
PULLAc	Pullulan acetate
PULLBr	Bromide-bearing pullulan (bromopropionylated pullulan)
PULLX	Pullulan–xanthate macro-chain transfer agent (macro-CTA)
PULL-g-PAA	Pullulan-graft-poly(acrylic acid)
PULL-g-PNVCL	Pullulan-graft-poly(N-vinylcaprolactam)
RAFT	Reversible addition-fragmentation chain transfer
SEM	Scanning electron microscopy
S _N 2	Bimolecular nucleophilic substitution
TEA	Triethylamine

TGA	Thermogravimetric analysis
UCST	Upper critical solution temperature

LIST OF SYMBOLS AND UNITS OF MEASUREMENT

$^{\circ}\text{C}$	Celsius temperature
Cm	Centimeter
$\bar{D}$	Dispersity (M_w/M_n)
ΔH	Enthalpy of mixing
ΔS	Entropy of mixing
$[\text{M}]_0$	Initial monomer concentration ($\text{mol}\cdot\text{L}^{-1}$)
$[\text{CTA}]_0$	Initial chain transfer agent concentration ($\text{mol}\cdot\text{L}^{-1}$)
ΔG	Gibbs free energy of mixing
g	Gram
g/mol	Gram per mole
T_g	Glass transition temperature
Z_{av}	Hydrodynamic average diameter
h	Hour
kDa	Kilodalton
kV	Kilovolt
kg/mol	Kilogram per mol
L	Liter
mg/ml	Mass concentration
MHz	Megahertz
mL	Milliliter

mL/h	Milliliter per hour
mg	Milligram
min	Minute
mmol	Millimole
mol	Mole
mol/L (M)	Molar concentration
T _m	Melting temperature
μL	Microliter
μm	Micrometer
p	Monomer conversion
M _{monomer}	Molar mass of monomer repeat unit
M _{CTA}	Molar mass of chain transfer agent
nm	nanometer
N ₂	Nitrogen gas
δ	NMR chemical shift
T _{onset}	Onset degradation temperature
ppm	Parts per million
%	Percentage
s	Second
T	Temperature
T _{max}	Temperature at maximum mass-loss rate
mL/h	Volumetric flow rate
v/v	Volume ratio

cm^{-1}

Wavenumber

wt%

Weight percent

INTRODUCTION

The development of advanced polymeric materials is increasingly shaped by two converging drivers: the need for high-performance functionalities and the imperative to rely on sustainable, bio-based feedstocks. Within this context, polysaccharides offer a compelling platform due to their renewability, inherent biocompatibility, and amenability to chemical derivatization (El Ghoul, Ammar, & Alsultan, 2025; Gopinath, Saravanan, Al-Maleki, Ramesh, & Vadivelu, 2018; Simsek, Asiyanbi-Hammed, Rasaq, & Hammed, 2023; Torres, Troncoso, Pisani, Gatto, & Bardi, 2019). Pullulan, a water-soluble polysaccharide composed of maltotriose repeating units, has attracted sustained attention as a bio-based macromolecular framework (de Souza et al., 2023; Singh, Kaur, Singh, Purewal, & Kennedy, 2023; Thakur, Sharma, Naman, & Baldi, 2023). Its high amount of hydroxyl groups enables diverse chemical modifications, which support its potential use, such as in biomedical and environmental technologies (Raychaudhuri et al., 2020; Singh, Kaur, Hassan, & Kennedy, 2021; Tiwari, Patil, Dubey, & Bahadur, 2019). At the same time, native pullulan remains limited by its strong hydrophilicity and rapid dissolution in aqueous media, as well as by the relatively narrow range of functions that can be accessed without structural modification. Expanding the application window of pullulan, therefore, requires strategies that introduce targeted and tunable physicochemical characteristics while preserving the advantages of the polysaccharide backbone. A powerful route toward such tunability is the synthesis of pullulan-based graft copolymers (Raychaudhuri et al., 2020; Tiwari et al., 2019). By covalently attaching synthetic side chains, grafting enables the molecular engineering of stimuli-responsive behavior (e.g., temperature- or pH-responsiveness). In recent years, controlled radical polymerization techniques have become central to this objective because they can, in principle, provide control over chain length, dispersity, and architecture (Corrigan et al., 2020; Moad & Rizzardo, 2020; Truong, Jones, Bradford, Konkolewicz, & Anastasaki, 2021). Among these approaches, reversible addition-fragmentation chain transfer (RAFT) polymerization is particularly attractive for polysaccharide functionalization, as it can be implemented through a “grafting from” strategy using a chain transfer agent anchored to the backbone (Hernández-Guerrero, Davis, Barner-Kowollik, & Stenzel, 2005; Hua, Tang, Cheng, Deng, & Zhu, 2008; Ikkene et al., 2020; Pal,

Sarkar, Karmakar, & Pal, 2018; Tang, Hua, Cheng, Jiang, & Zhu, 2008). However, translating controlled polymerization concepts to polysaccharide substrates remains non-trivial. In practice, the chemical accessibility and uniformity of functionalization along a polysaccharide backbone can be constrained by the initial molar mass and dispersity of the starting material, which may promote chain entanglement. As a result, establishing a robust macro-RAFT platform requires careful design of the backbone functionalization chemistry, the chain transfer agent, and the polymerization conditions, followed by rigorous structural characterization to distinguish true grafting versus competing side processes.

From an application standpoint, temperature- and pH-responsive polymers represent two widely exploited stimulus systems, particularly in controlled release, biosensing, and responsive coatings (Teixeira do Nascimento et al., 2025; Wei, Gao, Li, & Serpe, 2017). Poly(N-vinyl caprolactam) (PNVCL) is a prominent temperature-responsive polymer, and grafting it from pullulan provides a direct route to impart temperature-responsiveness to a renewable backbone and to access self-assembled particles in aqueous media. In parallel, poly(acrylic acid) (PAA) is a pH-responsive polymer in which ionization of carboxylic acid groups enables changes in solubility, swelling, and interchain interactions (Dalei & Das, 2022; Ofridam et al., 2021; Pourmadadi et al., 2023). In addition to molecular functionalization, the translation of pullulan-derived materials into practical formats often requires processes. Electrospinning is a versatile technique to fabricate micro- to nanoscale fibrous membranes with high surface area, interconnected porosity, and tunable architecture, features that are valuable for applications such as wound dressings, drug-loaded scaffolds, coatings, and filtration/separation membranes (Ahmadi Bonakdar & Rodrigue, 2024; Al-Abduljabbar & Farooq, 2023; Elfawal, Šišková, & Andicsová, 2025; Xue, Wu, Dai, & Xia, 2019). Nonetheless, electrospinning pullulan-derived fibers into water-resilient structures is intrinsically challenging. Chemical modification offers a logical solution that, by increasing the hydrophobic character, may make it possible to retain a fibrous architecture upon aqueous exposure. Acetylation is an especially practical modification because it can be implemented with different degrees of substitution (DS), enabling exploration of how substitution level governs both electrospinnability and water stability (Niu, Shao, Chen, & Sun, 2019; Teramoto & Shibata, 2006; Zhang et al., 2009). A careful, structure-processing-property perspective is

therefore required to define conditions under which pullulan acetate can be electrospun and can preserve a porous fibrous scaffold in water.

In this context, the central objective of this thesis is to evaluate and develop pullulan-based functional materials by tailoring graft copolymerization routes and producing electrospun membranes, while elucidating the resulting structure-property relationships. The work begins by establishing a pullulan-xanthate-based RAFT grafting platform intended to generate stimuli-responsive pullulan graft copolymers. Within this path, temperature-responsive pullulan-g-poly(N-vinyl caprolactam) (PULL-g-PNVCL) copolymers are designed and synthesized, and their molecular characteristics are correlated with temperature-responsive behavior in aqueous media. The platform is then challenged and extended to acrylic acid with the aim of producing pullulan-g-poly(acrylic acid) (PULL-g-PAA) copolymers and, critically, assessing the feasibility and limitations of xanthate-mediated grafting for an acidic monomer through structural analysis. The second path is a preliminary study dedicated to the development of pullulan-based electrospun membranes. Here, pullulan is acetylated to generate derivatives with various DS, and electrospinning formulations and processing windows are identified to produce uniform fibers, followed by evaluation of water stability and morphological evolution upon aqueous exposure.

Following this introduction, Chapter 1 presents a critical literature review and state-of-the-art overview, establishing the background on pullulan, RAFT polymerization, and electrospinning, and positioning the present work within current advances relevant to each thesis theme. Chapter 2 then outlines the main research objective and the specific objectives that guide the work. Chapter 3 details the overall methodology, including the research strategy, materials, synthesis procedures, processing steps, and characterization techniques. Chapters 4 through 6 present the research results and discussion: Chapter 4 addresses the synthesis and characterization of PULL-g-PNVCL via RAFT polymerization and the resulting temperature-responsive behavior; Chapter 5 examines the extension of this “grafting from” strategy to PULL-g-PAA; and Chapter 6 reports the acetylation of pullulan and the fabrication of electrospun pullulan acetate fibrous membranes. The thesis concludes with an integrated

discussion of the key findings, their implications for the design of pullulan-based functional materials, and perspectives for future development.

CHAPTER 1

LITERATURE REVIEW

This chapter is structured into two main sections: General Background and State of the Art. The first section outlines the key concepts supporting this work, including the structure and properties of pullulan and its modification strategies, the principles of reversible addition-fragmentation chain transfer (RAFT) polymerization, and the main characteristics of the electrospinning process. Building on this foundation, the second section provides a critical overview of recent literature on pullulan-based graft copolymers, electrospun membranes derived from modified pullulan, and the current and emerging applications of modified pullulan.

1.1 General Background

1.1.1 Pullulan

Pullulan is a biopolymer obtained from microbial fermentation of starch-based biomass that has garnered significant interest due to its biocompatibility, biodegradability, and low toxicity (de Souza et al., 2023; Singh, Kaur, Singh, Purewal, & Kennedy, 2023; Thakur, Sharma, Naman, & Baldi, 2023). Pullulan is mainly degraded through enzymatic cleavage of its glycosidic linkages in natural environments and in some biological media (Coltelli, Danti, De Clerck, Lazzeri, & Morganti, 2020; Naik et al., 2023; Wolf, Garleb, Choe, Humphrey, & Maki, 2003). Its synthesis can be supported by diverse carbon sources (e.g., glucose- and lactose-based media), and many renewable substrates have been investigated to enable more sustainable production routes. In particular, agro-industrial residues such as sweet potato, potato starch, and sugarcane bagasse have been reported as substrates for pullulan production (S. Wu, Jin, Tong, & Chen, 2009). Pullulan was first produced in 1958, and commercial-scale production was subsequently developed in the late 1970s by a Japan-based company, Nagase Vitta (formerly Hayashibara Co., Ltd.), later established its industrial production (Cheng, Demirci, & Catchmark, 2011; Prajapati, Jani, & Khanda, 2013; Raychaudhuri et al., 2020; R.

S. Singh, Kaur, Hassan, & Kennedy, 2021a). It is produced from starch syrup (Prajapati et al., 2013).

Structurally, pullulan is a polysaccharide consisting of repeating maltotriose units, each composed of three glucose units linked by α -(1 $\rightarrow$ 4) glycosidic bonds, with consecutive maltotriose units connected to one another through α -(1 $\rightarrow$ 6) glycosidic linkages. Its chemical structure is shown in Figure 1.1. Pullulan is frequently described as not interfering with drugs (R. S. Singh et al., 2023; Thakur et al., 2023). In addition, pullulan has been listed as a Generally Regarded as Safe (GRAS) material for food applications (US-FDA, 2002).

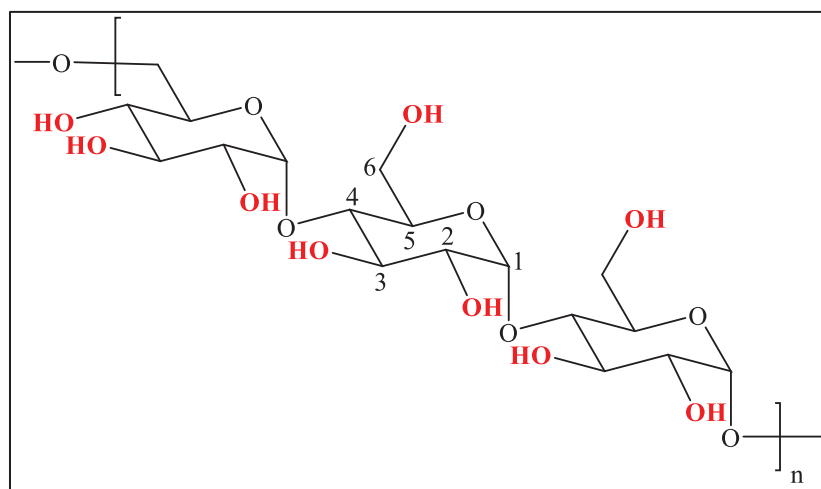


Figure 1.1 Chemical structure of pullulan

A defining characteristic of pullulan is its high water solubility, which can be interesting in various applications such as edible food coating (Erceg et al., 2024; Priyadarshi, Riahi, & Rhim, 2022), fast-dissolving films (Ponrasu, Chen, Chou, Wu, & Cheng, 2021; Ze-yu Qin, Jia, Liu, Kong, & Wang, 2019), and production of capsules (Ding et al., 2020). Although its hydrophilicity is beneficial for some applications, in the context of expanding the use of pullulan, such as in biomedical fields and drug delivery systems, water treatment materials, and packaging, such high solubility may cause undesired rapid dissolution, poor structural stability, and shortened functional lifetime of pullulan-based structures. Consequently, in applications that demand lower water solubility, chemical modification of pullulan is often

required to tailor pullulan's aqueous resilience and introduce application-specific functionalities, thereby broadening its processing window and expanding its performance envelope for targeted end uses.

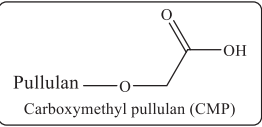
1.1.2 Chemical Modifications of Pullulan

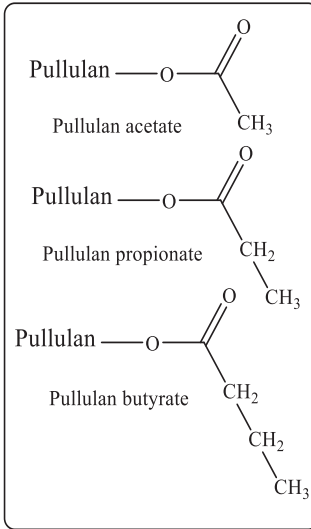
Pullulan contains nine hydroxyl groups along its backbone that serve as reactive sites for chemical functionalization aimed at tuning its properties. Such modifications enable the development of novel materials that valuably combine the properties of different materials for specific applications that are not accessible to native pullulan (Raychaudhuri et al., 2020; Singh, Kaur, Hassan, & Kennedy, 2021; Tiwari, Patil, Dubey, & Bahadur, 2019). The chemical modification of pullulan can be achieved by introducing functional groups or by grafting larger polymer chains, which are discussed in the following sections.

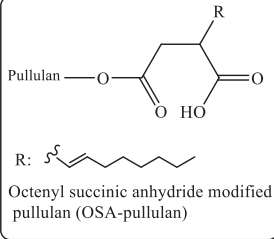
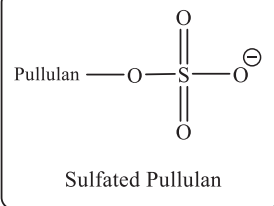
1.1.2.1 Functional Groups Introduction to Pullulan Structure

The functionalization of pullulan can be achieved through several chemical reactions that introduce desired functional groups by reacting with its hydroxyl groups. Reported approaches include etherification (e.g., carboxymethylation), esterification (e.g., acetylation, sulfation), and oxidation. These reactions are used to tailor structure-property relationships by adjusting parameters such as the hydrophilicity/hydrophobicity balance, charge density, and the availability of crosslinkable groups, thereby enabling changes in thermal and mechanical behavior (Agrawal, Budhwani, Gurjar, Telange, & Lambole, 2022; Ganie, Rather, Assiri, & Li, 2024; Singh et al., 2021; Tiwari et al., 2019). Table 1.1 lists some of the recent research papers on the derivatization of pullulan using these approaches to enable tailored applications in diverse fields.

Table 1.1 Summary of some recent studies on pullulan functional group modifications

Modification	Pullulan Modifier Agent	Aim of the study	Key Findings / Contributions	Chemical Structure/Other references
Etherification	Chloroacetic acid and NaOH	Chemical modification of pullulan (carboxymethyl pullulan (CMP)) and assess its coating properties to be used in blueberry preservation	Decreasing thermal stability and increasing the glass transition temperature, mechanical properties, and water vapor permeability in CMP compared to unmodified pullulan. Film forming and prolonging the shelf life of blueberries (Hernandez-Tenorio et al., 2024).	 Pullulan —O—CH₂—C(=O)OH Carboxymethyl pullulan (CMP) (Legros, Cardinael, Dulong, Picton, & Le Cerf, 2008; Shibata, Nozawa, Teramoto, & Yosomiya, 2002; Souguir, Roudesli, About-Jaudet, Cerf, & Picton, 2007)
	Chloroacetic acid and NaOH	Obtain a CMP / modified chitosan bilayer edible coating for sliced cheese	The bi-layer coating showed improved mechanical and water vapor barrier properties, and prolonged the shelf-life of sliced cheese by 50 % (Erceg et al., 2024).	
	Sodium chloroacetate and NaOH	Develop an injectable gel based on a physical mixture of CMP and poly (ethylene oxide)-poly (propylene glycol)-poly (ethylene oxide) (Known as Poloxamer)	CMP with a high degree of substitution was required to obtain miscible polymeric mixtures, whereas a low degree of substitution led to phase separation. CMP addition modulated gel strength and self-healing (Popescu et al., 2023).	

Modification	Pullulan Modifier Agent	Aim of the study	Key Findings / Contributions	Chemical Structure / Other references
Esterification	Acetic anhydride and pyridine	Prepare pullulan acetate in order to assess the impact of acetylation on thermal and film properties	Acetylation of degree of substitution 2.90 produced a more hydrophobic pullulan acetate with increased thermal stability, and decreased tensile strength and elongation than unmodified pullulan (Hernandez-Tenorio & Giraldo-Estrada, 2022).	 (Enomoto-Rogers, Iio, Takemura, & Iwata, 2015; Jung, Jeong, & Kim, 2003; Teramoto & Shibata, 2006; H. Zhang et al., 2009)
	Acetic anhydride, propionic anhydride, and butyric anhydride, besides pyridine	Synthesize a series of hydrophobic pullulan esters and evaluate their suitability as packaging films for fruit preservation	Esterification gave pullulan esters with various degrees of substitution; increasing degrees of substitution enhanced hydrophobicity but reduced tensile strength and elongation. Modified pullulan with a higher degree of substitution showed the lowest water-vapor and oxygen permeability and, as coating films, effectively delayed softening and weight loss of fruits (Niu et al., 2019).	

Modification	Pullulan Modifier Agent	Aim of the study	Key Findings / Contributions	Chemical Structure / Other references
Esterification	Octenyl succinic anhydride (OSA) and NaOH	Optimize the esterification of pullulan with OSA and evaluate how introducing hydrophobic octenyl groups alters emulsifying properties.	Esterification was confirmed by FTIR and ^1H NMR and led to higher solution viscosity, and markedly improved emulsifying and foaming capacity compared with native pullulan, showing OSA-pullulan as a surface-active pullulan derivative (Omar-Aziz et al., 2020a).	 Octenyl succinic anhydride modified pullulan (OSA-pullulan) (Shah, Vishwasrao, Singhal, & Ananthanarayan, 2016; Suenaga, Komuro, & Terao, 2025; Zhao et al., 2020)
	Chlorosulfuric Acid	Obtain sulfated pullulan and use it, together with an aminated pullulan, to form pullulan-based polyelectrolyte complex nanoparticles for protein delivery.	Sulfation of pullulan with $\text{SO}_3\cdot\text{DMF}$ yielded sulfated pullulan with a stable negative charge, which formed polyelectrolyte complexes with oppositely charged pullulan, giving nanoparticles for drug delivery applications (M. Dionísio et al., 2016).	 Sulfated Pullulan (Alban, Schauerte, & Franz, 2002; Marita Dionísio et al., 2013; Mähner, Lechner, & Nordmeier, 2001)
Oxidation	Sodium periodate (NaIO_4)	Prepare the oxidized pullulan and form chitosan/oxidized pullulan for oil encapsulation	Oxidized pullulan is demonstrated as a reactive crosslinker to prepare hydrogels that are porous with sustained oil-release and combined antioxidant/antimicrobial activity (Suflet et al., 2024).	

Modification	Pullulan Modifier Agent	Aim of the study	Key Findings / Contributions	Chemical Structure / Other references
Oxidation	Sodium periodate (NaIO ₄)	Determine whether oxidation can convert pullulan into an antibacterial polymer against <i>S. aureus</i>	Single-step oxidation (~28% aldehyde) produced oxidized-pullulan with ~97% bactericidal and strong activity against <i>S. aureus</i> , acting via disruption of its membrane integrity, while remaining low-toxic to mammalian cells (Roy et al., 2023).	Please see Figure 1.2 (Liang et al., 2019; Ma et al., 2025; Selvakumar & Lonchin, 2020; L. Zhang et al., 2019)
	Sodium periodate (NaIO ₄)	Tune the properties of oxidized pullulan/chitosan hydrogel	Showing oxidized pullulan as a key handle to tailor hydrogel performance for wound dressings by increasing storage modulus, cohesion, and tissue adhesion while preserving injectability and self-healing (Elangwe, Uspenskaya, & Olekhovich, 2025).	

Etherification introduces ether bonds into the pullulan backbone, which are commonly used to impart ionic character or to modulate solubility. The esterification of pullulan, which serves as a platform for introducing hydrophobic segments, often involves converting hydroxyl groups into ester groups, while retaining the original oxygen atoms in the backbone. It is a method for disturbing the polymer's hydrogen bond network, thereby increasing its hydrophobicity. Oxidation is used to selectively transform vicinal diols into more reactive groups, such as aldehydes, which can be useful in crosslinking (Agrawal et al., 2022; Ganie et al., 2024; Tiwari et al., 2019). Selected chemical structures of the modified pullulan through these functional group strategies are presented in Figure 1.2. Moreover, according to the literature, the degree

of substitution of the hydroxyl groups by the functional groups is the key control variable linking chemistry to properties, including solubility and charge density.

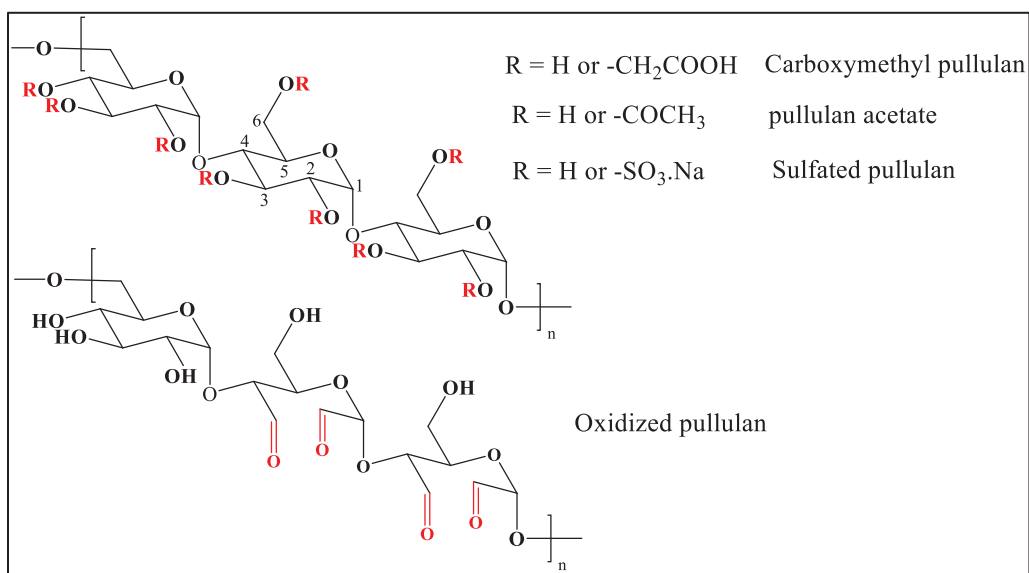


Figure 1.2 Chemical structures of selected pullulan derivatives mentioned in Table 1.1

Taken together, these functional group strategies provide a modular functionalization platform: they can (i) directly tune water solubility through hydrophobization/amphiphilization, and (ii) generate reactive handles that enable subsequent derivatization, such as graft copolymerization, thereby bridging native pullulan properties to application-driven requirements.

1.1.2.2 Grafting

Grafting polymer chains to pullulan is a widely adopted strategy to expand its functionality and application. This approach is attractive because it enables the incorporation of properties from the grafted component while preserving the primary structure of pullulan in the main chain (Bhattacharya & Misra, 2004; Raychaudhuri et al., 2020; Tiwari et al., 2019). In general, pullulan graft copolymers are prepared via two main routes, “grafting onto” and “grafting from”, which yield architectures consisting of a linear backbone bearing multiple side chains.

In the grafting onto strategy, pre-synthesized polymer chains bearing functional groups are coupled to the pullulan backbone. In contrast, in the “grafting from” approach, the polymer chains are grown from the functionalized pullulan backbone (Purohit, Bhatt, Mittal, Abdellattif, & Farghaly, 2023; Tiwari et al., 2019). These grafting approaches are presented in Figure 1.3.

Although “grafting onto” is conceptually straightforward, it often leads to relatively low grafting densities because the coupling of additional macromolecules becomes progressively hindered by steric crowding from chains already attached. In comparison, the “grafting from” method can achieve higher grafting efficiency and offers superior control over the final material's molar mass and distribution. Nevertheless, quantitative characterization of graft copolymers, particularly the determination of the exact number and size of the grafts, is challenging (Tiwari et al., 2019b). This difficulty may arise from the need to separate the contributions of the backbone and side chains in order to determine both the number of grafts per backbone and the molar mass of individual grafts with sufficient accuracy.

Importantly, graft copolymerization provides a versatile route to introduce *stimuli-responsive* segments onto the pullulan backbone. Therefore, the following section outlines the fundamentals of stimuli-responsive polymers.

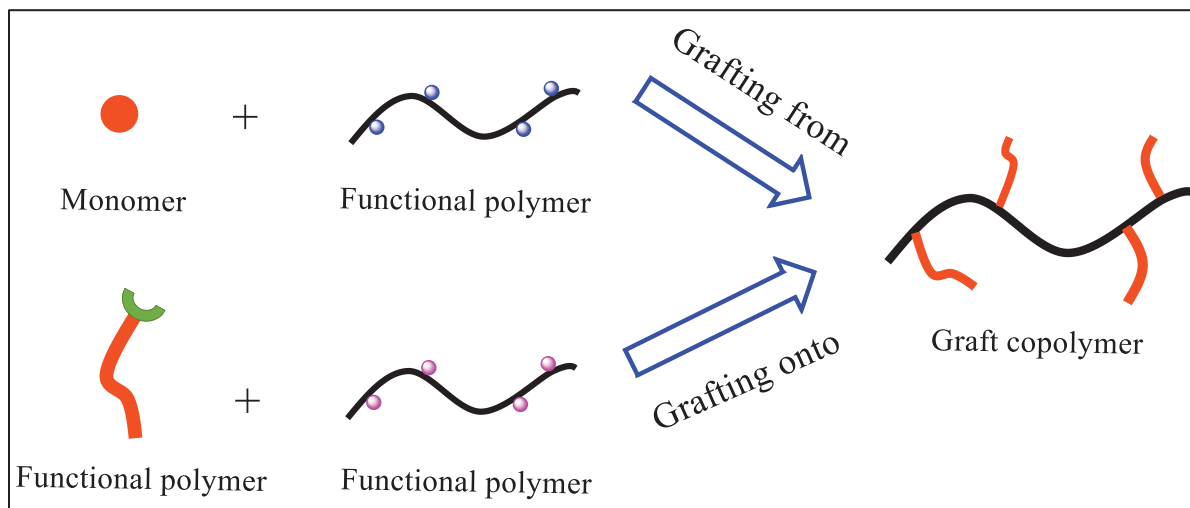


Figure 1.3 Generalized categories of graft copolymer synthesis approaches: Grafting from and Grafting onto. In the grafting from approach, chain-transfer sites are first introduced onto the backbone, and the side chains are then grown directly from these anchored sites during polymerization. In the grafting onto approach, pre-formed polymer chains bearing a reactive end group are covalently attached to complementary functional groups already present on the backbone

1.1.3 Stimuli-Responsive Polymers

Stimuli-responsive polymers (often known as smart polymers) are macromolecular systems that undergo changes in physicochemical properties in response to external triggers, which are frequently reversible. Depending on polymer chemistry and architecture, these responses may manifest as changes in solubility, chain conformation, hydrophilic-hydrophobic balance, or network swelling/shrinking. Typical stimuli include temperature, pH, light, and electric or magnetic fields (Medeiros, Santos, Fessi, & Elaissari, 2011; Wei, Gao, Li, & Serpe, 2017).

The interest in stimuli-responsive polymers stems from their ability to impart functionality to materials, enabling performance in applications such as controlled drug delivery, biosensing, tissue engineering, and smart coatings (Medeiros et al., 2011; Teixeira do Nascimento et al., 2025; Ward & Georgiou, 2011; Wei et al., 2017). In the context of this thesis, stimuli-responsiveness is pursued as a functionalization strategy to complement pullulan's intrinsic properties by integrating temperature- or pH-responsive polymer segments. Accordingly, the

following subsections focus on two widely used and practically relevant trigger modes, temperature-responsive and pH-responsive polymers, which are the focus of this work and can be integrated into pullulan-based systems.

1.1.3.1 Temperature-Responsive Polymers

Temperature-responsive polymers exhibit a characteristic transition temperature at which their affinity for water changes markedly, leading to a pronounced shift in solubility and chain conformation. Near this critical temperature, thermal variations change the balance between hydrophilic and hydrophobic interactions, driving transformations such as chain collapse/expansion and, in solution, changes in aggregation (Wei et al., 2017; Yuan et al., 2023).

Temperature-responsive polymers are commonly categorized according to whether they display a lower critical solution temperature (LCST) or an upper critical solution temperature (UCST) in a given solvent system. LCST-type polymers are soluble below a characteristic transition temperature, but upon heating above the LCST, they undergo a rapid loss of solubility that can manifest as collapsing chain or aggregation. In contrast, UCST-type polymers exhibit the opposite temperature dependence: they are poorly soluble at lower temperatures and become soluble upon heating above the UCST (Seuring & Agarwal, 2012; Yuan et al., 2023).

LCST-type temperature-responsive polymers, which are the target of interest in this thesis, are typically soluble in water at lower temperatures by making hydrogen bonds with the water molecules. As temperature increases, polymer hydration becomes less favorable and the balance shifts toward polymer-polymer interactions, particularly involving hydrophobic segments, resulting in chain collapse and aggregation above the LCST (Cortez-Lemus & Licea-Claverie, 2016; Pasparakis & Tsitsilianis, 2020; Wei et al., 2017; Yuan et al., 2023). This behavior can be interpreted in terms of the Gibbs free energy of mixing, $\Delta G = \Delta H - T\Delta S$: at temperatures above LCST, the increasingly positive $-T\Delta S$ term (due to negative ΔS)

outweighs the enthalpic gain, and drives ΔG toward positive values, thereby promoting dehydration and phase separation of the systems (Pasparakis & Tsitsilianis, 2020). This behavior is illustrated schematically in Figure 1.4.

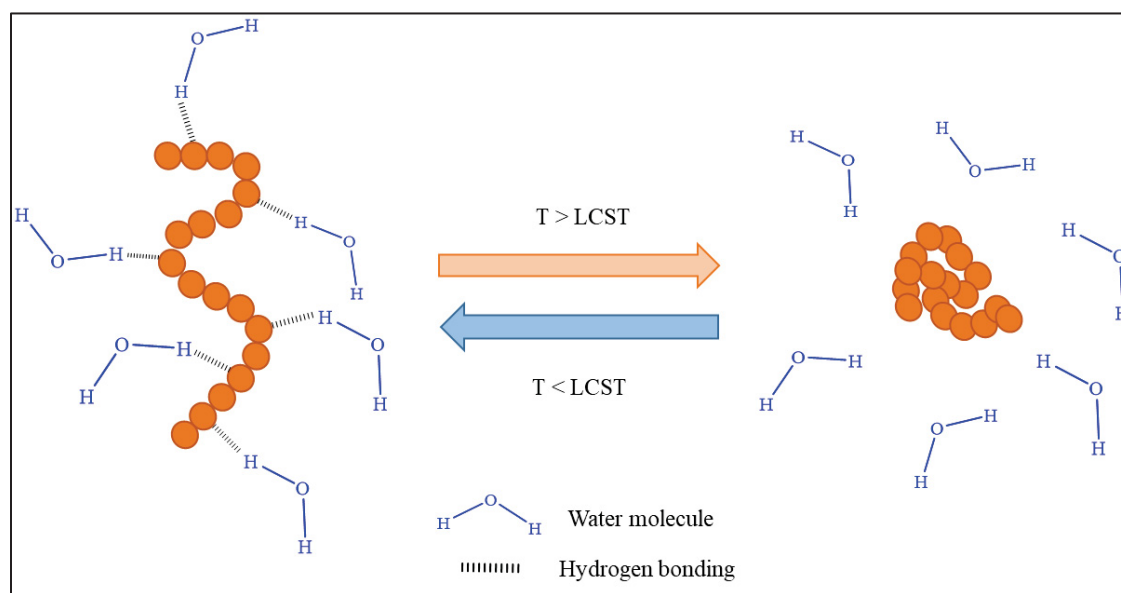


Figure 1.4 The effect of temperature on the phase transition of a thermosensitive polymer chain

A wide variety of temperature-responsive polymers have been reported, exhibiting transition temperatures that span a broad range (Liu, Fraylich, & Saunders, 2009; Medeiros et al., 2011; Pasparakis & Tsitsilianis, 2020). Among these materials, poly(N-vinylcaprolactam) (PNVCL) is frequently highlighted as a biocompatible LCST-type polymer whose transition temperature is close to physiological temperature (typically reported in the ~ 30 - 38 °C range), making it particularly attractive for biomedical technologies, including drug-delivery systems. However, because the LCST is near body temperature, it can limit the system's utility in the bloodstream or hyperthermic tumor environments.

PNVCL is obtained by polymerization of N-vinylcaprolactam (NVCL), an amphiphilic monomer bearing a hydrophilic carbonyl group and a comparatively hydrophobic ring and main chain environment (Figure 1.5). In addition, because the amide functionality is

incorporated within the lactam structure rather than as a backbone linkage, hydrolytic pathways are commonly described as yielding polymer-bound functionalities rather than generating small, potentially harmful amide by-products. Accordingly, PNVCL is regarded as a biocompatible polymer relative to several other temperature-responsive polymers (Cortez-Lemus & Licea-Claverie, 2016; González & Frey, 2017; Medeiros et al., 2011).

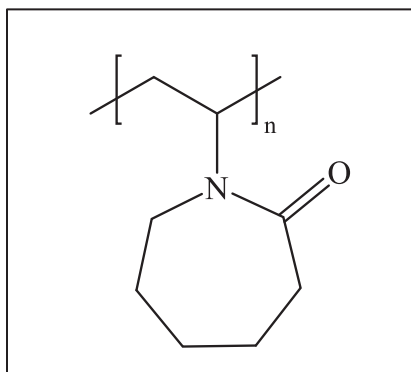


Figure 1.5 Chemical structure of PNVCL

Beyond temperature-responsive systems, pH-responsive polymers represent another major class of stimuli-responsive materials. The following section outlines their key characteristics and the mechanisms by which pH governs their behavior.

1.1.3.2 pH-Responsive Polymers

pH-responsive materials are designed to change their physicochemical behavior when the acidity/basicity of their environment varies. For pH-responsive polymers, this behavior arises from the presence of ionizable functional groups that can reversibly accept or donate protons as a function of pH. Ionization alters charge density along the macromolecular chain, which can modify intermolecular interactions and trigger changes in properties such as solubility, conformation, and swelling. In aqueous media, once ionization occurs, electrostatic repulsion between charges on the polymer can promote chain expansion (Ofriidam et al., 2021; J. Singh & Nayak, 2023).

Among widely studied pH-responsive polymers, poly(acrylic acid) (PAA) is a classic example with broad use in industrial and biomedical contexts, including thickening, dispersion stabilization, coatings, adhesion, and drug delivery systems. For dispersion stabilization, ionized groups provide electrostatic repulsion between particles, reducing aggregation. Moreover, in drug delivery systems, PAA's pH-dependent ionization and associated swelling (e.g., at physiological pH) can be leveraged to form controlled release carriers for therapeutics. At pH values above the pKa, a significant fraction of carboxylic acid groups deprotonate to a carboxylate; conversely, at pH values below pKa, the carboxyl groups are predominantly present in their protonated form (Figure 1.6). At pH = pKa, the concentrations of protonated and deprotonated groups are equal (Arkaban et al., 2022; Dalei & Das, 2022; Grunlan, Liu, & Kim, 2006; Ofriidam et al., 2021; Pourmadadi et al., 2023; Ran, Wu, Shen, Ran, & Shen, 2007; M. Wang et al., 2024).

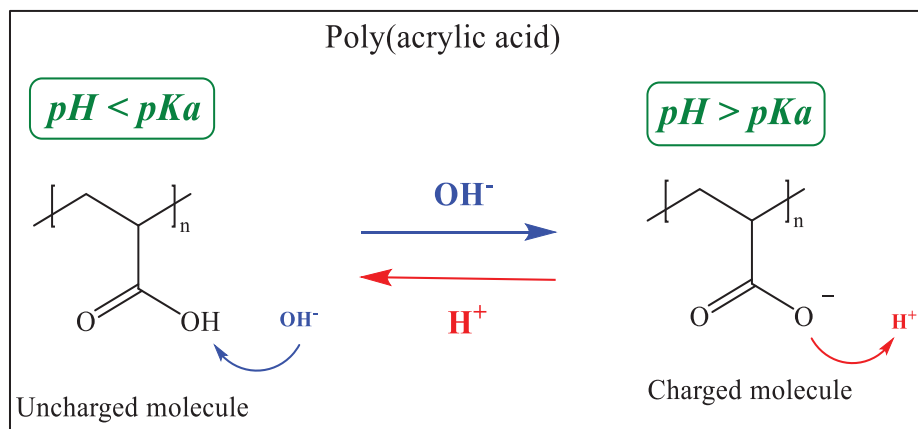


Figure 1.6 Schematic representation of the pH-dependent ionization of poly(acrylic acid) chains, highlighting the transition between protonated and deprotonated states as a function of pH

To achieve well-defined and tunable stimuli-responsive polymers, conventional free radical polymerization often provides broad molar mass distributions and limited control over chain architecture. Accordingly, this motivates the use of controlled radical polymerization (CRP) techniques, such as RAFT polymerization, which is introduced in the following section.

1.1.4 Reversible Addition-Fragmentation Chain Transfer Polymerization

Reversible addition-fragmentation chain transfer (RAFT) polymerization is a widely used form of controlled (living) radical polymerization that enables precise control over molar mass, molar mass distribution, and polymer architecture, such as stars, branched, block, and graft copolymers (Chiefari et al., 1998; Hawthorne, Moad, Rizzardo, & Thang, 1999; Moad et al., 2000; Moad, Rizzardo, & Thang, 2008; Moraes et al., 2020; Nakabayashi & Mori, 2013). In practice, RAFT is conducted under conventional radical polymerization conditions but in the presence of a chain transfer agent (CTA), which mediates a reversible chain transfer process. Chain transfer agents (CTAs) are the central mediating species in RAFT polymerization and are commonly represented by the general structure below (Figure 1.7 (a)). A broad family of CTAs has been developed, including dithioesters (dithiobenzoates), trithiocarbonates, xanthates, and dithiocarbamates, as shown in Figure 1.8 (b) (Opiyo & Jin, 2021; Perrier, 2017; Tian et al., 2018).

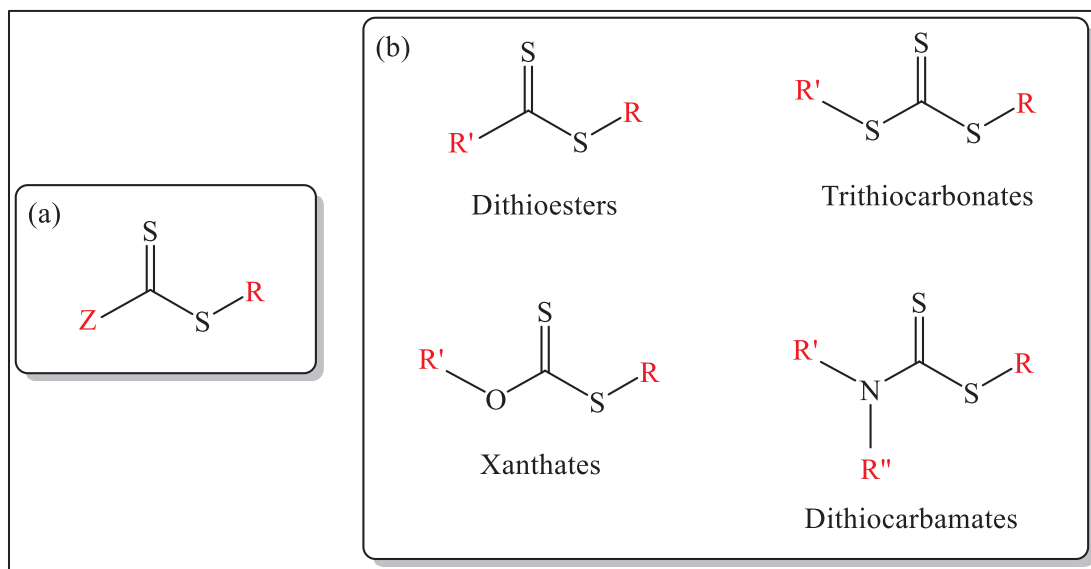


Figure 1.7 (a) general structure of a CTA; (b) structure of different classes of CTA (dithioesters, trithiocarbonates, xanthates, and dithiocarbamates)

Adopted from Opiyo & Jin, (2021); Tian et al., (2018)

The key mechanistic steps of RAFT polymerization are summarized in Figure 1.8 and described below.

1. Initiation: Radical polymerization is initiated by decomposition of an initiator, generating radicals ($I^\bullet$). These primary radicals provide the reactive species required to begin chain growth.
2. RAFT pre-equilibrium: An actively propagating radical chain reacts with the CTA by reversible addition to the C=S bond to form an intermediate radical. The intermediate subsequently undergoes fragmentation, regenerating a radical capable of reinitiating propagation while converting the CTA-derived functionality to the dormant polymer chain end.
3. Reinitiation: The radical released from the pre-equilibrium ($R^\bullet$) adds to the monomer and initiates a new growing chain.
4. Main RAFT equilibrium: RAFT control comes from a degenerate chain transfer equilibrium in which an active radical chain adds to the RAFT end group of a dormant chain, then fragments to release another radical chain, so chains take turns growing. The resulting rapid equilibrium between active and dormant chains equalizes chain growth across the population, thereby allowing uniform growth of the macromolecular chains and, typically, a low dispersity of the polymeric material.
5. Termination: As in all radical polymerizations, irreversible termination can occur via bimolecular reactions between radical chains (combination and/or disproportionation), yielding “dead” polymer chains. Although RAFT does not eliminate termination, efficient RAFT exchange reduces its relative impact by maintaining most chains in the dormant state and enabling frequent radical exchange (Chiefari et al., 1998; Matyjaszewski & Davis, 2002; Moad et al., 2000; Moad, Rizzardo, & Thang, 2008b; Perrier, 2017; Tian et al., 2018).

In RAFT polymerization, the number of polymer chains formed is primarily determined by the initial concentration of the CTA, since each CTA molecule ideally produces one polymer chain. Accordingly, the theoretical number-average molar mass ($\overline{M}_n$) can be estimated using equation (1.1), where $[M]_0$ and $[CTA]_0$ are the initial monomer and CTA concentrations, p is the monomer conversion, $M_{monomer}$ is the repeating unit molar mass, and M_{CTA} is the CTA molar mass. Thus, at a constant CTA concentration, increasing the initial monomer concentration (for a given conversion) increases the molar mass of the resulting polymer (Moad, Rizzardo, & Thang, 2005; Perrier, 2017).

$$\overline{M}_n \approx M_{CTA} + \left(\frac{[M]_0}{[CTA]_0} \times p \times M_{monomer} \right) \quad (1.1)$$

1.1.4.1 Chain Transfer Agents

In mechanistic terms, the structure of the CTA (Figure 1.7) determines how the Z group modulates the reactivity of the C=S bond toward radical addition and influences the stability and lifetime of the intermediate radicals formed in the pre-equilibrium and in the main equilibrium of the RAFT process. Excessive stabilization of this intermediate, however, can slow fragmentation, leading to polymerization retardation and increasing the likelihood of termination of intermediate species. The R group must act as an efficient leaving group upon fragmentation and generate a radical capable of rapidly reinitiating propagation, so that chains begin growing within a comparable time frame and narrow dispersities can be achieved. Consequently, the effectiveness of RAFT control depends on the balance imposed by the stabilizing and leaving groups and on how well the selected CTA class matches the radical reactivity of the target monomer (Moad et al., 2000; Opiyo & Jin, 2021; Perrier, 2017; Tian et al., 2018). Table 1.2 summarizes typical CTA classes used for RAFT polymerization of some monomers.

Table 1.2 Typical RAFT CTA classes for selected monomers

Chain Transfer Agent Type	Monomer
Dithioesters	Methyl methacrylate (Perrier, 2017)
Trithiocarbonates	Methyl methacrylate (Perrier, 2017; Robles Grana et al., 2021) Acrylic acid (Ladavière, Dörr, & Claverie, 2001; Loiseau et al., 2003; Yin, Hoffman, & Stayton, 2006) Methacrylic acid (Robles Grana et al., 2021) N-isopropyl acrylamide ((Yin et al., 2006))
Xanthates	N-Vinyl pyrrolidone (Perrier, 2017) N-vinyl caprolactam (Moraes et al., 2020; Wan, Zhou, Pu, & Yang, 2008) Vinyl acetate (Perrier, 2017)
Dithiocarbamates	Styrene (Zhou, Zhu, Zhu, & Yin, 2005)

This level of control makes RAFT particularly attractive for designing graft copolymers from polysaccharide backbones. In the case of pullulan, chemical modification is necessary because the native polymer, while highly water-soluble and biocompatible, lacks the stimulus-responsiveness and aqueous stability required for many advanced biomedical and functional-material applications. Introducing temperature- or pH-responsive behavior, therefore, requires the incorporation of synthetic segments whose composition governs the transition profile and performance. Accordingly, functionalizing pullulan with a suitable CTA enables RAFT polymerization from the backbone, providing access to pullulan-graft copolymers. Therefore, an appropriate synthetic sequence is required to translate the modified pullulan into practical, application-relevant formats.

1.1.5 Electrospinning

On the other hand, direct functionalization with hydrophobic groups can also alter the intrinsic hydrophilicity of pullulan. While such systems are not necessary stimuli-responsive, direct functionalization provides a quick and efficient way to access tailor-made materials suitable for specific applications. In this thesis, electrospinning is employed as a versatile processing

route to convert a modified pullulan system into continuous micro- to nanoscale fibrous membranes with high surface area, interconnected porosity, and tunable morphology. Such features are particularly attractive for biomedical and drug delivery platforms, textiles, as well as separation and filtration applications (Al-Abduljabbar & Farooq, 2023; Borban, Gohain, Yadav, Karki, & Ingole, 2023; Elfawal, Šišková, & Andicsová, 2025; Tan, Wang, Pan, & Chen, 2022; Xue, Wu, Dai, & Xia, 2019). Accordingly, the following section introduces the principles of electrospinning and discusses the key processing parameters that govern fiber formation and membrane properties.

1.1.5.1 Principle of the Technique

Electrospinning (ES) is a fiber-fabrication technique in which a strong electric field is applied between a metallic emitter (e.g., spinneret or needle) and a grounded collector to generate continuous polymer fibers from a solution or a melt. Upon extrusion of the polymer fluid from the needle tip (when a needle is used), electrostatic forces progressively deform the droplet. Once these forces exceed the solution's surface tension, a conical geometry, known as the Taylor cone, forms, and a charged jet is launched toward the collector. During flight, the jet undergoes elongation and thinning, accompanied by solvent evaporation, leading to solidification of the polymer into micro- to nanoscale fibers. The resulting fibers are collected as a nonwoven mat on the collector surface (Ahmadi Bonakdar & Rodrigue, 2024; Al-Abduljabbar & Farooq, 2023; Borban et al., 2023; Cho et al., 2025; Elfawal et al., 2025; C. Wang et al., 2025; Xue et al., 2019).

A typical vertical electrospinning apparatus (Figure 1.9) comprises three primary modules: (i) a solution-delivery unit (most commonly a syringe pump) that delivers the polymer solution at a controlled flow rate to the spinneret (typically a metallic needle), (ii) a high-voltage power supply that imposes the electric potential difference required to generate and sustain the charged jet, and (iii) a collector positioned opposite the spinneret, generally maintained at ground potential, on which the solidified fibers accumulate to form a nonwoven mat. Depending on the desired fiber organization, the collector may be configured as a stationary plate or as a rotating drum to promote partial alignment.

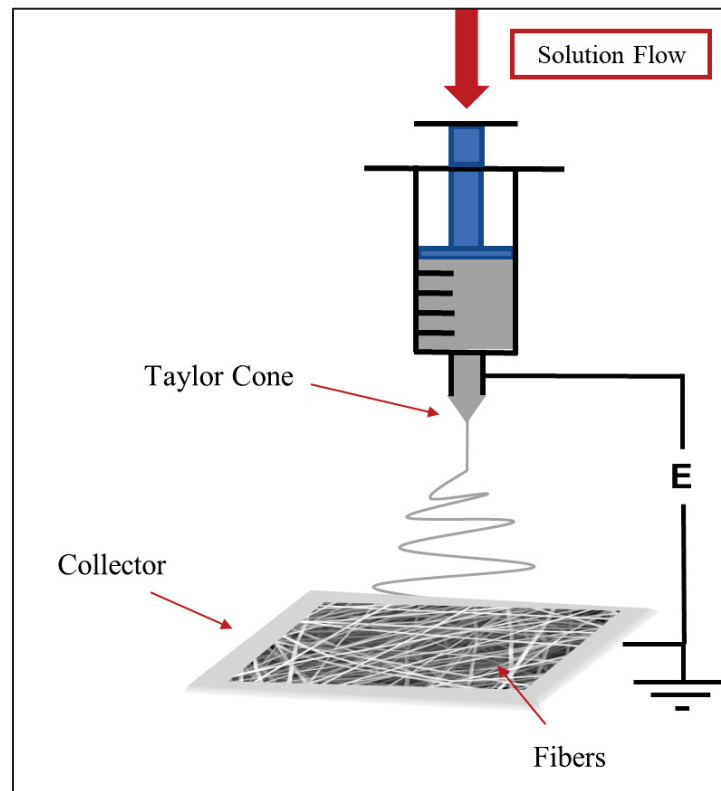


Figure 1.9 Schematic of a simple vertical electrospinning setup, comprising a syringe pump, spinneret, high-voltage power supply, and grounded collector

1.1.5.2 Electrospinning Parameters

Fiber diameter and morphology in electrospinning are governed by a coupled set of variables that are typically grouped into three domains: (i) solution-related parameters, (ii) processing parameters, and (iii) environmental conditions, as presented in Table 1.3. Solution parameters are primarily determined by the polymer-solvent system and include properties such as viscosity and surface tension. Processing parameters refer to the externally imposed controls, most notably the electric field, solution flow rate, and the tip-to-collector distance. Environmental conditions, including temperature and relative humidity, affect solvent evaporation and jet solidification (Ahmadi Bonakdar & Rodrigue, 2024; Al-Abduljabbar & Farooq, 2023; Elfawal et al., 2025). An understanding of how these factors interact is essential

for tuning fiber uniformity and morphology for a given application. The following sections summarize the influence of each parameter group on electrospinning outcomes.

Table 1.3 Summary of electrospinning variables

Parameters	
Solution	Viscosity
	Surface tension
	Electrical properties
	Solvent volatility
Process	Electric field
	Solution flow rate
	Needle-to-collector distance
Environmental	Temperature
	Humidity

Solution parameters: The stability of the Taylor cone and charged jet, and consequently, jet stretching, thinning, and solidification, depend strongly on the physicochemical properties of the spinning solution, including viscosity, surface tension, electrical conductivity, and volatility characteristics. Viscosity is primarily governed by the polymer molar mass and concentration (Ahmadi Bonakdar & Rodrigue, 2024; Elfawal et al., 2025). Moreover, electrospinnability has been shown to correlate with elongational viscosity, which depends on both molar mass and molar mass distribution of the polymer (Beaudoin, 2025). Polymer chemical functionality (e.g., bulky substituents or strongly interacting groups) further modulates polymer coil expansion and interchain interactions in a given solvent, thereby affecting viscosity and spinnability (Teraoka, 2002). In addition, higher surface tension generally reduces electrospinnability by opposing jet formation unless compensated by stronger electrostatic stresses (Ahmadi Bonakdar & Rodrigue, 2024; C. Wang et al., 2025).

Solvent volatility, and, more broadly, the solvent evaporation rate during flight, play a critical role in electrospinning because it governs when and where the jet transitions from a fluid to a solid filament. Excessively rapid evaporation can promote premature skin formation near the spinneret or at the Taylor cone, which may result in needle clogging and destabilization of the jet. Conversely, when evaporation is insufficient, the jet can arrive at the collector before adequate solidification, leading to fiber fusion, coalescence, or even the formation of a continuous film rather than a discrete fibrous mat (Ahmadi Bonakdar & Rodrigue, 2024; Haider, Haider, & Kang, 2018).

Processing parameters: The principal operating variables in electrospinning together govern the electric field intensity, jet stability, and the time available for stretching and solvent removal. The electric field provides the electrostatic driving force at the needle tip; at a fixed needle-to-collector distance, increasing the electric field generally enhances jet elongation and can reduce fiber diameter. However, beyond an appropriate range, overly high electric field may promote jet instabilities, including the formation of multiple jets and fluctuating deposition behavior. The flow rate, imposed by the syringe pump, must be balanced with the electrically driven throughput of the jet to sustain a stable Taylor cone. If the flow rate is too high relative to the rate of jet drawing and solvent evaporation, incomplete drying can occur, and defects such as beads, fused fibers, or larger diameters are typically observed. The tip-to-collector distance controls jet flight time and thus influences both elongational thinning and solvent evaporation; it should be sufficient to allow adequate solidification before collection. Excessively large distances can broaden the deposition area and may require a higher applied voltage to maintain an effective electric field at the spinneret (Ahmadi Bonakdar & Rodrigue, 2024; Al-Abduljabbar & Farooq, 2023; Basu, Agrawal, & Jassal, 2011; Bhardwaj & Kundu, 2010; Ibrahim & Klingner, 2020; Xue et al., 2019). Overall, stable electrospinning is typically achieved by identifying an operating window in which voltage, flow rate, and needle-to-collector distance collectively support a sustained cone-jet and reproducible fiber formation.

Environmental conditions: Higher temperatures generally accelerate solvent removal, which may change viscosity during flight and alter jet stretching. Under overly warm conditions,

premature drying near the spinneret can occur, increasing the risk of needle clogging and process instability (De Vrieze et al., 2009; Haider et al., 2018).

The influence of humidity is strongly dependent on the solvent system. In water-based electrospinning, elevated relative humidity tends to delay drying, allowing the jet to remain fluid for longer and increasing the likelihood of defects such as beads, and smaller average fiber diameters can be observed (Pelipenko, Kristl, Janković, Baumgartner, & Kocbek, 2013). However, low humidity can accelerate solidification but may promote clogging at the needle tip (Ibrahim & Klingner, 2020). For non-aqueous solvent systems, the effect of humidity is more system-specific and depends on whether water acts as a non-solvent for the polymer solution, which can alter fiber morphology (Rezabeigi & Demarquette, 2019). When moisture uptake induces phase separation or surface gelation, higher humidity can trigger premature skin formation of the jet and yield thicker fibers or incompletely dried deposits (Ahmadi Bonakdar & Rodrigue, 2024; De Vrieze et al., 2009; İÇOĞLU & OĞULATA, 2013).

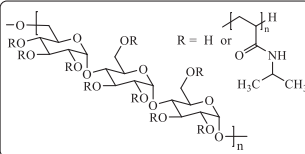
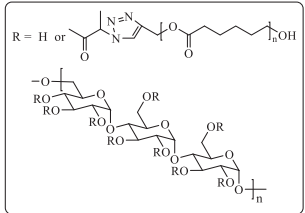
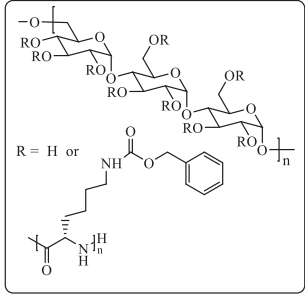
1.2 State of the Art

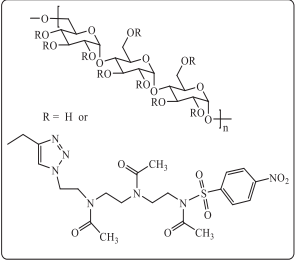
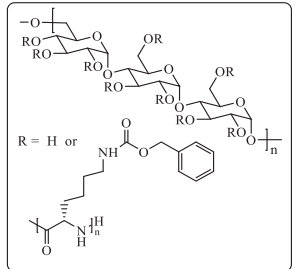
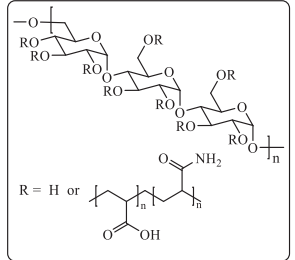
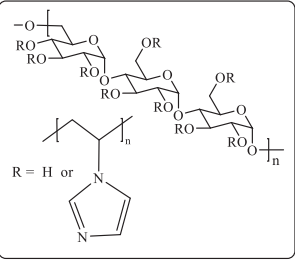
The previous section established the foundational concepts relevant to this thesis, including pullulan as a bio-based polysaccharide, major routes for its chemical functionalization, the principles of RAFT/MADIX (macromolecular design via interchange of xanthate) polymerization, and the basic features of electrospinning. Building on this foundation, the present section examines the current state of the art in two research areas that mirror the specific objectives of this thesis: (1) pullulan graft copolymers designed for temperature- and pH-responsive behavior, and (2) electrospun fibrous membranes produced from modified pullulan. Together, these subsections highlight existing advances, identify critical knowledge gaps, and provide the scientific context for the research strategy implemented in the following Chapters.

1.2.1 Pullulan Graft Copolymers: Molecular Engineering for Temperature- and pH-Responsive Behavior

The development of pullulan-based graft copolymers has become an effective strategy to broaden the functional scope of a polysaccharide that is intrinsically highly hydrophilic. A wide range of pullulan graft copolymer systems, using pullulan as the backbone and accessing diverse architectures, have been reported. These studies are summarized in Table 1.4.

Table 1.4 Summary of research articles on pullulan-based graft copolymers

Studied Material	Grafting Approach	Polymerization Type	Key Findings / Contributions	Chemical Structure
Pullulan-g-poly(N-isopropylacrylamide)	Grafting from	Free radical polymerization	Preparation of temperature-responsive nanoparticles enabling indomethacin loading and temperature-triggered release (Bucătariu, Stoica, & Fundueanu, 2017).	
Pullulan-g-poly(ϵ -caprolactone)	Grafting to	Ring-opening polymerization	Synthesis of an amphiphilic copolymer using click chemistry, enabling self-assembly in aqueous media (Carvalho et al., 2020).	
Pullulan-g-poly(Z-L-lysine)	Grafting from	Ring-opening polymerization	Preparation of an amphiphilic copolymer and evaluation of in vitro cytocompatibility (no detectable cytotoxicity under the tested conditions) (Carvalho et al., 2022)	

Studied Material	Grafting Approach	Polymerization Type	Key Findings / Contributions	Chemical Structure
Pullulan-g-poly(2-methyl-2-oxazoline)	Grafting to	Ring-opening polymerization	Modification of pullulan by poly(2-methyl-2-oxazoline) side chains of varying lengths via click chemistry, enabling systematic tuning of graft length (Zorin et al., 2023).	
Pullulan-g-poly(Z-L-lysine)	Grafting from	Ring-opening polymerization	Preparation of an amphiphilic copolymer forming self-assembled particles, investigated for indomethacin encapsulation (Vieira et al., 2024).	
Pullulan-g-poly(acrylic acid) and pullulan-g-poly(acrylic acid-co-acrylamide)	Grafting from	Free radical polymerization	Preparation of hydrogels from the grafted copolymers and demonstration of their use as reusable metal-ion adsorbents (Sonmez & Celikkol, 2021).	
Pullulan-g-poly(N-vinylimidazole)	Grafting from	Free radical polymerization	Synthesis of a water-soluble copolymer proposed as a platform for biomedical use, including drug conjugation/carrier applications (Hezarkhani & Yilmaz, 2019)	

Many studies on pullulan grafting relied on conventional free-radical approaches to introduce synthetic side chains such as poly(methyl acrylates), polyacrylamide, or poly(*N*-isopropylacrylamide) (Bucătariu et al., 2017; Saber-Samandari & Gazi, 2015; Wu, Jin, Kim, et al., 2009). While these routes demonstrated the feasibility of producing amphiphilic graft architectures and, in some cases, self-assembled nano-objects, they generally resulted in broad distributions of graft length, with limited control over molar mass and dispersity of the copolymer. This lack of architectural precision obscures the ability to correlate composition and architecture with properties and performance metrics. Consequently, establishing clear structure-property relationships for these materials has remained challenging.

In parallel, increasing attention has been directed toward combining pullulan with stimuli-responsive synthetic polymers to create “smart” materials that respond to environmental cues such as temperature or pH (Bucătariu et al., 2017; Constantin, Cosman, Bercea, Ailiesei, & Fundueanu, 2021; Hezarkhani, Ustürk, Özbilenler, & Yilmaz, 2024). In this context, the advent of RAFT-controlled radical polymerization can significantly improve the ability to design well-defined graft copolymers (Mori, Ookuma, Nakano, & Endo, 2006; Perrier, 2017). Nevertheless, the controlled polymerization of non-conjugated NVCL monomers still represents a challenge due to the high reactivity of the radical species derived from these monomers. This reactivity is attributed to the absence of resonance stabilization and the presence of strongly electron-donating groups in these radicals (Cortez-Lemus & Licea-Claverie, 2016; Nakabayashi & Mori, 2013). Accordingly, a CTA capable of stabilizing the process, thus ensuring adequate control over the polymerization process, is required. In this study, a xanthate-based CTA was chosen due to its proven ability to effectively manage NVCL polymerization for grafting applications, a process specifically referred to as MADIX (macromolecular design by the interchange of xanthate) (Pal, Sarkar, Karmakar, & Pal, 2018; Winninger et al., 2019). Beyond synthesis, the temperature-responsive behavior of PNVCL-based systems in an aqueous solution is known to be tunable through copolymer composition and polymer architecture (e.g., incorporation of hydrophilic/hydrophobic characters and variation of PNVCL chain length) (Etchenausia et al., 2017; Moraes et al., 2020; Yuan et al., 2023; Zhao et al., 2015).

Concomitantly, several studies have investigated the polymerization of acrylic acid (AA) using xanthate-based CTAs (Gupta & Washburn, 2014; Ladavière et al., 2001; Sütökin & Güven, 2018; Taton et al., 2006; Taton, Wilczewska, & Destarac, 2001). In this context, evaluating AA within the same pullulan-xanthate-based CTA framework used for NVCL provides a rigorous test of how broadly the method applies and a limitation study of this platform. Holding the pullulan backbone functionalized with xanthate CTA relatively unchanged while testing different monomers enables a clearer interpretation of how monomer influences grafting efficiency and the degree of control. This comparative study, therefore, contributes as a rigorous assessment and applicability of the pullulan-xanthate RAFT/MADIX approach.

Several studies have explored RAFT polymerization on a polysaccharide backbone (Fleet et al., 2008; Hernández-Guerrero, Davis, Barner-Kowollik, & Stenzel, 2005; Hua, Tang, Cheng, Deng, & Zhu, 2008; Ikkene et al., 2020; Karmakar & Pal, 2021; Lu, Xiao, & Sun, 2012; Pal et al., 2018; Tang, Hua, Cheng, Jiang, & Zhu, 2008). Although pullulan functionalized with trithiocarbonate RAFT agents has been reported for grafting applications with N-isopropylacrylamide (Morimoto, Qiu, Winnik, & Akiyoshi, 2008), to the best of our knowledge, reports specifically describing the controlled polymerization on a pullulan substrate derivatized with a RAFT/MADIX chain transfer agent remain limited. In this context, the present work investigates the first synthesis of pullulan-graft-poly(N-vinylcaprolactam) (PULL-g-PNVCL) and pullulan-graft-poly(acrylic acid) (PULL-g-PAA) via RAFT polymerization of NVCL or AA mediated by a pullulan-based macro-CTA, to integrate pullulan's intrinsic attributes with temperature-responsive (PNVCL) or pH-responsive (PAA) behavior.

Accordingly, Chapter 4 focuses on establishing a pullulan-xanthate RAFT/MADIX platform for the synthesis of PULL-g-PNVCL copolymers, and on demonstrating how their molecular characteristics can modulate their temperature-responsive behavior. Chapter 5 then extends the same macro-CTA framework to AA, thereby probing the feasibility and limitations of xanthate-mediated graft polymerization of AA from a pullulan backbone.

1.2.2 Electrospun Pullulan-Derived Fibrous Membranes

Electrospinning has become a key technique for producing polymer fibers. However, most electrospinning studies have historically focused on synthetic polymers (Al-Abduljabbar & Farooq, 2023; Xue et al., 2019). The translation of electrospinning to biobased systems has expanded strongly in more recent years and raised specific challenges related to solubility in solvents to be electrospun and poor aqueous stability of the resulting fibers.

Pullulan is one of the polysaccharides that can be readily electrospun from aqueous solutions. Several studies have reported uniform pullulan nanofibers using water as a solvent. In addition, pullulan is frequently incorporated as a processing aid to certain polymers, such as starch and chitosan (Karim et al., 2009; Kong & Ziegler, 2014; R. Li et al., 2017; S. Li, Kong, & Ziegler, 2021; X. Li et al., 2023; Zeyu Qin, Jia, Liu, Kong, & Wang, 2020; Ze-yu Qin et al., 2019). Nonetheless, its rapid dissolution in water severely limits its practical use unless hydrophobic group introduction strategies are implemented. Crosslinking with reactive agents and physical blending with amphiphilic or hydrophobic polymers have been explored to improve water resistance, but these approaches can complicate processing and introduce potentially residual reagents or additives (Ai et al., 2024; Amjadi et al., 2022; Zeyu Qin et al., 2020; Ze-yu Qin et al., 2019).

Non-crosslinking chemical modifications offer alternative routes to stabilize pullulan fibers by directly modulating the hydrophilicity of the backbone. Among the possible modifications, acetylation stands out because the solubility of its pullulan derivatives can be tuned through the degree of substitution (DS), defined in this thesis as the number of substituted groups per pullulan monomer unit. However, the literature on pullulan acetate and its use in electrospinning remains comparatively limited. Existing reports have emphasized the synthesis and characterization of pullulan acetate, often in the form of films or particles (Hernandez-Tenorio & Giraldo-Estrada, 2022; Jung et al., 2003; Na, Jeong, & Lee, 1997; Niu et al., 2019; H. Zhang et al., 2009). Notably, Enomoto-Rogers et al. demonstrated electrospinning of fully acetylated pullulan (reported DS $\approx$ 9) from 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), as the

solvent (Enomoto-Rogers et al., 2015a). Nevertheless, systematic insight into how the chemical modification (DS) governs electrospinnability, fiber morphology, and subsequent stability in aqueous environments has not yet been explored.

These gaps motivate the second research pathway of this thesis, developed in Chapter 6, focusing on a preliminary investigation of pullulan acetylation and evaluating the electrospinnability of the resulting derivatives, aiming to identify processing windows that produce continuous fibers with minimal defects. In addition, the work assesses whether, and to what extent, acetylation mitigates the rapid dissolution characteristic of native pullulan fibers.

CHAPTER 2

OBJECTIVES

The main objective of this work is to evaluate and develop pullulan-based functional materials by tailoring graft copolymerization routes and producing electrospun membranes, while elucidating the resulting structure-property relationships. To achieve this, the specific objectives are organized into two complementary parts, outlined below:

A. RAFT/MADIX-based grafting platform for stimuli-responsive pullulan copolymers,

A1. To design and synthesize pullulan-g-poly (N-vinyl caprolactam) (PULL-g-PNVCL) copolymers, and to demonstrate how their molecular characteristics (molar mass and grafting percentage) influence their temperature-responsive behavior.

A2. To extend the pullulan-xanthate RAFT/MADIX grafting strategy toward pullulan-g-poly (acrylic acid) (PULL-g-PAA) copolymers, and to assess the feasibility and limitations of xanthate-mediated acrylic acid grafting through detailed structural analyses.

B. Development of pullulan-based electrospun membranes, through the following step:

B1. Preliminary study to evaluate the electrospinnability of pullulan acetate derivatives to produce uniform fibers and to assess their stability in water.

CHAPTER 3

METHODOLOGY

This chapter describes the methodological framework adopted in this thesis. It begins by presenting the overall research plan, explaining how the specific objectives are articulated into complementary lines of investigation that converge toward the central goal of developing pullulan-based functional materials. Subsequently, the materials used and the corresponding experimental procedures are detailed. Finally, the chapter summarizes the set of analytical techniques applied to characterize the physical and chemical properties of the modified pullulan in the different research stages.

3.1 Research Strategy

A schematic representation of the overall methodological framework is provided in Figure 3.1, illustrating its alignment with the stated sub-objectives and depicting how the sequential experiments contribute to the targeted development of pullulan-based functional materials.

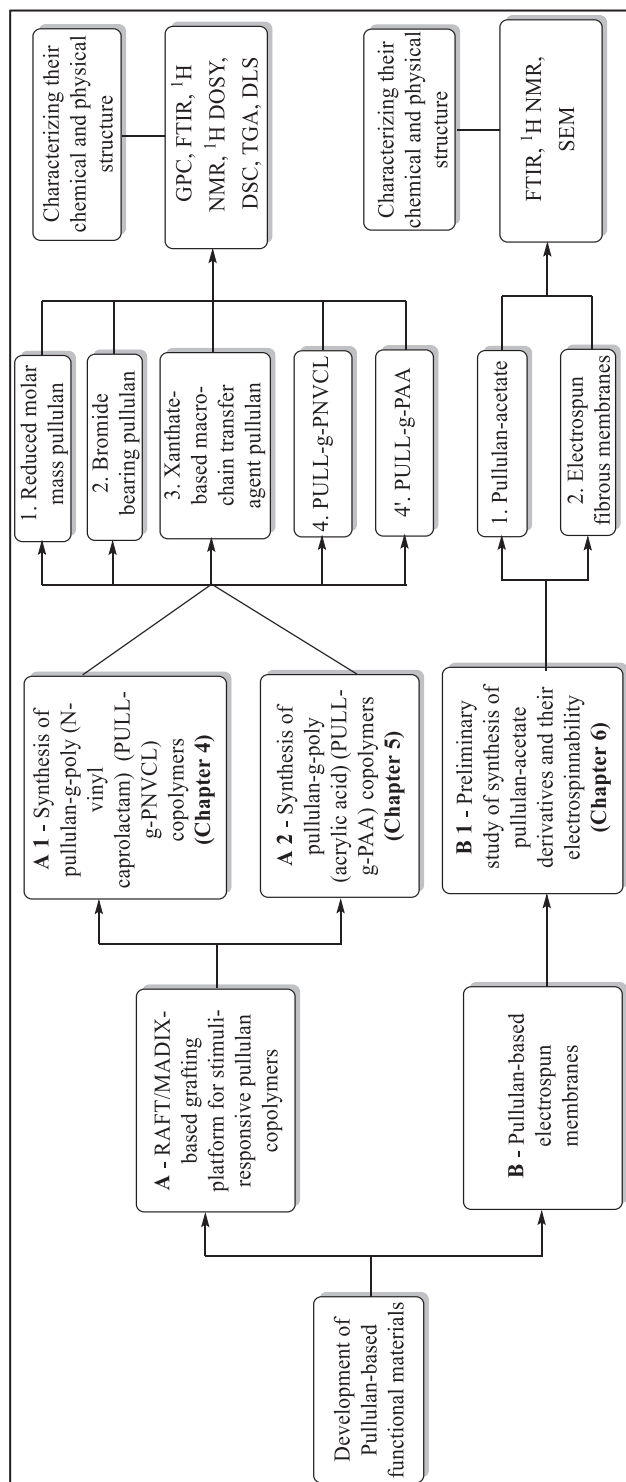


Figure 3.1 Methodological framework for the development of pullulan-based functional materials, outlining experimental steps and thesis chapters for each approach

3.2 Materials

The polymers and monomers employed in this study included pullulan (food grade, 95% purity (5 % mono- and oligo-saccharides), 98.2 total carbohydrates (1.8 % water), $\overline{M}_n = 96000$ g/mol, $\overline{M}_w = 288000$ g/mol, and $\overline{D} = 3.01$ in DMF/LiBr) obtained from Hayashibara Co., Ltd., Okayama, Japan; distributed by Nagase Biochemical Laboratory, as well as N-vinyl caprolactam (NVCL, 98%, Sigma-Aldrich), and anhydrous acrylic acid (AA, 99%, Sigma-Aldrich), both purchased from Sigma-Aldrich and used as received.

Additional reagents, along with their purity and suppliers, are summarized in Table 3.1, while the solvents used in this study, including their respective purities and sources, are presented in Table 3.2.

Table 3.1 List of additional reagents selected for this study with their corresponding purity and supplier

Reagents*	Purity	Supplier
Acetic anhydride	$\geq 98\%$	Sigma-Aldrich, USA
Anhydrous magnesium sulfate (MgSO_4)	-	Thermo Fisher Scientific, USA
Anhydrous pyridine	99.8%	Sigma-Aldrich, USA
2,2'-Azobis(2-methylpropionitrile) (AIBN)	98%	Sigma-Aldrich, USA
2-Bromopropionyl bromide (BPB)	97%	Sigma-Aldrich, USA
4-(Chloromethyl) benzyl alcohol	99%	Sigma-Aldrich, USA
Potassium ethyl xanthogenate (KEX)	96%	Sigma-Aldrich, USA
Sodium hydroxide (NaOH)	-	Sigma-Aldrich, USA
Triethylamine (TEA)	99.5%	Sigma-Aldrich, USA

* All reagents were used as received and stored appropriately (in designated cabinets or refrigerated), protected from light as required.

Table 3.2 List of solvents used in this study, including the purity and supplier

Solvents	Purity	Supplier
Anhydrous dichloromethane	$\geq 99.8\%$	Sigma-Aldrich, USA
Anhydrous ethanol	$\leq 0.003\%$ water	Sigma-Aldrich, USA
Anhydrous N, N-dimethylformamide (DMF)	99.8%	Sigma-Aldrich, USA
Deionized water	-	-
Deuterated water (D ₂ O)	-	Cambridge Isotope Laboratories, Inc., USA
Diethyl ether	99%	Thermo Fisher Scientific, USA
Dimethyl sulfoxide-d ₆ (DMSO-d ₆)	-	Cambridge Isotope Laboratories, Inc., USA
Dried dimethyl sulfoxide (DMSO)	$\leq 0.02\%$ water	Sigma-Aldrich, USA
Hydrochloric acid	37%	Sigma-Aldrich, USA
Methanol	$\geq 99.8\%$	Thermo Fisher Scientific, USA

3.3 Experimental Procedures

The overall synthetic pathway employed to prepare pullulan-graft-poly (N-vinyl caprolactam) (PULL-g-PNVCL) and pullulan-graft-poly (acrylic acid) (PULL-g-PAA) is illustrated in Figure 3.2. A four-step strategy was followed. Initially, the molar mass of the commercially sourced pullulan was decreased through acid hydrolysis, according to procedures adapted from previous reports (Guerrini et al., 2021a, 2021b; Ilić, Jeremić, & Jovanović, 1991; Lupo, Boulos, & Nyström, 2020; Shady et al., 2013). In the second step, hydroxyl groups of pullulan were partially acylated with 2-bromopropionyl bromide (BPB), resulting in bromide-bearing pullulan (PULLBr). Subsequently, the bromopropionyl groups were partially replaced with a pre-synthesized hydroxyl-functionalized chain transfer agent (xanthate-based, CTA-OH),

affording the pullulan-xanthate-based macro-chain transfer agent (PULLX). In the final stage, graft copolymers, either PULL-g-PNVCL or PULL-g-PAA, were obtained from PULLX. The molecular structure and composition of the obtained (co)polymers were investigated using the analytical techniques described in the following section.

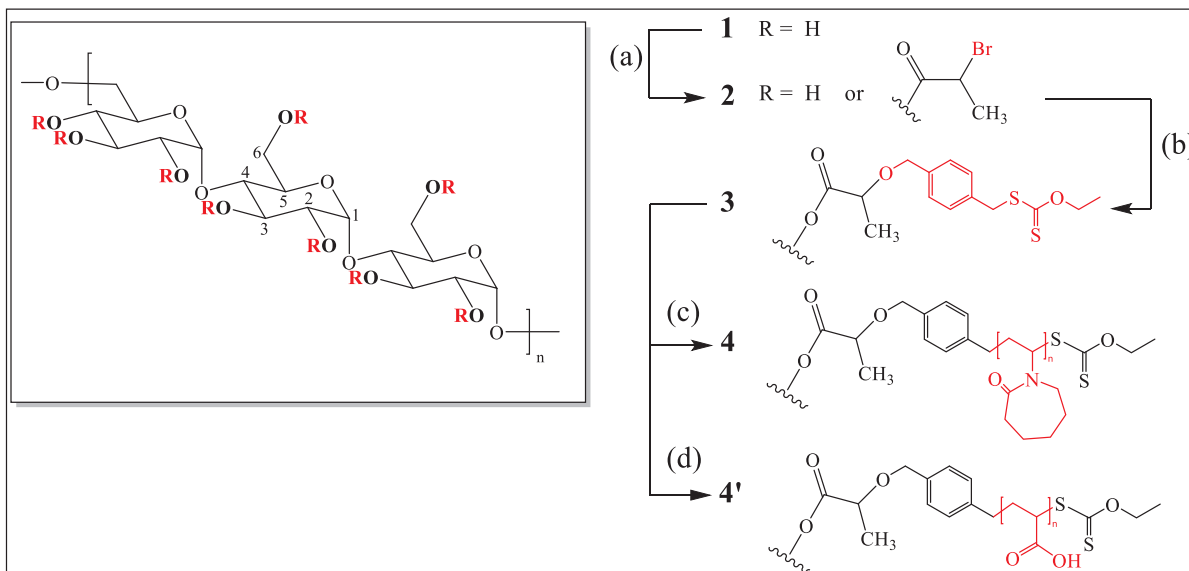


Figure 3.2 Chemical structures and route for the synthesis of PULL-g-PNVCL or PULL-g-PAA (a) BPB, DMF, TEA, 25 °C, 72 h; (b) CTA-OH, DMSO, Pyridine, 50 °C, 24 h; (c) NVCL, DMSO, AIBN, 70 °C, 24 h; (d) AA, DMSO, AIBN, 70 °C, 6 h; **1**: Reduced molar mass pullulan (Yield: 92 %); **2**: Bromide-bearing pullulan (Yield: 58 - 64 %); **3**: Xanthate-based macro-chain transfer agent pullulan (Yield: 30 - 73 %); **4**: PULL-g-PNVCL (Yield: 37 - 38 %); **4'**: PULL-g-PAA (Yield: 40 %)

3.3.1 Pullulan Molar Mass Reduction

Low-molecular-weight pullulan was prepared through acid-catalyzed hydrolysis, adapted from established procedures reported in the literature (Guerrini et al., 2021b; Ilić et al., 1991; Shady et al., 2013). Briefly, pullulan (6 g) was dissolved in deionized water (120 mL) in a 250 mL oven-dried round-bottom flask at 60 °C under continuous stirring for 4 h. Once complete dissolution was achieved, hydrochloric acid (250 μL , 0.025 M) was introduced into the solution. The reaction vessel was then sealed and maintained at 85 °C for 1.5 h. To terminate the reaction, the flask was immediately cooled in an ice bath. The hydrolyzed product was

isolated by dropwise precipitation of the reaction mixture into ice-cold methanol under vigorous stirring. The resulting solid was washed repeatedly with cold methanol and subsequently dried under vacuum at 40 °C for 24 h. The overall synthetic route for the preparation of reduced molar mass pullulan is schematically illustrated in Figure 3.3.

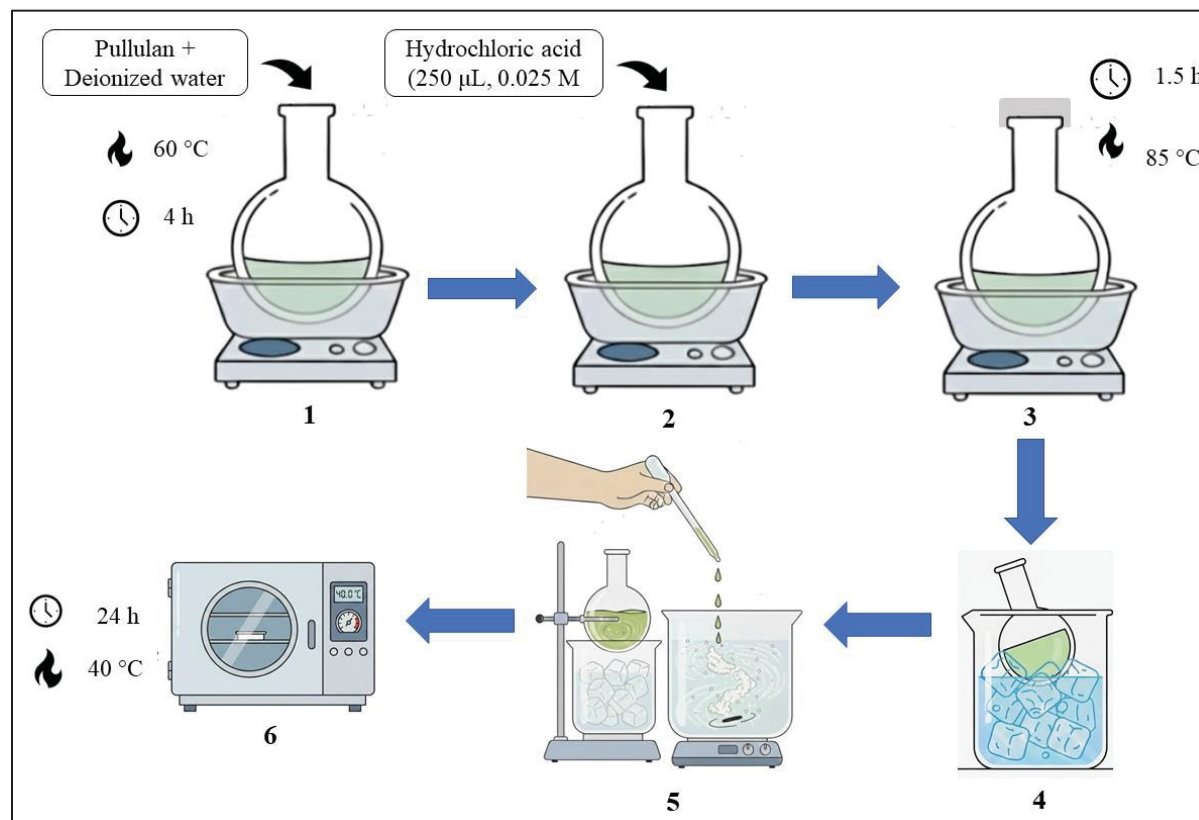


Figure 3.3 Schematic representation of the molar-mass reduction of pullulan: (1) dissolution of pullulan in deionized water at 60 °C for 4 h; (2) addition of hydrochloric acid (250 µL, 0.025 M); (3) acid hydrolysis at 85 °C for 1.5 h; (4) rapid cooling of the reaction mixture in an ice bath to quench the reaction; (5) dropwise precipitation into cold methanol under vigorous stirring; and (6) collection and drying under vacuum at 40 °C for 24 h. (Cartoons generated by gemini-3.0-pro, 2025)

3.3.2 Synthesis of Bromide-Bearing Pullulan

The preparation of bromide-bearing pullulan (PULLBr) was carried out following a procedure adapted from Bontempo et al. (Bontempo et al., 2006). A total of five different molar ratios of 2-bromopropionyl bromide (BPB) relative to the pullulan monomer unit (0.5, 1, 2, 5, and 7)

were investigated, yielding the corresponding derivatives PULLBr-0.5, PULLBr-1, PULLBr-2, PULLBr-5, and PULLBr-7. This screening step was necessary because the degree of bromide substitution governs the number of sites available for subsequent xanthate-based chain transfer agent installation and graft polymerization, while excessive substitution may adversely affect solubility and further modification. The detailed experimental procedure is described for the PULLBr-5 synthesis, while the reagent quantities for the other reactions were adjusted proportionally according to their respective molar ratios. Reduced-molar-mass pullulan (0.250 g, 0.514 mmol of monomer units) was dissolved in DMF (5 mL) in a round-bottom flask at room temperature. Triethylamine (TEA, 0.716 mL, 5.14 mmol), corresponding to twice the molar quantity of 2-bromopropionyl bromide (BPB), was added under a nitrogen atmosphere with vigorous stirring. After 30 min, the solution was cooled in an ice bath, and BPB (0.269 mL, 2.57 mmol, corresponding to a 5 molar ratio relative to a pullulan monomer unit) was introduced dropwise. The reaction mixture was maintained under nitrogen and stirred at room temperature for 72 h. The resulting product, denoted PULLBr-5 (0.227 g, 64% yield), was purified by dialysis against distilled water (molecular weight cutoff: 14 kDa) for 3 days, followed by precipitation in a diethyl ether/methanol mixture (1:4, v/v). The precipitate was collected and dried under vacuum at 40 °C for 24 h.

3.3.3 Synthesis of Pullulan-Xanthate-Based Macro-Chain Transfer Agent

First, O-ethyl S-4-(hydroxymethyl)benzyl carbonodithioate (CTA-OH) was synthesized according to the procedure reported by Binauld et al. (Binauld, Delafresnaye, Charleux, D'Agosto, & Lansalot, 2014). A solution of 4-(chloromethyl) benzyl alcohol (2.452 g, 15.50 mmol) in ethanol (10 mL) was first prepared. Separately, potassium ethyl xanthogenate (KEX, 3.882 g, 23.25 mmol) was dissolved in ethanol (35 mL) and then added dropwise to the reaction flask. The mixture was stirred at room temperature for 24 h. The white precipitate of potassium chloride formed during the reaction was removed by filtration (repeated twice). The solvent was subsequently evaporated under reduced pressure using a rotary evaporator. The resulting concentrate was dissolved in dichloromethane (25 mL) and subjected to repeated washing with water (10 × 20 mL). The organic phase was dried over anhydrous magnesium sulfate overnight,

filtered, and concentrated under vacuum to yield the desired product as a white powder (2.420 g, 64 % isolated yield).

The pullulan-xanthate-based macro-chain transfer agents (PULLX) were obtained by reacting PULLBr-5 and PULLBr-7 with CTA-OH in the presence of pyridine, using DMSO as the solvent. This reaction afforded the corresponding derivatives, PULLX-5 and PULLX-7. The experimental procedure is described in detail for the synthesis of PULLX-5, while the preparation of PULLX-7 followed the same protocol. The molar feed ratio of [bromide groups in PULLBr]:[CTA-OH]:[pyridine] was fixed at 1:9:90. In the procedure, PULLBr-5 (0.1 g, 0.0314 mmol) and CTA-OH (0.068 g, 0.283 mmol) were introduced into an oven-dried round-bottom flask and dissolved in DMSO (5 mL) under nitrogen over 6 h. Pyridine (0.224 g, 2.830 mmol) was subsequently added, and the reaction mixture was stirred at 50 °C for 24 h under an inert atmosphere.

The crude product was purified by dialysis (MW cutoff 14 kDa) against distilled water for 24 h, followed by precipitation in cold methanol. To remove excess CTA-OH, the precipitate was suspended in ethanol, filtered, and dried under vacuum at room temperature for 24 h. Further purification was performed by dissolving the crude material in a DMSO/ethanol mixture (10:90, v/v) and subjecting it to dialysis (molecular weight cutoff: 14 kDa) against the same solvent system for 24 h, followed by an additional 24 h of dialysis against absolute ethanol. The final product was obtained after drying under vacuum at room temperature for 24 h, yielding PULLX-5 (0.033 g, 30% isolated yield).

3.3.4 Synthesis of PULL-g-PNVCL or PULL-g-PAA Copolymers

To ensure sufficient material for the subsequent copolymerization reactions, PULLBr-5 and PULLBr-7 were synthesized on a larger laboratory scale. Reactions were performed using 2 g of reduced molar mass pullulan, instead of 0.250 g, following the procedure described previously. The resulting PULLBr samples were subsequently converted into pullulan-xanthate-based macro-chain transfer agents (PULLX-5 and PULLX-7) using the established method. In this step, 1.3 g of PULLBr-5 and 1.9 g of PULLBr-7 were employed instead of 0.1

g. These macro-chain transfer agents were then used for the synthesis of pullulan-g-poly (N-vinyl caprolactam) (PULL-g-PNVCL) and pullulan-g-poly (acrylic acid) (PULL-g-PAA) copolymers. The detailed experimental procedure is provided for the copolymerization derived from PULLX-5, while those based on PULLX-7 were carried out according to the same protocol.

The graft copolymers PULL-g-PNVCL and PULL-g-PAA were synthesized using PULLX-5 as the macro-chain transfer agent. Two feed molar ratios were employed for both systems: $[CTA]:[AIBN]:[NVCL] = 1:0.5:150$ and $1:0.5:300$, and $[CTA]:[AIBN]:[AA] = 1:0.5:150$ and $1:0.5:300$, respectively. These two monomer-to-CTA feed ratios were selected to examine how increasing the monomer loading, at constant CTA and initiator proportions, influences the length of grafted chains and the resulting copolymer properties. In a typical procedure, PULLX-5, AIBN, and DMSO were introduced into an oven-dried round-bottom flask. AIBN was added as a stock solution in DMSO to ensure accurate initiator addition at a small scale. The stock solution was prepared by dissolving AIBN (70 mg, 0.43 mmol) in DMSO (10.0 mL); an aliquot of this solution was used for each polymerization, and additional DMSO was added to reach a total volume. After complete dissolution of the solids, NVCL or AA was added, and the solution was purged with nitrogen for 30 min at room temperature. The polymerization mixtures were maintained at 70 °C for 24 h in the case of PULL-g-PNVCL syntheses, and for 6 h for the PULL-g-PAA syntheses. Notably, using a single solvent (DMSO) simplified the process, as both PULLX-5 and the NVCL monomer are soluble in DMSO, making it preferable over mixed solvents often used in block or graft copolymerization.

The resulting graft copolymers, PULL-g-PNVCL (150) (38% isolated yield), PULL-g-PAA (150) (40% isolated yield), PULL-g-PNVCL (300) (37% isolated yield), and PULL-g-PAA (300) (yield not determined) were purified by dialysis (molecular weight cutoff: 14 kDa) against deionized water for 24 h, followed by drying under vacuum at 40 °C for 24 h. The specific reagent and solvent quantities used in this synthesis, along with the corresponding sample labels, are summarized in Table 3.3. The overall synthetic route for the preparation of PULL-g-PNVCL and PULL-g-PAA is schematically illustrated in Figure 3.4.

Table 3.3 Amounts of reagents used in the synthesis of PULL-g-PNVCL and PULL-g-PAA and corresponding sample labels

Copolymer name	Amount of PULLX (g, mmol of the xanthate group)	Amount of AIBN (mg, mmol)	Amount of NVCL (g, mmol)	Amount of AA (g, mmol)	Amount of DMSO
PULL-g-PNVCL (150)	0.100 g, 0.006 mmol	0.5 mg, 0.003 mmol	0.125 g, 0.901 mmol	-----	240 μ L
PULL-g-PNVCL (300)	0.100 g, 0.006 mmol	0.5 mg, 0.003 mmol	0.250 g, 1.802 mmol	-----	240 μ L
PULL-g-PAA (150)	0.200 g, 0.008 mmol	0.7 mg, 0.004 mmol	-----	0.095 g, 1.315 mmol	440 μ L
PULL-g-PAA (300)	0.200 g, 0.008 mmol	0.7 mg, 0.004 mmol	-----	0.190 g, 2.63 mmol	440 μ L

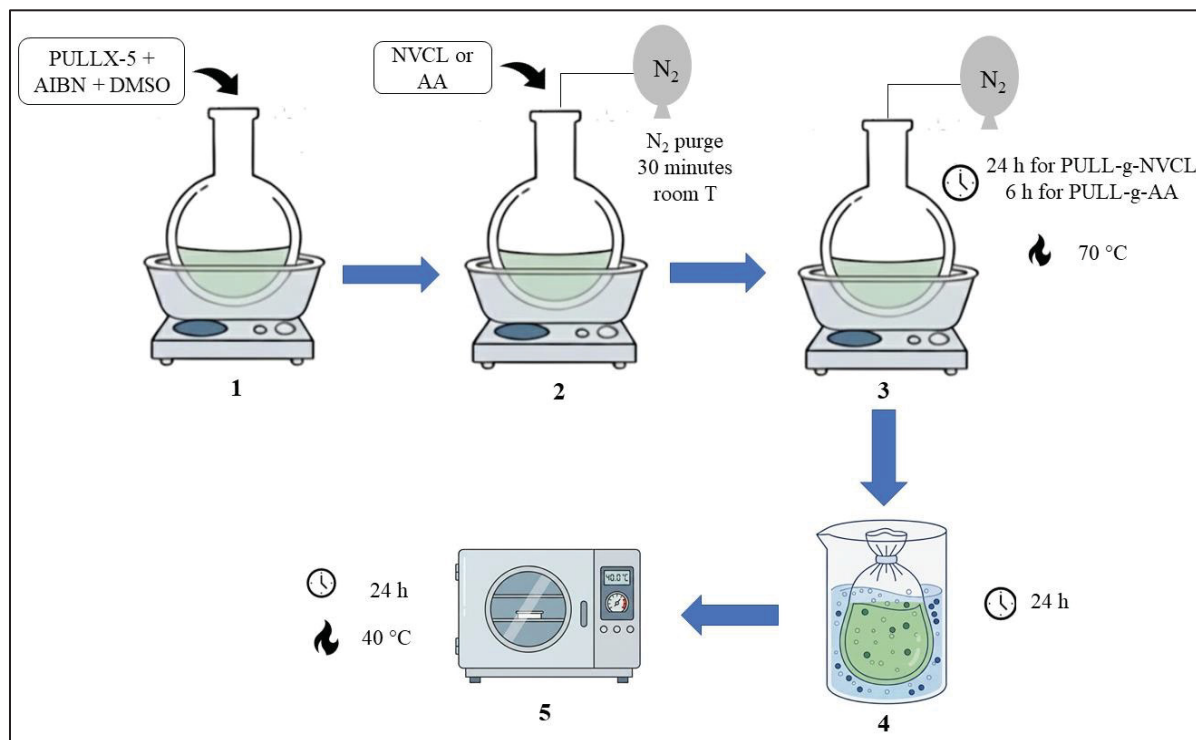


Figure 3.4 Schematic representation of the synthesis of PULL-g-PNVCL and PULL-g-PAA: (1) dissolution of PULLX-5 and AIBN in DMSO; (2) addition of NVCL or AA and purging with N₂ at room temperature; (3) graft polymerization at 70 °C (24 h for PULL-g-PNVCL and 6 h for PULL-g-PAA); (4) purification by dialysis against water for 24 h; and (5) drying of the obtained materials in a vacuum oven at 40 °C for 24 h. (Cartoons generated by gemini-3.0-pro, 2025)

3.3.5 Polymeric Particle Preparation

Polymeric particles were prepared from PULL-g-PNVCL (150) and PULL-g-PNVCL (300) via a self-assembly approach (Kang et al., 2006; Li, Zheng, Zhu, Wu, & Lu, 2015). This step was performed to evaluate whether the graft copolymers could spontaneously organize into particles in water and thereby express their temperature-responsive behavior in a dispersed form. In the procedure, each copolymer (15 mg) was dissolved in DMSO (1 mL). The resulting solutions were then added dropwise into deionized water (3 mL) under gentle stirring at room temperature. To remove the organic solvent, the mixtures were subjected to dialysis (MW cutoff 14 kDa) against deionized water for 24 h. The resulting dispersions were collected,

transferred into 15 mL volumetric flasks, and diluted to the calibration mark with deionized water, giving a final particle concentration of 1 mg/mL.

3.3.6 Synthesis of Pullulan Acetate

Pullulan acetate (PULLAc) was synthesized according to procedures adapted from the literature (Jung, Jeong, & Kim, 2003; Motozato, Ihara, Tomoda, & Hirayama, 1986; Na, Jeong, & Lee, 1997; Niu, Shao, Chen, & Sun, 2019). In a typical reaction, 2.0 g of pullulan was dissolved in 20 mL of DMF under magnetic stirring until a clear solution was obtained. Pyridine was then added under a nitrogen atmosphere in a molar amount equivalent to that of acetic anhydride. To obtain different degrees of substitution, three acetic anhydride to pullulan monomer unit molar feed ratios (5:1, 9:1, and 15:1) were introduced dropwise into the pullulan solution, and the corresponding products were designated PULLAc-5, PULLAc-9, and PULLAc-15, respectively. The reaction mixture was maintained at 54 °C and continuously stirred for 48 h.

After completion of the reaction, the crude mixture was dialyzed (molecular weight cutoff: 14 kDa) against deionized water for 24 h, with 3 water changes. The dialyzed dispersion yielded a white solid, which was collected, cut into smaller pieces, and washed repeatedly with deionized water on a funnel under vacuum to further remove residual acetic acid formed during the reaction and DMF. The product was then dried under vacuum at 40 °C for 24 h. To further ensure the removal of residual acetic acid and DMF, an additional dissolution-precipitation purification step was performed. PULLAc-5 was dissolved in acetone, whereas PULLAc-9 and PULLAc-15 were dissolved in an acetone/DMSO mixture (50/50 % v/v). Each polymer solution was added dropwise into cold deionized water under vigorous stirring, leading to the precipitation of PULLAc. The precipitated polymers were collected by filtration and dried under vacuum at 40 °C for 24 h. The detailed amounts of reagents employed in these syntheses and the associated sample labels are presented in Table 3.4. The overall synthetic route for the preparation of pullulan acetate is schematically illustrated in Figure 3.5.

Table 3.4 Amounts of reagents used in the synthesis of PULLAc and corresponding sample labels

Sample	Amount of pyridine (mL, mmol)	Amount of acetic anhydride (mL, mmol)
PULLAc-5	1.7 mL, 21 mmol	2.0 mL, 21 mmol
PULLAc-9	3.0 mL, 37 mmol	3.5 mL, 37 mmol
PULLAc-15	5.0 mL, 62 mmol	5.8 mL, 62 mmol

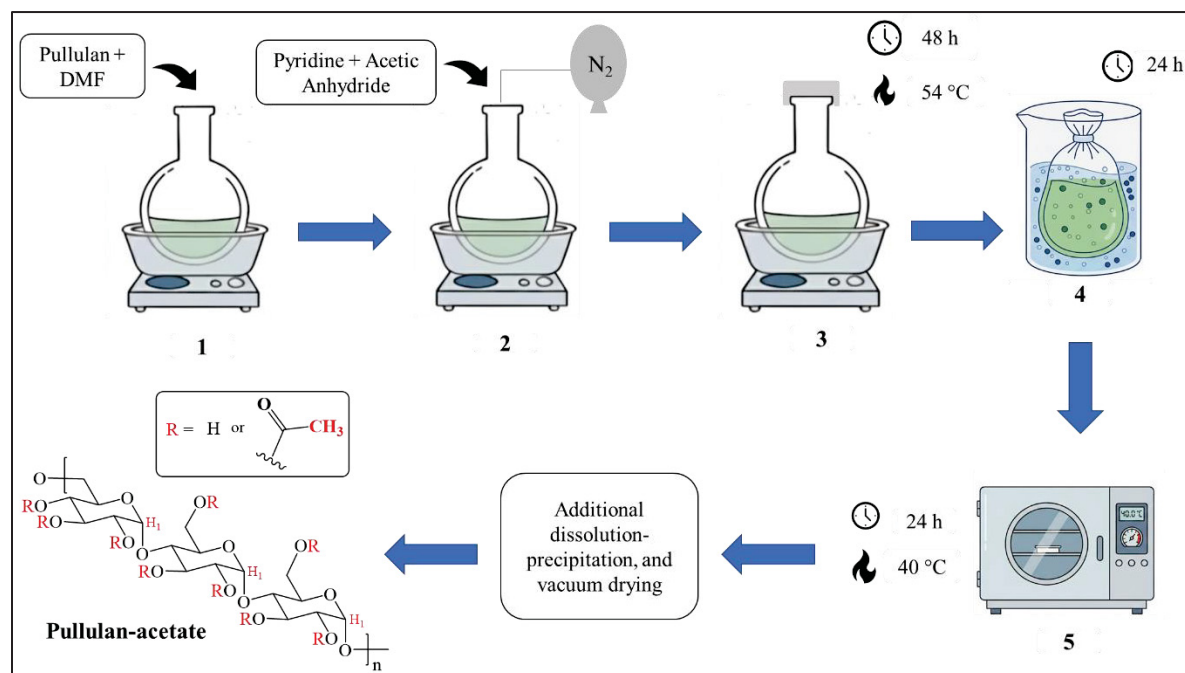


Figure 3.5 Schematic representation of the pullulan acetate synthesis: (1) dissolution of pullulan in DMF; (2) addition of pyridine, followed by acetic anhydride, under N_2 purge at room temperature; (3) reaction at $54\text{ }^{\circ}\text{C}$ for around 48 h; (4) purification by dialysis against water for 24 h; and (5) vacuum drying at $40\text{ }^{\circ}\text{C}$ for 24 h, followed by an additional dissolution-precipitation step and a second vacuum-drying step. (Cartoons generated by gemini-3.0-pro, 2025)

3.3.7 Electrospinning

Fibrous membranes of pullulan and PULLAc were produced using a FLUIDNATEK L-100 electrospinning system (Bionicia, Paterna, Valencia, Spain). A schematic representation of the setup is presented in Figure 3.6. For electrospinning, the polymer solutions were loaded into a syringe mounted on the integrated syringe pump and delivered through 25-gauge stainless-steel needles (diameter $\approx 0.51\text{ mm}$). High voltages were applied between the needle and the collector. The fibers were collected on aluminum foil or parchment paper affixed to the flat collector plate. All experiments were performed inside the closed electrospinning chamber, where temperature and relative humidity were controlled using the integrated environmental control unit.

Pullulan solutions were prepared in deionized water, whereas PULLAc samples were dissolved in mixtures of DMSO and acetone. Figure 3.7 lists the pullulan and PULLAc solution formulations (solvent systems) evaluated for electrospinning. All polymer solutions were magnetically stirred at 50 °C for 3 h to ensure complete dissolution and then allowed to equilibrate to room temperature for 30 minutes before processing. Electrospinning parameters, including solution concentrations, applied voltage, needle-collector distance, flow rate, and chamber conditions, is provided in Chapter 6.

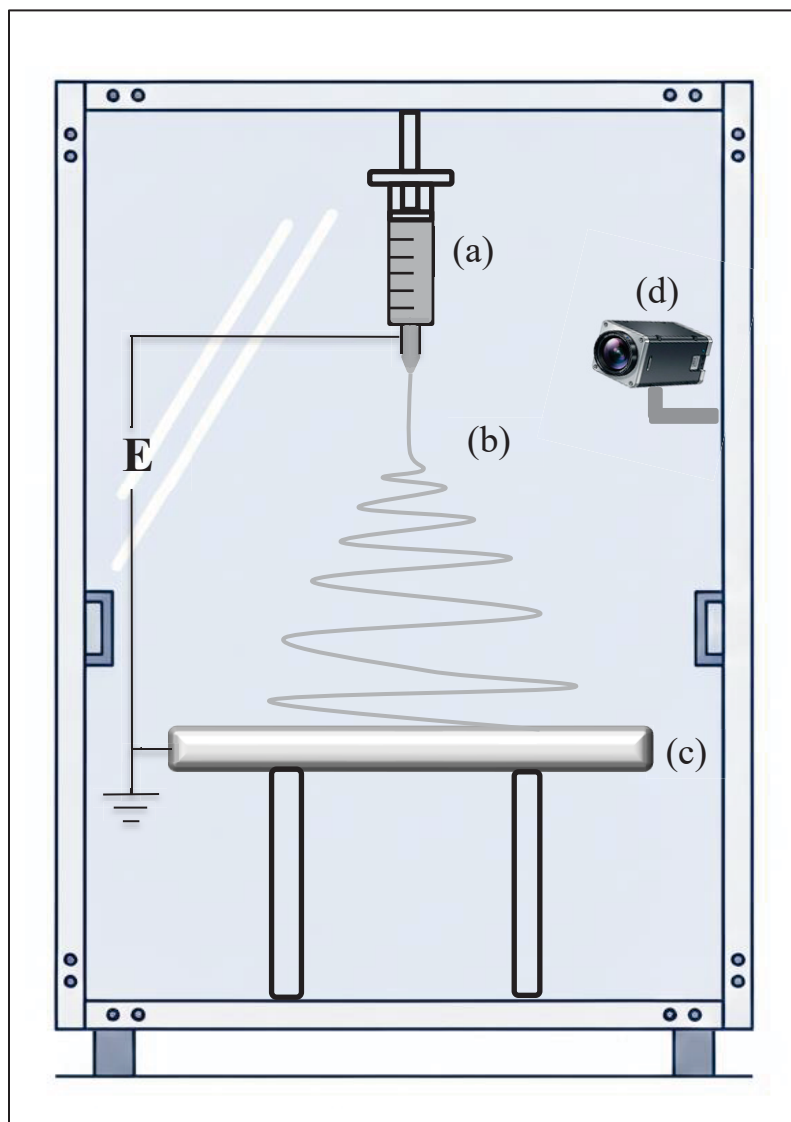


Figure 3.6 Schematic of the Electrospinning set-up. (a) syringe; (b) solution jet; (c) collector; (d) camera to observe the Taylor cone. (Camera generated by gemini-3.0-pro, 2025)

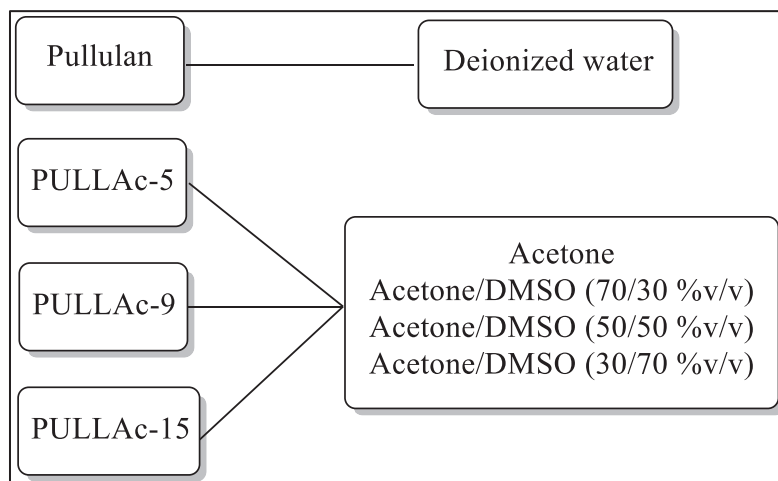


Figure 3.7 Polymer solution formulations evaluated for electrospinning. PULLAc-5, PULLAc-9, and PULLAc-15 denote pullulan acetate samples prepared using initial acetic anhydride to pullulan monomer unit molar feed ratios of 5:1, 9:1, and 15:1, respectively

3.4 Characterization Techniques

This section describes the analytical methods used to evaluate the materials and intermediates throughout the development of the proposed pullulan-based modifications. Certain techniques were applied at multiple stages to maintain methodological uniformity, whereas others were employed selectively, depending on the specific requirements or goals of each experimental step. By combining these approaches, a comprehensive understanding of the structural and chemical features of the modified pullulan was achieved. To provide a clear overview of the techniques, Table 3.5 summarizes the characterization techniques employed, the rationale for their use, and the chapters in which each technique was implemented.

Table 3.5 Summary of characterization techniques employed in this study, their purposes, and the chapters in which they are applied

Characterization Techniques	Chapter 4	Chapter 5	Chapter 6
GPC	Monitor the molar mass and dispersity evolution from pullulan to reduced pullulan, bromide-bearing pullulan, pullulan-xanthate macro-CTA, and PULL-g-PNVCL	Determine the molar mass and dispersity of the pullulan-xanthate macro-CTA and PULL-g-PAA	-----
^1H NMR	Assess the chemical structure of modified pullulan and quantify the degree of modification	Assess the chemical structure of modified pullulan and quantify the degree of modification	Assess the chemical structure of modified pullulan and quantify the degree of modification
^1H DOSY	Characterize unmodified pullulan, homopolymers, and grafted copolymers in solution by their diffusion behavior	Characterize unmodified pullulan, homopolymers, and grafted copolymers in solution by their diffusion behavior	-----
FTIR	Determine changes in characteristic bands and the appearance of new peaks associated with pullulan modification	Determine changes in characteristic bands and the appearance of new peaks associated with pullulan modification	Determine changes in characteristic bands and the appearance of new peaks associated with pullulan modification
TGA	Assess the thermal stability and mass-loss behavior of pullulan and its modified derivatives	-----	-----
DSC	Evaluate changes in thermal transitions upon pullulan modification and investigate the temperature-responsive behavior of PULL-g-PNVCL copolymers	-----	-----

Characterization Techniques	Chapter 4	Chapter 5	Chapter 6
DLS	Determine particle size and monitor temperature-induced changes in the hydrodynamic dimensions of PULL-g-PNVCL copolymers.	-----	-----
SEM	-----	-----	Analyze the morphology of the electrospun membranes

The following subsections describe each characterization technique in detail, together with the conditions and parameters employed.

3.4.1 Gel Permeation Chromatography

The weight-average molar mass ($\overline{M}_w$), number-average molar mass ($\overline{M}_n$), and dispersity ($\mathcal{D} = \overline{M}_w/\overline{M}_n$) of pullulan and its modified derivatives were determined by gel permeation chromatography (GPC). Analyses were performed on a Tosoh ECOSEC Elite GPC 8420 system equipped with an automatic injector and a refractive index detector. Separation was achieved with two Tosoh Bioscience TSKgel Alpha-M columns (7.8 mm $\times$ 30 cm, 13 μ m particle size) calibrated using pullulan standards (molar mass range of 667–107,000 g/mol). The mobile phase consisted of dimethylformamide (DMF) containing 0.1% (v/v) lithium bromide (LiBr) and was maintained at 60 $^{\circ}$ C, with a flow rate of 0.8 mL/min and an injection volume of 100 μ L. Prior to analysis, all samples were dissolved in the mobile phase and filtered through a 0.2 μ m PTFE membrane.

3.4.2 Nuclear Magnetic Resonance

The chemical structures of the materials were examined using nuclear magnetic resonance (NMR) spectroscopy. Proton NMR (^1H NMR) and ^1H - ^1H correlation spectroscopy (COSY) were recorded on a Bruker Avance III spectrometer operating at 300 MHz, while diffusion-

ordered spectroscopy (^1H DOSY) was performed on a Bruker Avance III HD system at 600 MHz. All measurements were conducted at room temperature using either deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated water (D_2O) as solvents. The residual proton signals of DMSO-d_6 ($\delta = 2.50$ ppm) and D_2O ($\delta = 4.79$ ppm) served as internal references, and all chemical shifts are reported in parts per million (ppm). ^1H NMR spectra of the modified pullulan obtained at each synthetic stage were used to calculate the degree of substitution of hydroxyl groups along the pullulan backbone. ^1H NMR was employed to confirm the successful introduction of new functional groups, while COSY was used to identify proton–proton spin systems and verify the preservation of the pullulan backbone structure. COSY is a two-dimensional ^1H NMR experiment that shows which protons are coupled to nearby protons through chemical bonds. Data processing was performed using MestreNova software, with baseline and phase corrections applied to all spectra prior to analysis.

^1H DOSY experiments were conducted to provide information on the diffusion coefficients of the polymer species, enabling the distinction between homopolymers, unmodified pullulan, and the grafted copolymers in solution. DOSY separates NMR signals according to the diffusion behavior of the species in solution, allowing compounds with different hydrodynamic sizes to be distinguished even when some resonances overlap in the conventional ^1H NMR spectrum. The technique applies pulsed field gradients to determine diffusion coefficients, with larger molecular species generally diffusing more slowly than smaller ones in solution. In the present work, this made DOSY particularly useful for comparing unmodified pullulan, free homopolymer, and grafted copolymer species in the same solution. Measurements were carried out using a bipolar longitudinal eddy current delay (BPP-LED) pulse sequence with spoil gradients (D. H. Wu, Chen, & Johnson, 1995). The gradient duration (δ) was set to 4 ms, the diffusion time (Δ) to 175 ms, and the gradient strength (g) was varied linearly from 0.96 G/cm to 47.19 G/cm over 64 increments. These settings were chosen to achieve at least 50% signal attenuation for the slowest diffusing components. A recycle delay of 5 s was applied. Data processing was conducted with MestreNova software, and DOSY spectra were generated using the peak fit method to produce two-dimensional plots, with NMR chemical shifts along one axis and the calculated diffusion coefficients along the other. It should be noted that no external

standard was used to calibrate the diffusion coefficients; thus, the values in the spectra are provided for relative comparison only.

Approximately 10–15 mg of sample was dissolved in 0.4–0.5 mL of DMSO- d_6 or D_2O and was transferred to NMR tubes before recording the spectra.

3.4.3 Fourier Transform Infrared Spectroscopy

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) analysis was employed to identify characteristic functional groups and to confirm the chemical modifications introduced into the pullulan backbone during synthesis. FTIR spectra of the samples were recorded using a Perkin-Elmer FTIR spectrometer (Spectrum Two™) equipped with a diamond crystal. Measurements were carried out over the wavenumber range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} . Samples were finely ground and placed directly on the crystal. A uniform pressure was applied to ensure good contact between the sample and the crystal before collecting the spectra.

3.4.4 Thermogravimetric Analysis

The thermal stability of both homo- and copolymers was evaluated using thermogravimetric analysis (TGA) on a Pyris Diamond TG/DTA instrument (PerkinElmer® Inc.). Measurements were conducted over a temperature range of 30–600 °C under a nitrogen atmosphere (100 mL/min) at a heating rate of 10 °C/min. TGA was employed to determine the degradation profile and assess the thermal stability of the synthesized materials.

3.4.5 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were carried out using a TA Instruments DSC 2500 system equipped with a cooling accessory. Approximately 4 mg of each sample was placed in a T-Zero premium lid-punctured pan, with an empty pan serving as the reference. Samples were heated at a rate of 10 °C/min. The first heating scan, from 40 °C to 240 °C, was performed to remove any thermal history and moisture, followed by a second

heating scan from $-40\text{ }^{\circ}\text{C}$ to $250\text{ }^{\circ}\text{C}$ and subsequent cooling back to $40\text{ }^{\circ}\text{C}$. All measurements were conducted under a nitrogen flow of 50 mL/min , maintaining a consistent heating and cooling rate of $10\text{ }^{\circ}\text{C/min}$, and repeated three times. DSC was employed to analyze the thermal transitions of the materials, including glass transition, providing insight into the structural and thermal properties of the synthesized polymers.

DSC was also employed to investigate the temperature-responsive behavior of the PULL-g-PNVCL copolymers, providing insight into their temperature-induced phase transitions, such as the LCST, and confirming their thermal sensitivity. The pullulan-g-Poly (N-vinyl caprolactam) copolymers and modified pullulan before copolymerization samples were analyzed using DSC (Diab, Akiyama, Kataoka, & Winnik, 2004; S. Gupta, Singh, & Matsumi, 2019; Ko et al., 2020; Laukkanen, Valtola, Winnik, & Tenhu, 2005; K. Wei, Wang, Li, & Zheng, 2015). Approximately 4 mg of each sample, combined with $40\text{ }\mu\text{L}$ of water, was placed in a T-Zero premium hermetic unpunctured pan, with an empty pan serving as the reference. Measurements were conducted over a temperature range of $20\text{--}50\text{ }^{\circ}\text{C}$ at a heating and cooling rate of $1\text{ }^{\circ}\text{C/min}$. All runs were performed under a nitrogen flow of 50 mL/min and repeated three times. DSC curves were processed and analyzed using Trios software from TA Instruments.

3.4.6 Dynamic Light Scattering

Dynamic light scattering (DLS) was employed to determine particle size and monitor the temperature-responsive behavior of the PULL-g-PNVCL copolymers. The hydrodynamic average diameter (Z_{av}) and particle size distribution (polydispersity index, PdI) of the PULL-g-PNVCL copolymer samples dispersed in deionized water were measured using a Malvern Zetasizer Nano ZS instrument. Particle dispersions at a concentration of 1 mg/mL were analyzed in polystyrene cuvettes at $24\text{ }^{\circ}\text{C}$, and samples were allowed to equilibrate for 2 minutes prior to measurement. To assess the temperature sensitivity of the copolymer samples, Z_{av} and PdI were recorded across a temperature range of $20\text{--}44\text{ }^{\circ}\text{C}$ in $2\text{ }^{\circ}\text{C}$ increments. The transition temperature was identified as the point at which an increase in particle size occurred. All measurements were performed in triplicate. Additionally, heating and cooling cycles below

and above or close to the LCST were performed to investigate the reversible thermal phase transition of the copolymer.

3.4.7 Scanning Electron Microscopy

The morphology of the electrospun fibers was examined by scanning electron microscopy (SEM) using a TM3000 tabletop microscope (Hitachi High-Technologies, Tokyo, Japan). Before imaging, the samples were gold-sputtered using an Emitech K550X sputter coater (Quorum Technologies, Laughton, UK) operated at 30 mA for 2 min to improve surface conductivity. Fiber diameters were determined from SEM micrographs with ImageJ software (version 1.53a, NIH, Bethesda, MD, USA). For each membrane, approximately 70–100 individual fibers were measured across several representative regions, and the average diameter and standard deviation were calculated.

CHAPTER 4

SYNTHESIS AND CHARACTERIZATION OF PULLULAN-g-POLY (N-VINYL CAPROLACTAM) VIA RAFT/MADIX POLYMERIZATION

4.1 Introduction

This chapter presents experimental results addressing the first sub-objective of this thesis: investigating the design and synthesis procedure of pullulan-g-poly (N-vinyl caprolactam) (PULL-g-PNVCL) copolymers, and demonstrating their structure-property relationships for temperature-responsive applications.

Building on the methodology described in Chapter 3, this work focuses on developing a novel grafting strategy that combines the renewable, biocompatible, and biodegradable nature of pullulan with the temperature-responsive properties of poly (N-vinyl caprolactam) (PNVCL). The use of RAFT/MADIX polymerization represents a key advancement, enabling controlled grafting of PNVCL chains from a pullulan backbone. The synthetic approach involved three main steps: (i) partial functionalization of reduced molar mass pullulan with 2-bromopropionyl bromide (BPB), (ii) substitution with a xanthate-based chain transfer agent, and (iii) controlled polymerization of N-vinyl caprolactam (NVCL) from the resulting macro-RAFT agent. The complete synthetic route is shown in Figure 4.1.

This chapter presents a comprehensive analysis of the synthesis, characterization, and performance of PULL-g-PNVCL copolymers, highlighting their potential for expanding pullulan applications, particularly in stimuli-responsive drug delivery systems.

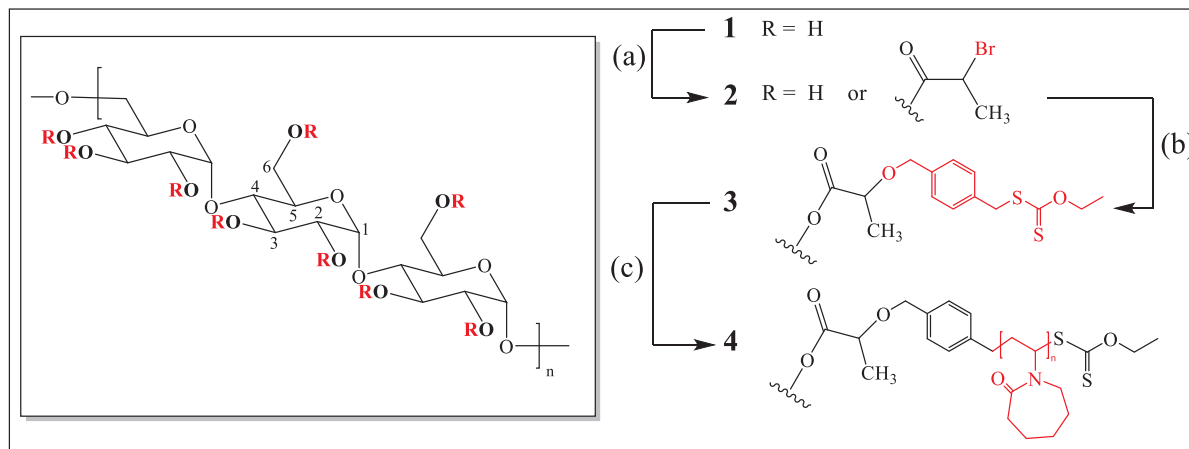


Figure 4.1 Chemical structures and route for the synthesis of PULL-g-PNVCL (a) BPB, DMF, TEA, 25 °C, 72 h; (b) CTA-OH, DMSO, Pyridine, 50 °C, 24 h; (c) NVCL, DMSO, AIBN, 70 °C, 24 h; **1**: Reduced molar mass pullulan; **2**: Bromide-bearing pullulan; **3**: Xanthate-based macro-chain transfer agent pullulan; **4**: PULL-g-PNVCL

4.2 Results and Discussion

4.2.1 Reduced Molar Mass Pullulan Characterization

Figure 4.2 presents the GPC chromatograms of commercial pullulan and the reduced molar mass pullulan. The commercial pullulan exhibits a high average molar mass and a broad distribution. Such high-molar-mass polymers are expected to display significant chain entanglement, which can limit the effective accessibility of hydroxyl groups and result in less efficient and more heterogeneous chemical modification along the polymer backbone (Maia-Obi & Bazito, 2025). To improve both the reactivity and dispersity of pullulan, it was therefore necessary to reduce its molar mass. Our objective was to obtain shorter, better-defined polymer chains compared to the commercial pullulan. This was accomplished through a 1.5-hour acid hydrolysis, which cleaves the maltotriose units in pullulan to result in a lower molar mass pullulan as the starting point of the synthetic sequence depicted in Figure 4.1. The GPC data in Table 4.3 show a decrease in molar mass and dispersity compared to the commercial pullulan following acid hydrolysis. Figure 4.3 presents the ^1H NMR spectrum of pullulan after the molar mass reduction reaction. The spectrum (300 MHz, D_2O) displays the characteristic resonances

of pullulan, notably the anomeric proton signals at $\delta = 5.25$ - 5.50 ppm and $\delta = 4.95$ ppm, corresponding to the α -(1 $\rightarrow$ 4) and α -(1 $\rightarrow$ 6) linkages of the maltotriose subunits, respectively. The presence and consistency of these characteristic peaks across the spectrum indicate that the maltotriose repeating units remain largely unaltered throughout the polymer following the molar mass reduction process.

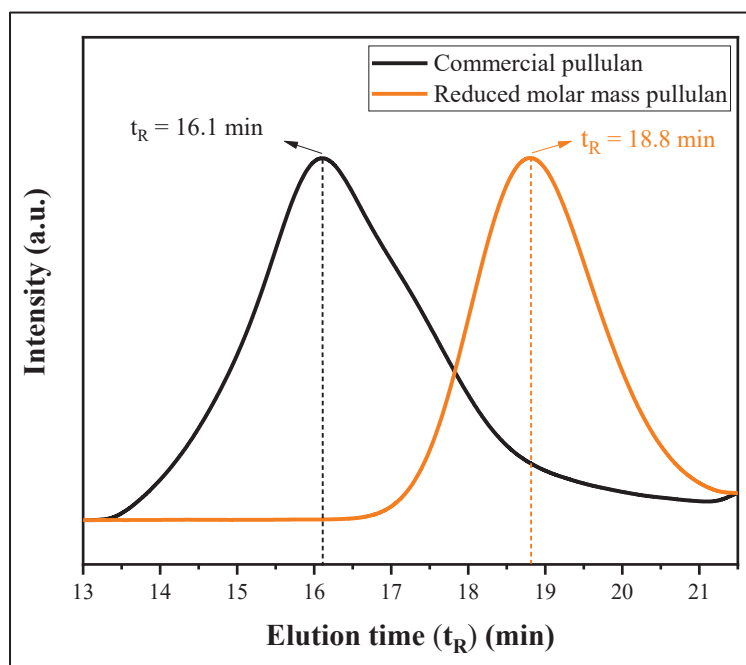


Figure 4.2 GPC chromatograms of commercial pullulan and reduced molar mass pullulan in DMF/LiBr

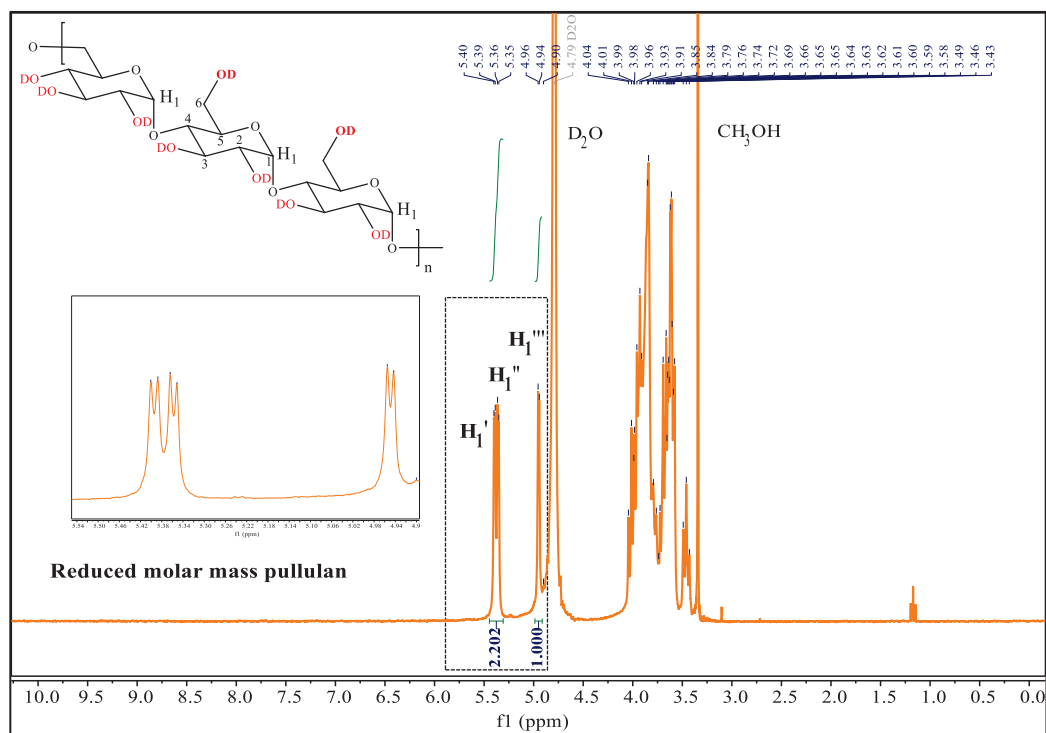


Figure 4.3 ^1H NMR spectra of reduced molar mass pullulan in D_2O

4.2.2 Bromide-Bearing Pullulan Characterization

Aiming to synthesize PULL-g-PNVCL, the precursor reduced molar mass pullulan was first acylated with 2-bromopropionyl bromide (BPB) at five different molar ratios of BPB relative to the pullulan monomer unit (0.5, 1, 2, 5, and 7). This modification yielded a series of bromide-functionalized derivatives, referred to as PULLBr-0.5, PULLBr-1, PULLBr-2, PULLBr-5, and PULLBr-7, respectively. The chemical structure of the PULLBr samples was confirmed using ^1H NMR spectroscopy, with representative spectra shown in Figure 4.4 for PULLBr-5 and in Figure-A I-1 for all samples (see APPENDIX I).

The ^1H NMR spectrum of PULLBr-5 displays additional signals in the region of 1.25–2 ppm and 4.60–4.70 ppm (see the outlined region in Figure 4.3, Figure-A I-1, and Figure-A I-2). New signals in ^1H NMR spectra are related to the protons of the methyl and methine groups in the bromopropionyl unit, respectively, thus confirming the successful chemical modification

of the reduced molar mass pullulan. This is consistent with observations from other studies on cellulose or pullulan with BPB (Carvalho et al., 2020; Sui et al., 2008).

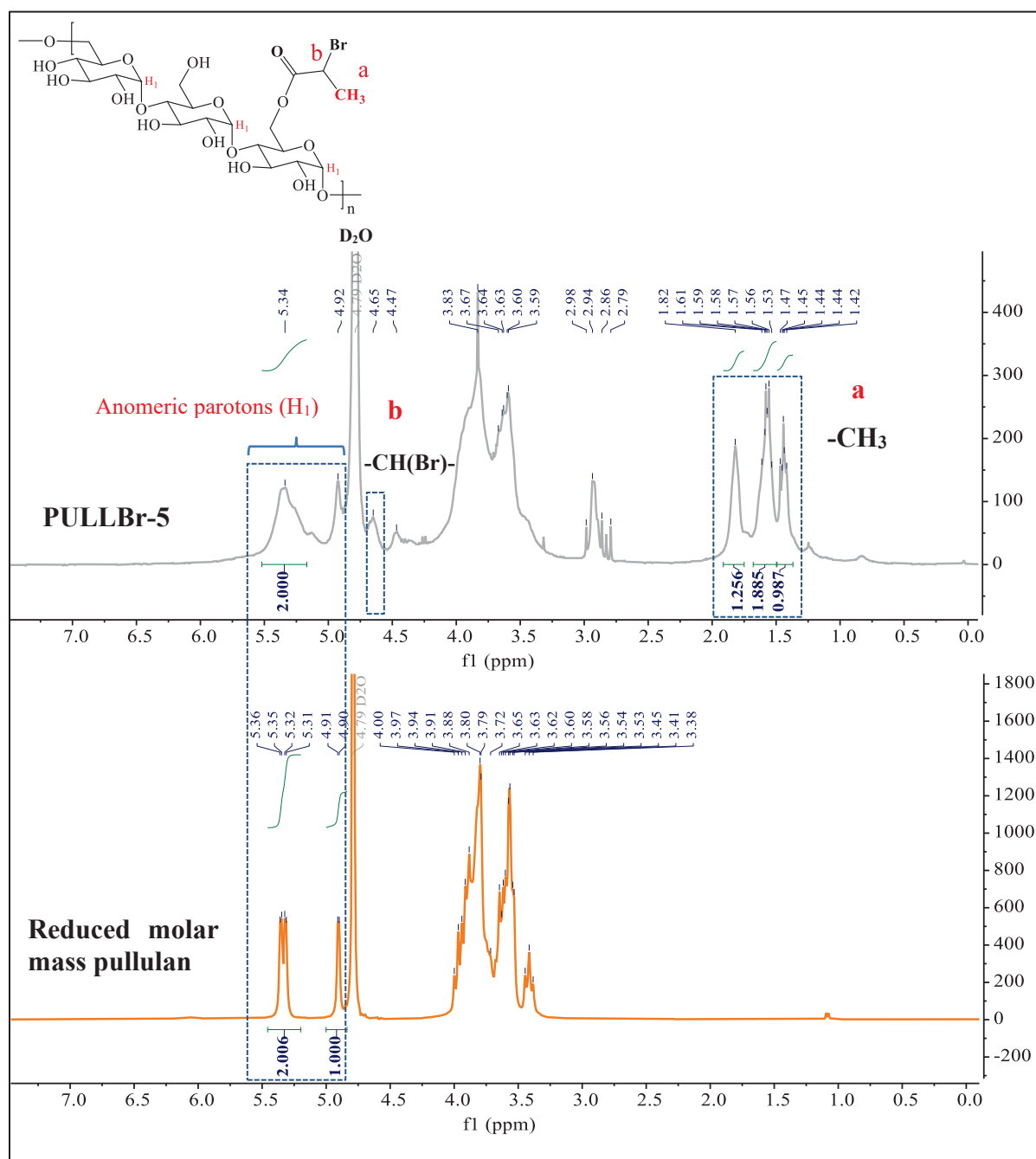


Figure 4.4 ¹H NMR spectra of reduced molar mass pullulan and PULLBr-5 in D₂O

Moreover, the influence of varying the BPB equivalents on the degree of substitution (DS) was examined. DS represents the number of bromide groups per pullulan monomer unit (maltotriose unit); here, it is converted to the number of bromide groups per 100 monomer units to facilitate comparison and make the values easier to interpret. To evaluate the modification efficiency, the DS was calculated by comparing the integral values of the peaks from 1.25 to 2 ppm with those corresponding to the anomeric protons (5.25 to 5.5 ppm) of pullulan, serving as the reference integrals. Details of the DS calculations can be found in APPENDIX I (Figure-A I-3). As was expected, and as Table 4.1 presents, higher DS is achieved by increasing the concentration of the BPB in the reaction media. The DS on hydroxyl groups of pullulan by bromopropionyl units in PULLBr-5, chosen for further synthesis, was determined to be 136 per 100 monomer units of pullulan. PULLBr-5 was also synthesized on a larger scale, and the DS for this sample was determined to be 82 per 100 monomer units of pullulan (See APPENDIX I, Table-A I-1).

Table 4.1 The degree of substitution for all PULLBr samples with different equivalents of BPB

Sample	Solvent for NMR	Mol of BPB relative to pullulan monomer units	Bromide groups in 100 monomers (DS) **
PULLBr-0.5	D ₂ O	0.5	4.5
PULLBr-1	D ₂ O	1	4.5
PULLBr-2	D ₂ O	2	36
PULLBr-5	D ₂ O	5	136
PULLBr-7	DMSO-d ₆ *	7	≈ 318

* DMSO-d₆ was used instead of D₂O due to the poor solubility of the substrate in water.

** The DS was calculated using the equation described in Appendix I.

Additionally, two-dimensional nuclear magnetic resonance correlation spectroscopy (2D NMR-COSY) (see Figure-A I-4 and Figure-A I-5 that show the COSY spectra of pullulan and PULLBr-5) was performed. The spectra displayed the same coupling patterns for the glucose ring protons as observed prior to modification, confirming the preservation of the maltotriose unit.

Figure 4.5 presents the ATR-FTIR spectra of the reduced molar mass pullulan and PULLBr-5. The characteristic peaks are listed in Table 4.2. In this case, a new band appearing at 1740 cm^{-1} (C=O stretch) provides further indications of the bromopropionyl grafting onto the reduced molar mass pullulan backbone.

The samples PULLBr-5 and PULLBr-7 were selected for the subsequent synthetic step; however, as the synthesis route derived from PULLBr-5 showed better results, it is presented in detail in this chapter. The other tested reactions derived from PULLBr-7 encountered challenges either during synthesis or characterization. These results are provided in APPENDIX I. Similar difficulties with alternative conditions have been reported by other researchers (Teramoto & Shibata, 2006a; Zorin et al., 2023).

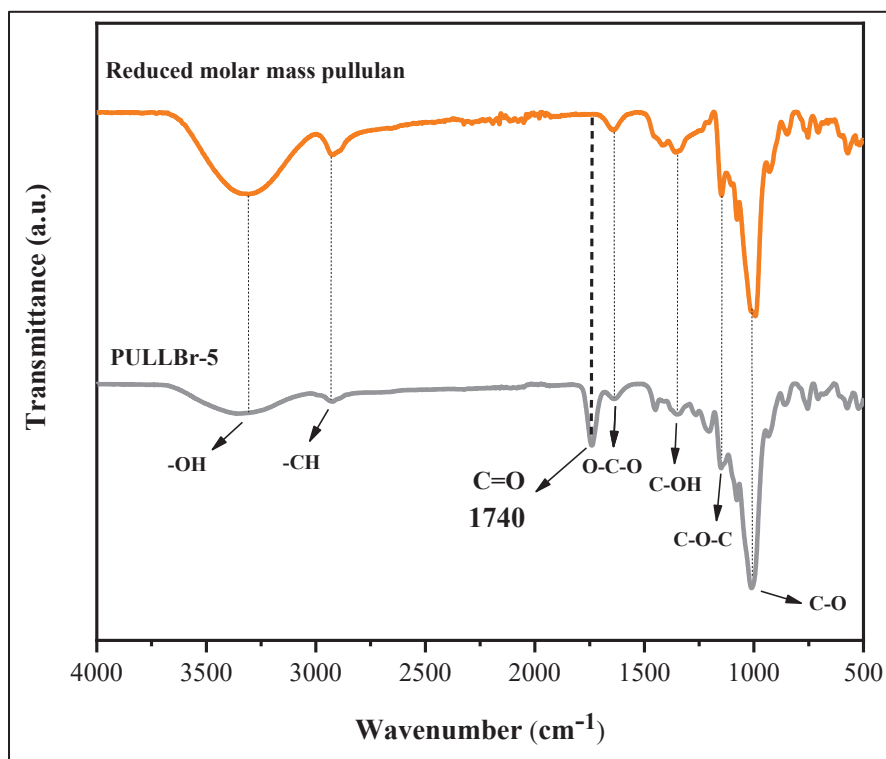


Figure 4.5 ATR-FTIR spectra of reduced molar mass pullulan and PULLBr-5

Table 4.2 Characteristic ATR-FTIR bands of reduced molar mass pullulan or PULLBr-5

Wavenumber (cm ⁻¹)	Vibration type and functional group
3308	Stretching of O-H
2925	Stretching of C-H
1740	Stretching of C=O
1642	Stretching of O-C-O
1354	Bending of C-OH
1146	Stretching of the C-O-C
1006	Stretching of C-O

4.2.3 Pullulan-Xanthate-Based Macro-Chain Transfer Agent Characterization

First, the xanthate-based chain transfer agent (CTA-OH) was synthesized, and its ¹H NMR spectrum is provided in APPENDIX I (Figure-A I-6). This spectrum was subsequently used as a reference in the analysis of the pullulan-xanthate-based macro-chain transfer agent, referred to as PULLX. Since the synthesis route here was derived from PULLBr-5, the sample is designated as PULLX-5.

Figure 4.6 shows the ¹H NMR spectra of PULLBr-5 and PULLX-5. New resonances between 7.10 and 7.36 ppm, attributed to the aromatic protons of the CTA group, confirmed the successful synthesis of PULLX-5. This functionalization reduced the hydrophilicity of the resulting PULLX-5. To evaluate the reaction efficiency, the number of CTA molecules on PULLX-5 was determined (see APPENDIX I, Figure-A I-7 for detailed calculations). The integration value for the combined resonances at 4.87–5.25 ppm (anomeric protons, 2H) was used as the reference to estimate the DS based on the peak integration from 7.10 to 7.36 ppm. The difference in anomeric-proton chemical shifts between PULLX-5 and PULLBr-5 spectra arises from the use of different NMR solvents. Due to the partial overlap of signals in the anomeric protons region of PULLX-5 in DMSO-d₆, the DS was determined approximately and is an underestimate of the actual DS. The DS on bromopropionyl units by CTA-OH in PULLX-5 was semi-quantified at 4.5 per 100 monomer units. The results showed partial substitution of the bromopropionyl groups on the PULLBr-5 backbone by the CTA-OH, thus a lower number of CTA-OH units were observed in PULLX-5 compared to the bromopropionyl groups in PULLBr-5, despite using CTA-OH in significant excess (9 equivalents). Similar findings

were reported by Pal et al. (Pal et al., 2018), who modified dextrin by incorporating 2-bromo-2-methyl propionyl bromide, followed by reacting with potassium ethyl xanthate to graft polymerize NVCL from a dextrin-based macro-RAFT chain transfer agent. Therefore, in our case, a lower DS obtained for CTA-derived pullulan compared to the bromopropionyl group is not surprising.

Given these findings, PULLX-5 was synthesized in larger batches to continue the synthetic sequence. A similar result, albeit with a slightly lower DS (3 CTA moieties in 100 monomers), was obtained in this reaction. Regardless of the relatively low DS, the CTA moieties in PULLX-5 effectively promoted RAFT/MADIX polymerization in the subsequent synthetic key step.

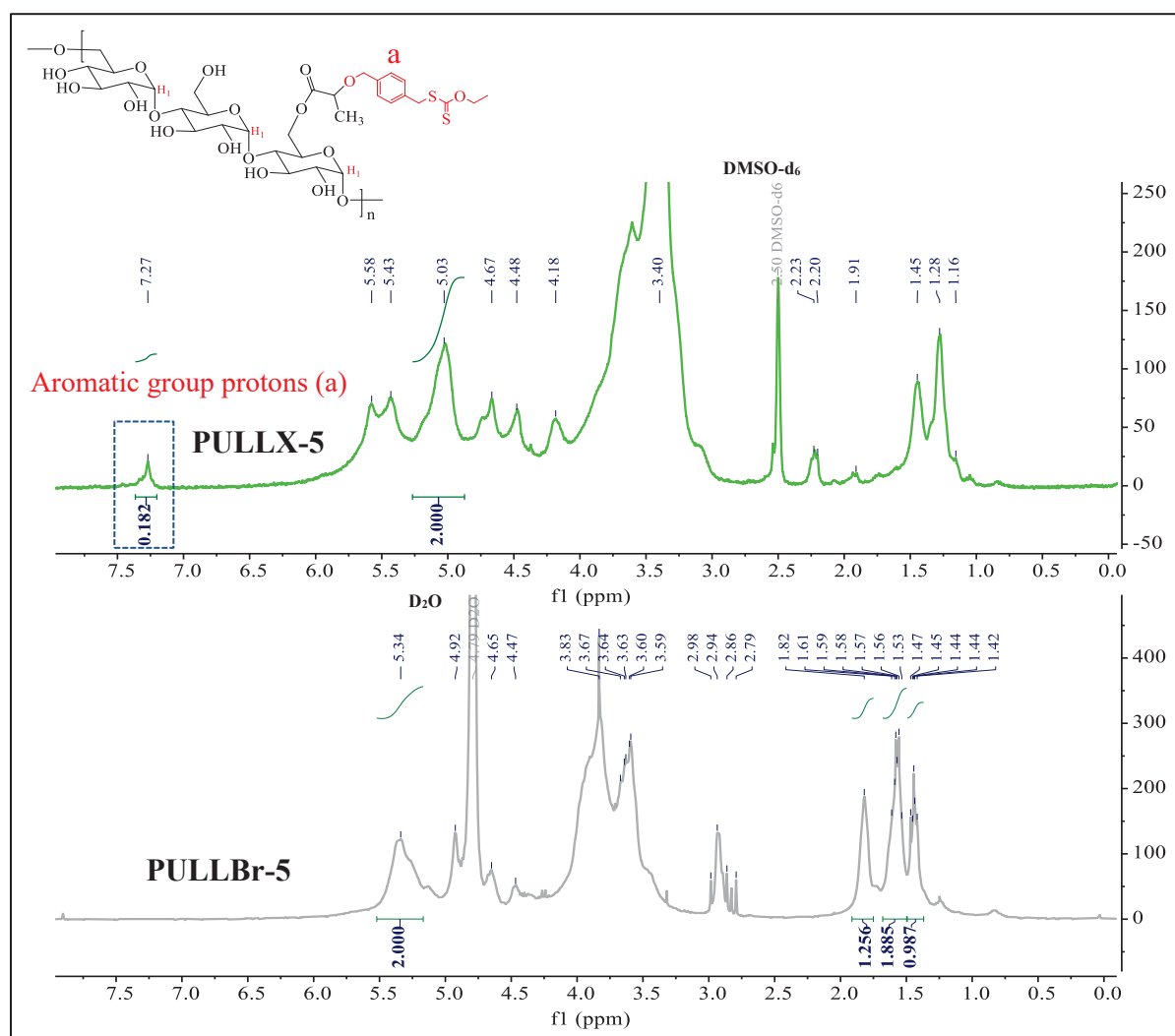


Figure 4.6 ^1H NMR spectra of PULLBr-5 in D_2O and PULLX-5 in DMSO-d_6 . The main feature is the appearance of new resonances at 7.10–7.36 ppm in PULLX-5, assigned to the aromatic protons of the CTA group, which are absent in PULLBr-5. The anomeric proton region (4.87–5.25 ppm) was used as the pullulan reference for approximate DS determination

4.2.4 PULL-g-PNVCL Characterization

Figure 4.7 shows the ^1H NMR spectra of PULL-g-PNVCL (150) and PULL-g-PNVCL (300). The characteristic resonances of PNVCL are observed between 1.50–2.00 ppm and 2.20–2.40 ppm (the caprolactam ring and the backbone chain of the polymer), in addition to the typical

resonances of the maltotriose unit of pullulan. Therefore, these findings confirmed the successful polymerization of NVCL from the pullulan backbone. Additionally, it was observed by ^1H NMR analysis that increasing the concentration of NVCL monomer in the reaction media generates longer PNVCL chains as evidenced by higher integral values for the PNVCL signals: integral values of 1.210 (1.50–2.00 ppm) and 0.456 (2.20–2.40 ppm) for the PNVCL signals in PULL-g-PNVCL (300); and 0.452 (1.50–2.00 ppm) and 0.180 (2.20–2.40 ppm) for PULL-g-PNVCL (150) with anomeric resonances as the reference peaks.

A DOSY NMR experiment was performed to further evaluate the effective formation of the PULL-g-PNVCL copolymer. Figure 4.8 (a) and (b) presents the DOSY spectra of PULLX-5 and PULL-g-PNVCL (150). First, the PULLX-5 was investigated, and it was found that this material was contaminated with a small amount of ungrafted CTA-OH that could not be purified further in the previous step without negatively impacting the isolated yield. Even though the presence of CTA-OH molecules may compete as initiation points with pullulan-grafted xanthate groups during the NVCL polymerization step, we envisioned that the resulting ungrafted polymer would be filtered out in the dialysis step, given the 14 kDa M_w cutoff of the membrane. Indeed, the dialysis step performed after polymerization effectively removed any ungrafted, smaller polymer chains, as shown in Figure 4.8 (b). Therefore, the dominant species align with a single diffusion coefficient that is correlated to the signals corresponding to both PNVCL and pullulan, indicating that both components are part of the same copolymer structure. Overall, the DOSY spectra of PULL-g-PNVCL further corroborate the successful grafting of PNVCL from the PULLX-5 chains.

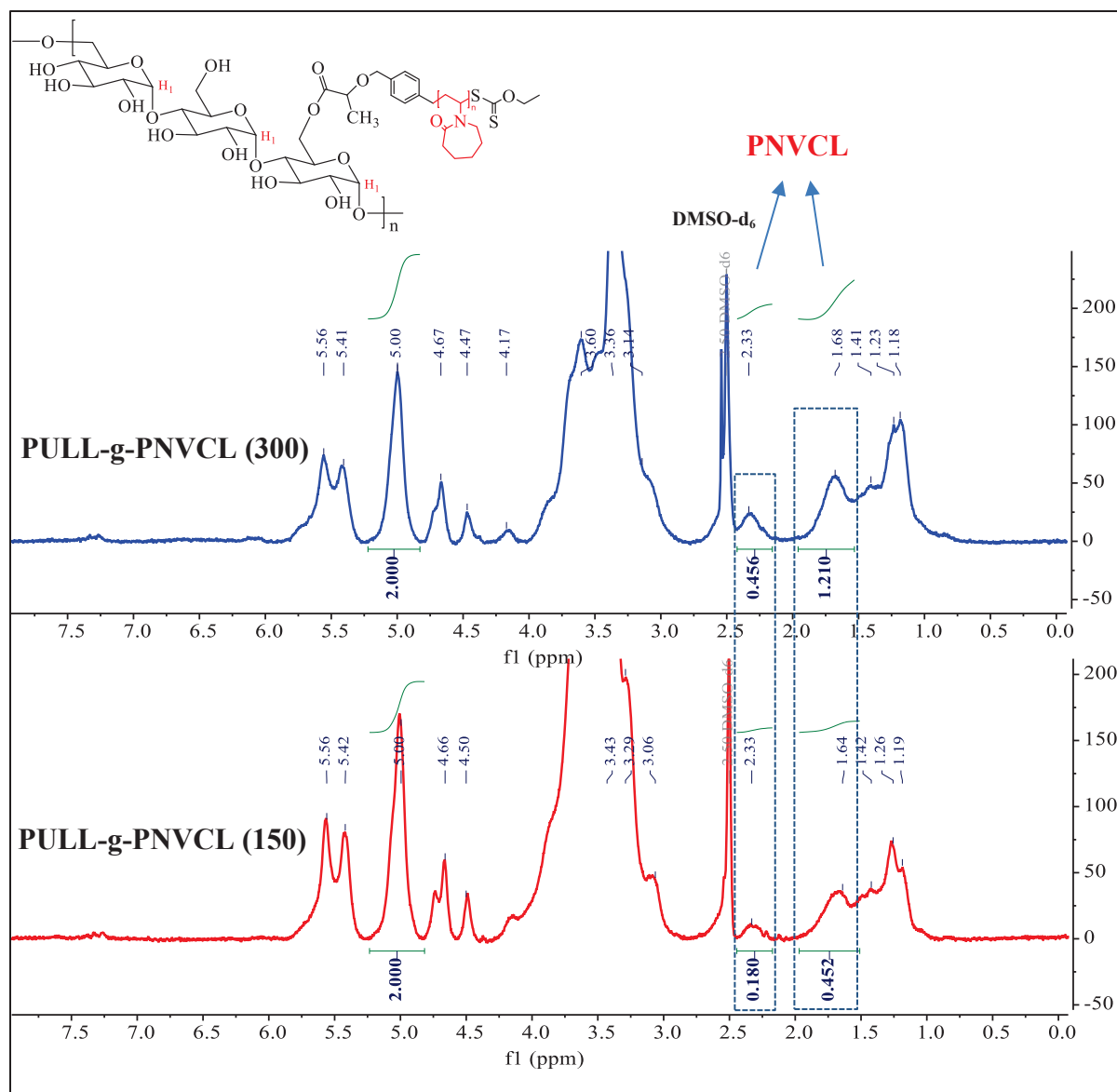


Figure 4.7 ^1H NMR spectra of PULL-g-PNVCL in DMSO-d_6

The ATR-FTIR spectra for PULLX-5, PULL-g-PNVCL (150), PULL-g-PNVCL (300), and the NVCL monomer are shown in Figure 4.9. Extending the spectral range from 1500 to 1700 cm^{-1} reveals a wavenumber shift and an increased intensity for carbonyl peaks (C=O) characteristic of the caprolactam function, probably due to stacking contributions of grafted PNVCCL chains with the O-C-O peak from pullulan. These findings align with the observations from the NMR analysis.

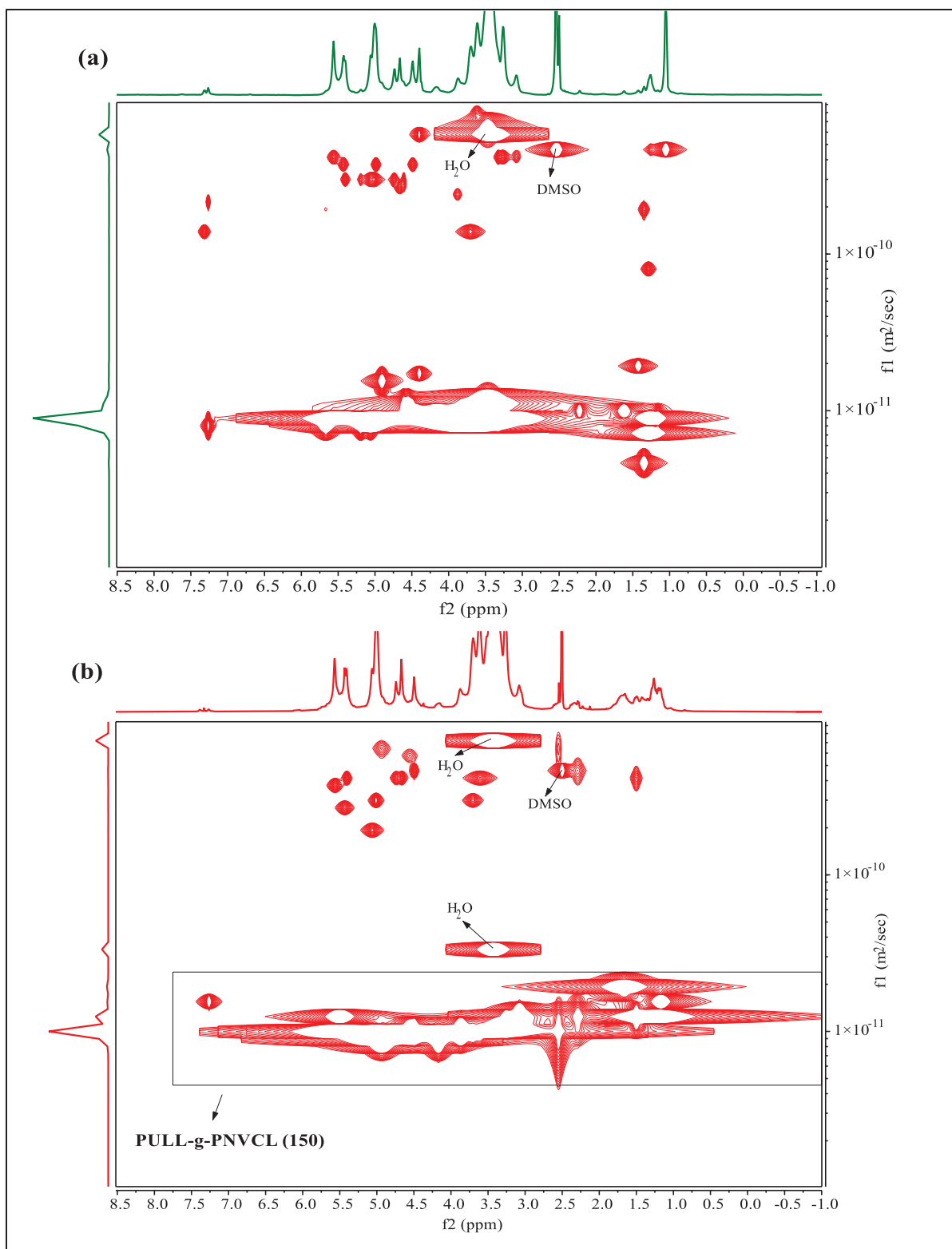


Figure 4.8 DOSY spectra of (a) PULLX-5, and (b) PULL-g-PNVCL (150) in DMSO- d_6

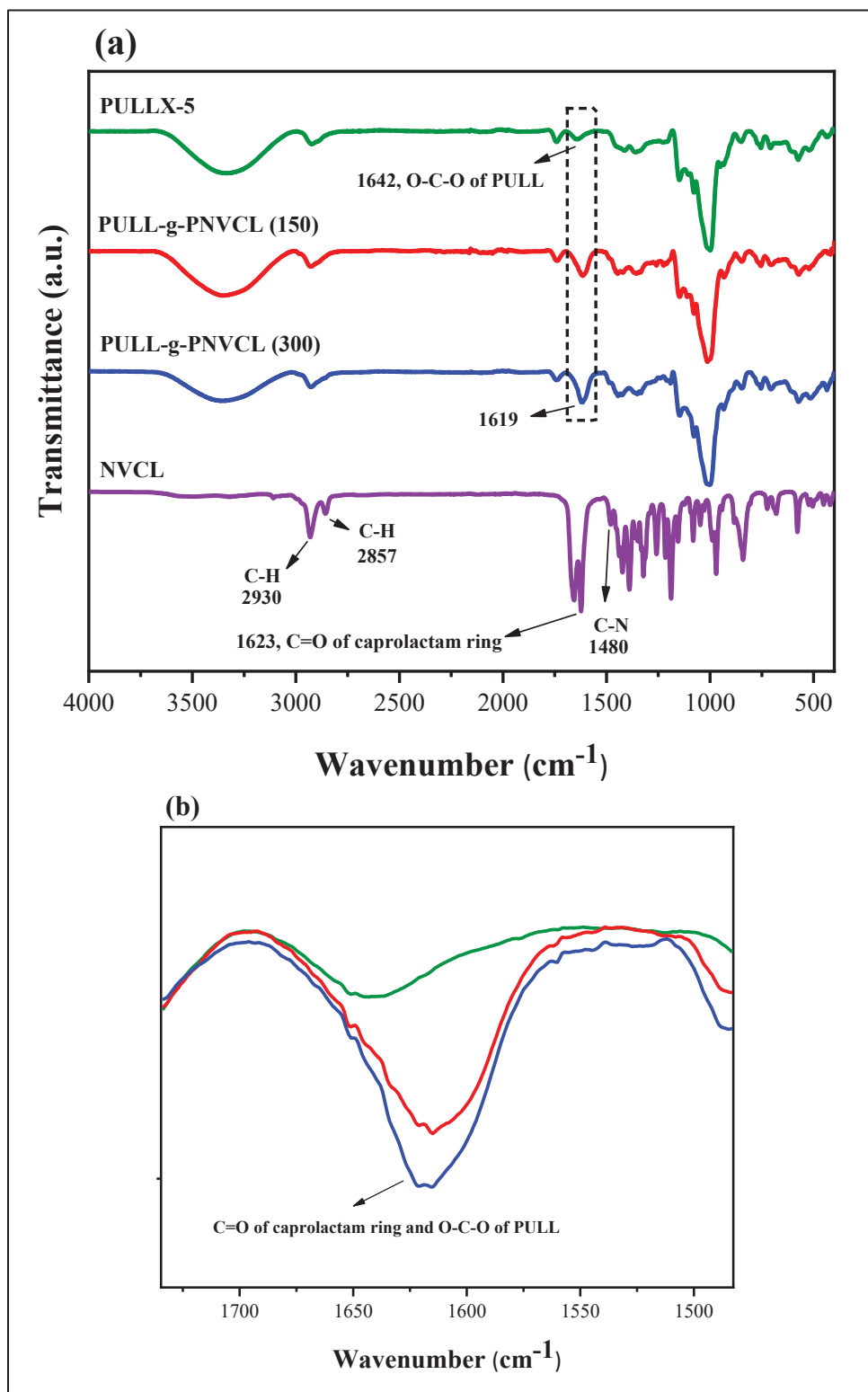


Figure 4.9 ATR-FTIR spectra of PULLX-5, PULL-g-PNVCL (150), PULL-g-PNVCL (300), and NVCL monomer, (a) full spectra, and (b) zoom between 1500 cm^{-1} and 1700 cm^{-1}

GPC analyses of all grafted materials were conducted to further investigate the NVCL polymerization from the pullulan backbone. Figure 4.10 presents the GPC chromatograms of PULLX-5, PULL-g-PNVCL (150), and PULL-g-PNVCL (300) in DMF/LiBr. It is shown that the GPC chromatograms for all copolymer samples shifted towards lower retention times, indicating the successful grafting of PNVCCL from the PULLX-5 (see APPENDIX I, Figure-A I-7 for the grafted PNVCCL growth from the PULLX-7).

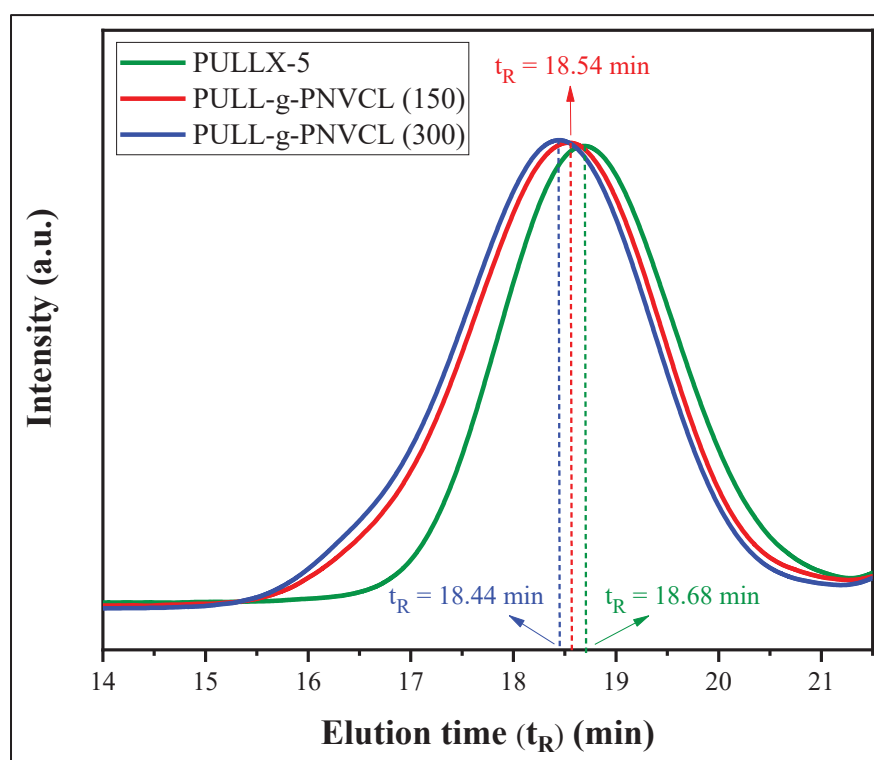


Figure 4.10 GPC chromatograms of PULLX-5, PULL-g-PNVCL (150), and PULL-g-PNVCL (300) in DMF/LiBr

The molar masses, dispersity, and PNVCCL percentage in copolymers values are summarized in Table 4.3 and Table-A I-4 (APPENDIX I). Results depicted that the molar masses of all graft copolymers exceed those of the pullulan-based macro-RAFT chain transfer agent, adding evidence about the successful RAFT grafted polymerization of NVCL. Furthermore, all grafted copolymers exhibit unimodal and symmetrical GPC curves with narrower molar mass distributions ($\bar{D} \leq 1.83$), when compared to the typical dispersity values observed for branched copolymers synthesized via non-controlled polymerization methods (often more than 5) (J.

Liu, Wang, Fu, Zhu, & Shi, 2008; X. Liu et al., 2014; Slark, Sherrington, Titterton, & Martin, 2003; R.-X. Zhao et al., 2012). Furthermore, taking into account the inherent dispersity of the pullulan backbone, the dispersity values obtained for the PULL-g-PNVCL copolymers are consistent with expectations for such grafted systems. This suggests effective mediation of NVCL polymerization by the CTA, with most chains reacting at similar rates, and can be further evidence that homopolymerization of NVCL, if produced in such experimental conditions, was removed by dialysis. Additionally, an increase in molar mass and grafting efficiency was observed with a higher NVCL monomer concentration, rising from 150 to 300 mol ratio relative to the CTA, with a more pronounced effect in the PULLX-5 sample than in PULLX-7 (APPENDIX I, Table-A I-4).

Table 4.3 Molar masses ($\overline{M}_n$ and $\overline{M}_w$), dispersity, and percentage of PNVCL in the copolymer for pullulan, reduced molar mass pullulan, PULLBr-5, PULLX-5, and PULL-g-PNVCL (150) and PULL-g-PNVCL (300) in DMF/LiBr

Samples	$\overline{M}_n$ (kg/mol) ^a	$\overline{M}_w$ (kg/mol) ^a	$\overline{D}$ ^a	PNVCL in copolymer (%) ^{b*}
Pullulan	96	288	3.01	-----
Reduced molar mass pullulan	14	28	1.96	-----
PULLBr-5	20	45	2.21	-----
PULLX-5	24	41	1.68	-----
PULL-g-PNVCL (150)	30	53	1.78	23
PULL-g-PNVCL (300)	33	57	1.74	35

^a Determined by GPC in DMF/LiBr (0.1% v/v) at 60 °C, ^b PNVCL in copolymer (%) = $(\overline{M}_n_{\text{(PULL-g-PNVCL)}} - \overline{M}_n_{\text{(PULLX)}}) / \overline{M}_n_{\text{(PULLX)}} \times 100$ (Gürdağ & Sarmad, 2013), * The percentage of PNVCL in the copolymers was estimated from the difference in molar masses between the copolymers and PULLX as determined by GPC. Although these values are not absolute, the increases were significant enough to confirm the presence of PNVCL in the copolymer structure.

This trend aligns with prior observations from the ^1H NMR analysis of PULL-g-PNVCL (150) and PULL-g-PNVCL (300). More interestingly, an increase in molar mass from reduced molar mass pullulan to PULLBr-5 and PULLX-5 samples indicates successful grafting of bromopropionyl and CTA groups side chains, consistent with the ^1H NMR and ATR-FTIR analyses. This trend is consistent with other studies on polysaccharide modifications (e.g., pullulan and dextran), which report stepwise increases in the molar mass following small organic modifications (e.g., acetyl chloride and bromoacetyl bromide) (Karmakar & Pal, 2021; Teramoto & Shibata, 2006). Although isolated yields of modified pullulan (PULLBr-5, PULLX-5, PULL-g-PNVCL (150), and PULL-g-PNVCL (300)) were in the 37-73% range (Table-A I-5), we were delighted to obtain the PULL-g-PNVCL (300) copolymer in appreciable quantities to evaluate its physical and chemical properties as well as compare its temperature sensitivity to PULL-g-PNVCL (150). However, analysis of monomer conversion during the polymerization process will be required to properly evaluate reaction efficiency and support the synthetic methodology.

4.2.5 Thermal Analyses

Figure 4.11 (a) presents the thermograms obtained by DSC for pullulan, reduced molar mass pullulan, PULLX-5, and PULL-g-PNVCL (300), which reveal distinct thermal behaviors for each sample. As observed in other studies, it was not possible to determine the glass transition temperature (T_g) of pullulan (Carvalho et al., 2020; Teramoto & Shibata, 2006). Determining transition temperatures for polysaccharides sourced from industry and nature is often challenging due to their high molar mass distribution. This lack of a distinct T_g is also observed in cellulose (Carvalho et al., 2020; Wang, Jiang, He, & Kang, 2017). Regardless of this inherent behavior for polysaccharides, the T_g for reduced molar mass pullulan, PULLX-5, and PULL-g-PNVCL (300) are listed in Table 4.4. The noticeable T_g for all pullulan-derivatives also provided evidence of the successful synthetic sequence. The T_g of PULLX-5 is 233 °C, and the T_g of PULL-g-PNVCL (300) is 189 °C. This decrease can be explained by the branched structure of PNVCL, which may diminish intermolecular interactions and enhance chain

mobility. A similar trend was reported by T. Carvalho et al. (Carvalho et al., 2022) for pullulan-g-poly(Z-L-lysine).

The TGA results for pullulan, reduced molar mass pullulan, PULLX-5, and PULL-g-PNVCL (300) are presented in Figure 4.11 (b). All samples displayed multistep weight loss during thermal decomposition. The initial weight loss, occurring between 30 °C and 150 °C, is attributed to the loss of water (Constantin, Mihalcea, Oanea, Harabagiu, & Fundueanu, 2011; Fernández-Quiroz et al., 2015). The main weight loss observed between 200 °C and 300 °C corresponds to the degradation of the biopolymers (Constantin et al., 2011; Fernández-Quiroz et al., 2015; Singh, Saini, & Kennedy, 2021). As shown in Table 4.4, pullulan exhibits an initial degradation temperature (T_{onset}) of 288 °C, higher than that of reduced molar mass pullulan and the modified pullulan samples, indicating superior thermal stability. The T_{onset} for reduced molar mass pullulan, PULLX-5, and PULL-g-PNVCL (300) decreased to 281, 261, and 251 °C, respectively. This decrease may be linked to diminished cooperative hydrogen bonding along the pullulan backbone due to PNVCL grafting. Furthermore, similar trends in the reduction of the temperature of maximum degradation rate (T_{max}) for reduced molar mass pullulan, PULLX-5, and PULL-g-PNVCL (300) compared to pullulan are shown in Table 4.4. These findings align with Fernández-Quiroz et al. (Fernández-Quiroz et al., 2015), who reported reduced thermal stability after grafting PNVCL onto chitosan, and Omar-Aziz et al. (Omar-Aziz et al., 2020b), who observed similar effects when modifying PULL with octenyl succinic anhydride. As illustrated in Figure 4.10 (b), the residue for final pullulan, reduced molar mass pullulan, and PULLX-5 ranged between 7% and 10%, whereas the ultimate carbon residue for the copolymer sample was approximately 18%. This increase in carbon residue for PULL-g-PNVCL (300) can be mainly attributed to the higher carbon content ratio of the copolymer compared to that of the PULLX-5, reduced molar mass pullulan, and pullulan alone. This provides additional evidence of the successful incorporation of PNVCL onto the pullulan chain.

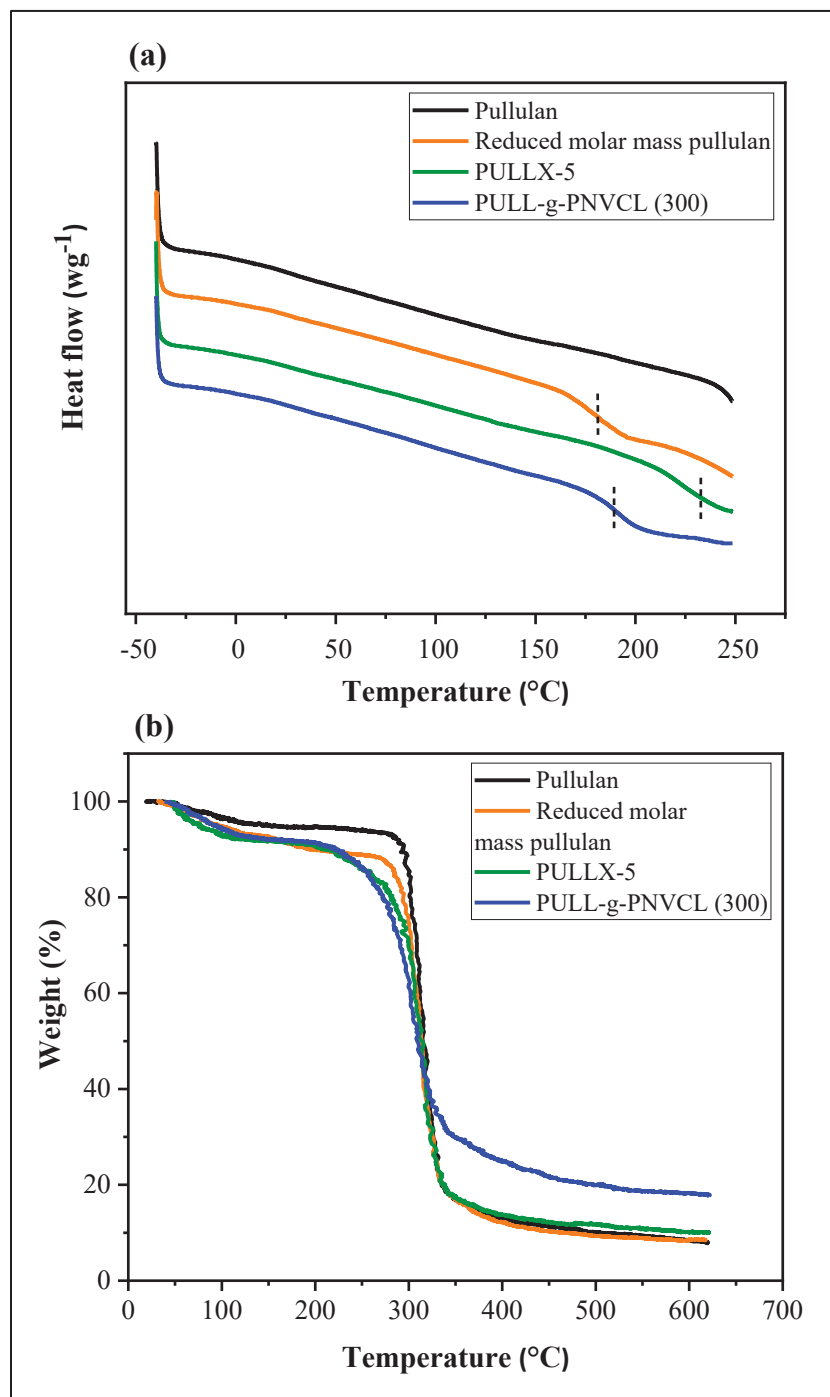


Figure 4.11 (a) DSC, and (b) TGA thermograms of pullulan, reduced molar mass pullulan, PULLX-5, and PULL-g-PNVCL (300)

Table 4.4 Glass transition temperature (T_g), the temperature of initial degradation (T_{onset}), the temperature of maximum degradation rate (T_{max}), and the percentage of residue for pullulan, reduced molar mass pullulan, PULLX-5, and PULL-g-PNVCL (300)

Sample	T_g (C)	T_{onset} (C)	T_{max} (C)	Residue (%)
Pullulan	-----	288	316	8
Reduced molar mass pullulan	181	281	313	9
PULLX-5	233	261	310	10
PULL-g-PNVCL (300)	189	251	303	18

4.2.6 Particle Size Characterization and Temperature-Responsive Phase Transition Behavior of PULL-g-PNVCL

To evaluate the effect of copolymerization on the average particle size distribution, PULL-g-PNVCL (150) and PULL-g-PNVCL (300) copolymer particles were prepared via a self-assembly process and were analyzed by dynamic light scattering (DLS) at 24 °C. Figures 4.12 (a) and (b) show the hydrodynamic average diameter (Z_{av}) of the particles. The Z_{av} of PULL-g-PNVCL (150) and PULL-g-PNVCL (300) particles (1 mg/mL) was approximately $(10.1 \pm 0.9) \times 10^2$ nm and $(14.7 \pm 0.8) \times 10^2$ nm, respectively. Both particles exhibited a bimodal size distribution with PDI values of around 0.7. As expected (B. Jiang et al., 2014), larger particle sizes were observed for the PULL-g-PNVCL (300) sample compared to PULL-g-PNVCL (150). This increase in size in DLS measurements, performed at a temperature below the LCST of PNVCL, indicates that increasing hydrophilic chain length in PULL-g-PNVCL (300) leads to larger sizes due to the extended hydrated layer around the particles, which leads to greater hydrodynamic diameter.

Subsequently, the temperature-responsive behavior of the PULL-g-PNVCL (150) and PULL-g-PNVCL (300) particles was investigated by DLS by increasing the temperature from 20 °C to 45 °C. The effect of temperature on the hydrodynamic diameter of the particles in aqueous dispersion is depicted in Figure 4.12 (c). At 24 °C, the hydrodynamic diameters of the particles were around $(10.1 \pm 0.9) \times 10^2$ nm and $(14.7 \pm 0.8) \times 10^2$ nm for PULL-g-PNVCL (150) and PULL-g-PNVCL (300), respectively. However, as the temperature reached the

LCST, particle aggregation occurred, leading to the formation of large flocculates, characterized by an abrupt increase in hydrodynamic diameter and a dramatic rise in the turbidity of the dispersion, as illustrated in Figure 4.13. The determined LCST values for PULL-g-PNVCL (150) and PULL-g-PNVCL (300) were 30 °C and 34 °C, respectively, which aligns well with the range (27 °C – 42 °C) reported for various PNVCL copolymers (X. Jiang, Li, Lu, & Huang, 2013; Pal et al., 2018; Ponce-Vargas, Cortez-Lemus, & Licea-Claverie, 2013). The LCST values for the copolymers are summarized in Table 4.5. At their respective LCST, the hydrodynamic diameters of the PULL-g-PNVCL (150) and PULL-g-PNVCL (300) particles reached approximately $(14 \pm 0.2) \times 10^2$ nm and $(26 \pm 2) \times 10^2$ nm, respectively. Then, by increasing the temperature past their LCST, the particle size began to slightly decrease, which may be attributed to the further expulsion of water molecules within the flocculates. Such behavior has been reported for PNVCL or PNIPAM homo- and copolymers (Cortez-Lemus & Licea-Claverie, 2016; Dong, Luo, Li, Chi, & Zhang, 2018; Hou & Wu, 2015). The lower LCST value for PULL-g-PNVCL (150) compared to PULL-g-PNVCL (300) can be attributed to the less pronounced interactions with water due to shorter PNVCL chains, thereby lowering the energy required for phase transition. Additionally, the smaller hydrophilic domains in PULL-g-PNVCL (150) resulted in less aggregation, leading to a moderate increase in size compared to PULL-g-PNVCL (300), which showed a more pronounced hydrophilic to hydrophobic transition and larger aggregation. The average particle size distributions of PULL-g-PNVCL (150) and PULL-g-PNVCL (300) at their respective LCST are shown as dotted lines in Figures 12 (a) and (b).

At their respective LCST, the PDI of the particles increased to 1, as anticipated. The particle flocculation observed at the LCST likely contributed to the increased dispersity of the particles.

Table 4.5 LCST values of the synthesized PULL-g-PNVCL samples

Copolymer	LCST (°C)
PULL-g-PNVCL (150)	30
PULL-g-PNVCL (300)	34

* Although turbidity measurements are commonly used to determine the LCST, DLS was selected as the most suitable method for the present system.

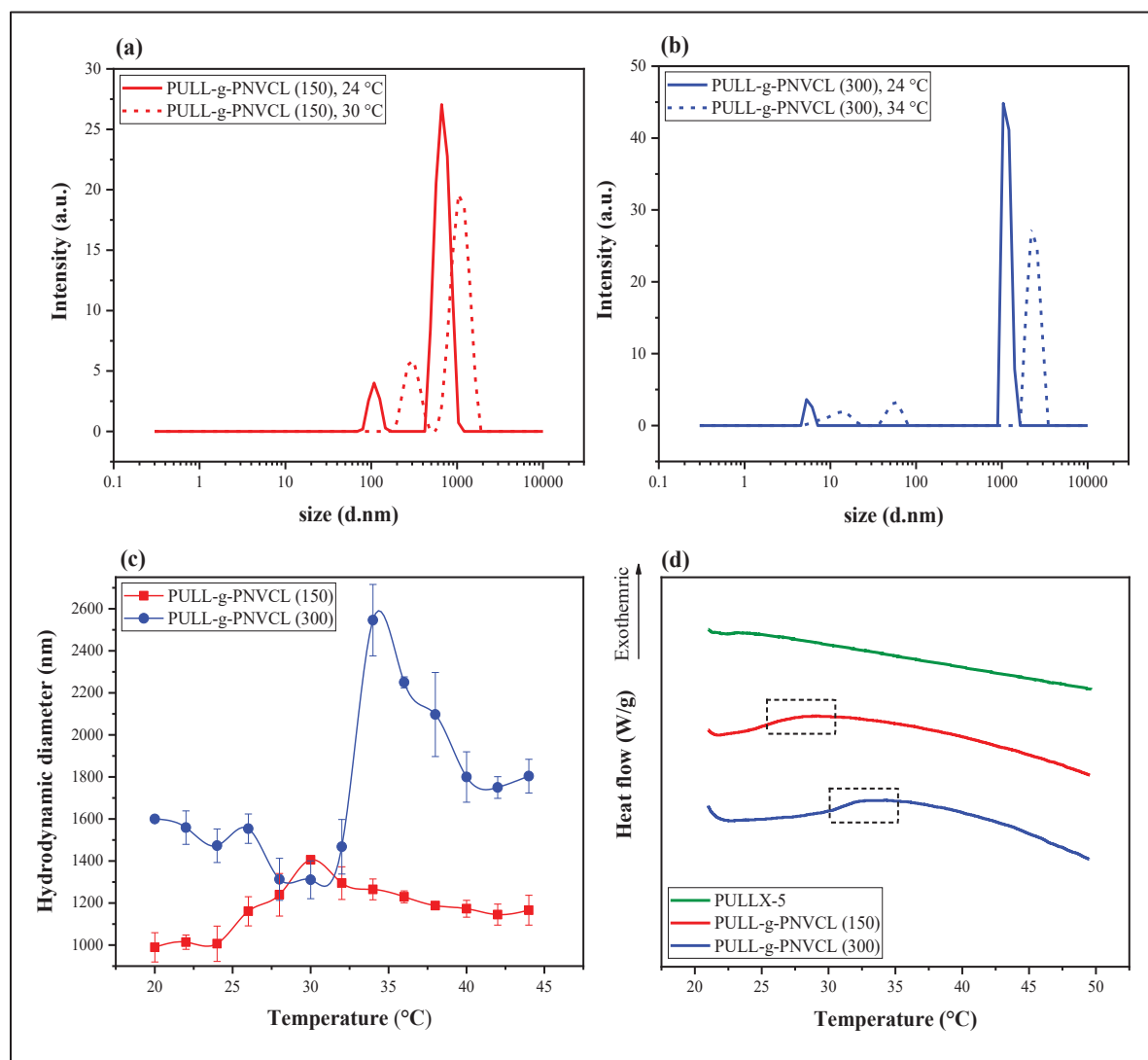


Figure 4.12 DLS analyses: (a) size distribution of PULL-g-PNVCL (150) particles at 1 mg/mL at selected temperatures of 24 °C and 30 °C; (b) size distribution of PULL-g-PNVCL (300) particles at 1 mg/mL at selected temperatures of 24 °C and 34 °C; (c) hydrodynamic diameters as a function of temperature for the PULL-g-PNVCL (150) and PULL-g-PNVCL (300) particles at 1 mg/mL; (d) DSC thermograms of PULLX-5, PULL-g-PNVCL (150), and PULL-g-PNVCL (300) aqueous dispersions

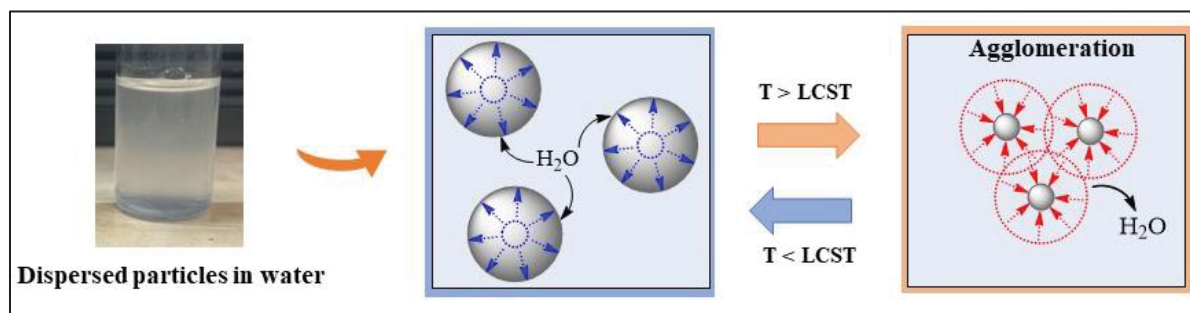


Figure 4.13 Schematic representation of temperature-responsive behavior and particle aggregation

To confirm the reversible thermal phase transition of both PULL-g-PNVCL (150) and PULL-g-PNVCL (300) particles, DLS analyses with heating/cooling cycles were conducted. Figures 4.14 (a) and (b) display the hydrodynamic diameter of the particle dispersions, transitioning between temperatures below and above or close to the LCST. The hydrodynamic diameter increased at a higher temperature, returning to nearly the initial value upon cooling, as reported in similar studies (Akiyoshi et al., 2000; Etchenausia, Khoukh, Deniau Lejeune, & Save, 2017; X. Jiang et al., 2013; Moraes et al., 2020; X. Zhao et al., 2015).

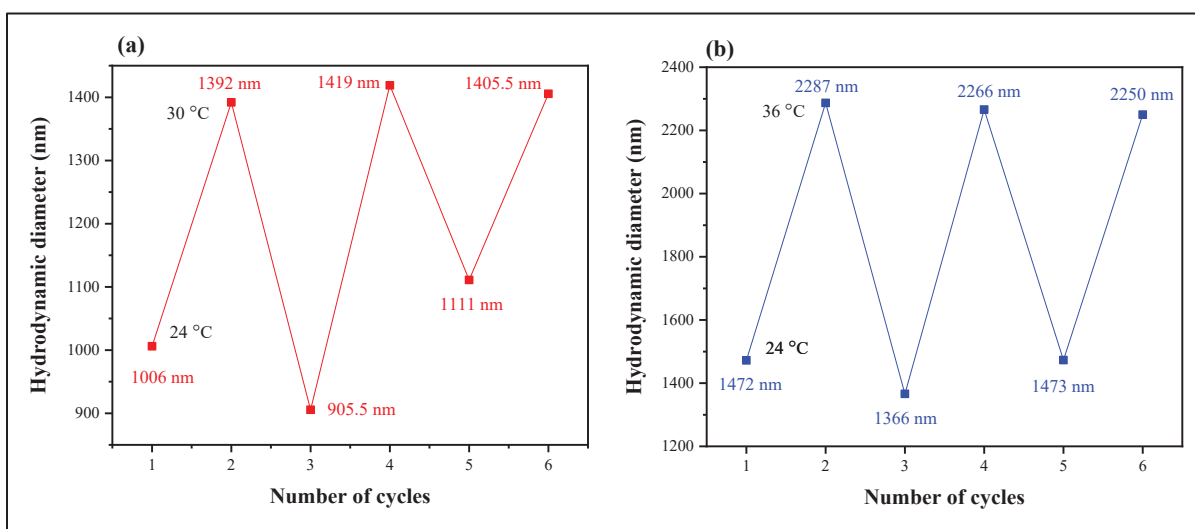


Figure 4.14 (a) Hydrodynamic diameter of PULL-g-PNVCL (150) particles at 1 mg/mL as a function of temperature cycling between 24 °C and 30 °C, and (b) hydrodynamic diameter of PULL-g-PNVCL (300) particles at 1 mg/mL as a function of temperature cycling between 24 °C and 36 °C

To further validate the LCST values obtained, DSC analyses were performed on PULLX-5, PULL-g-PNVCL (150), and PULL-g-PNVCL (300) samples. Figure 4.12 (d) illustrates the thermograms of these samples. A change in heat capacity with temperature for the PULL-g-PNVCL (150) and PULL-g-PNVCL (300) compared to PULLX-5 was observed. This can be due to phase separation, resulting from the collapse of the PNVCL chains, during the transition temperature. The obtained phase transition temperature range of 25 °C - 30 °C for PULL-g-PNVCL (150) and 30 °C - 35 °C for PULL-g-PNVCL (300) confirms that the LCST values obtained from DLS analysis fall within this range. Notably, a shift toward lower temperatures in the phase transition range for PULL-g-PNVCL (150) was observed, which is consistent with the previous results and with reduced incorporation of hydrophilic PNVCL chains in PULL-g-PNVCL (150) compared to PULL-g-PNVCL (300). The cooling thermograms (Figure-A I-10) further support the occurrence of a reversible phase transition in both PULL-g-PNVCL (150) and PULL-g-PNVCL (300). These results demonstrate that the phase transition of the copolymers can be modulated within a specific temperature range by adjusting the PNVCL functionalization of the copolymers.

4.3 Conclusion

Following the successful synthesis of PULL-g-PNVCL copolymers with two different PNVCL chain lengths, our findings about the ability to modulate the properties of pullulan enable the development of functional polysaccharide-based materials and future applications such as in biomedical fields and drug delivery systems. Notably, the polydispersity of the PULL-g-PNVCL copolymer is similar to the pullulan-xanthate-based macro-CTA, indicating the high efficiency of the xanthate-based CTA in controlling NVCL polymerization. The grafting of PNVCL impacted the thermal properties of pullulan, leading to a slightly reduced but acceptable thermal stability after copolymerization. Furthermore, dynamic light scattering (DLS) measurements showed that the average diameter of the copolymer particles increased with longer PNVCL chain length. Additionally, the lower critical solution temperature (LCST) decreased with shorter PNVCL chains. This confirms that the thermosensitive behavior of the

synthesized PULL-g-PNVCL copolymer is directly linked to the PNVCL content, allowing the LCST to be tailored to specific temperature and application requirements.

In conclusion, this study demonstrates that the successful graft polymerization of NVCL controlled by a PULL-based macro-RAFT chain transfer agent represents a significant advancement. This synthetic route also allows for future investigations into graft copolymerization of other desired monomers from pullulan that may lead to a wider range of modulation of physical and chemical properties. Specifically, the ability of the synthesized PULL-g-PNVCL to form particles opens possibilities for encapsulation and controlled release of drugs or active substances for the biomedical industry.

This research broadens the applications of pullulan and opens avenues for designing sustainable, high-value bio-based materials. More broadly, this study advances bio-based materials research by demonstrating an approach to upgrade renewable carbohydrate resources into higher-value functional polymers. Accordingly, the developed grafting platform offers a modular foundation that can be adapted in future studies toward industrial, product-oriented applications. Therefore, the procedures established in this chapter will be applied in the following chapter to polymerize acrylic acid from the pullulan backbone to access a material with pH-responsive behavior.

CHAPTER 5

SYNTHESIS AND CHARACTERIZATION OF PULLULAN-g-POLY (ACRYLIC ACID) VIA RAFT/MADIX POLYMERIZATION

5.1 Introduction

This chapter addresses the second sub-objective of this thesis: Extending the grafting from RAFT/MADIX approach towards pullulan-g-poly(acrylic acid) (PULL-g-PAA) copolymers and conducting a detailed analysis of its structure.

As described in the literature review (Chapter 1), several studies have explored acrylic acid polymerization using xanthate-based chain transfer agents (Sütekin & Güven, 2018; Taton et al., 2006; Taton, Wilczewska, & Destarac, 2001). Building on Chapter 4 and in light of their results, we envisioned that pullulan-xanthate macro chain-transfer agent (PULLX) could engage in RAFT polymerization of acrylic acid (AA). Although xanthate-based chain transfer agents are not the preferred choice for acrylic acid, we decided to evaluate them within a pullulan-anchored macro-chain transfer agent framework. This can help to test whether the grafting platform established in Chapter 4 can be generalized to other monomers, isolating the effect of monomer chemistry while holding the macro-chain transfer agent architecture constant. A systematic evaluation of the final materials via ^1H NMR, DOSY NMR, cleavage of PAA from the PULL-g-PAA, and complementary GPC can reveal to what extent xanthate-mediated grafting is feasible.

This chapter presents a comprehensive analysis of the synthesis of PULL-g-PAA copolymers. The complete synthetic route is shown in Figure 5.1. The workflow proceeds through polymerization of AA from the resulting macro-RAFT agent PULLX. The goal is not only to report the analysis, but to shed light on how far a pullulan-xanthate-based RAFT/MADIX strategy can be exploited toward poly(acrylic acid) (PAA) grafting.

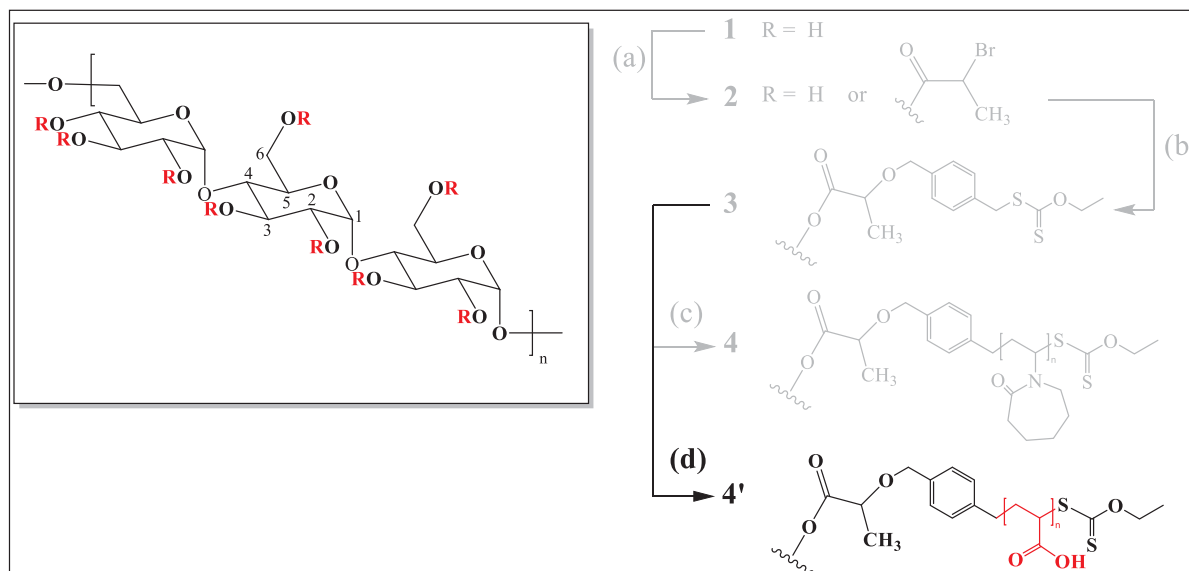


Figure 5.1 Synthetic route to PULL-g-PAA, (d) AA, DMSO, AIBN, 70 °C, 6 h. Earlier steps in grey are described in Chapter 4, and step (d) highlights the acrylic acid polymerization discussed in this chapter

5.2 Results and Discussion

5.2.1 Bromide-Bearing Pullulan and Pullulan-Xanthate-Based Macro-Chain Transfer Agent Characterization

Bromide-bearing pullulan (PULLBr-5) was prepared again from the reduced molar mass pullulan described in Chapter 4, using the acylation condition (2-bromopropionyl bromide, BPB, 5 molar ratio relative to a pullulan monomer unit), which had shown superior performance. Successful bromopropionyl functionalization was confirmed by ^1H NMR (new resonances at 0.94–2.00 ppm, Figure 5.2) and by ATR-FTIR ($\text{C}=\text{O}$ stretch at 1742 cm^{-1} , APPENDIX II). The CTA-OH was resynthesized and attached to PULLBr-5 to afford PULLX-5; ^1H NMR aromatic signals at 7.20–7.38 ppm verified CTA incorporation (Figure 5.2). The degree of substitution (DS) of these samples was determined as detailed in APPENDIX II.

The DS values obtained here, alongside those reported in Chapter 4 for the larger scale synthesis, are summarized in Table 5.1. The close agreement between the values demonstrates the reproducibility of the synthesis route.

Table 5.1 DS values reported in Chapter 4 versus Chapter 5

Sample	DS values in Chapter 4 per 100 monomer pullulan units	DS values in Chapter 5 per 100 monomer pullulan units
PULLBr-5	82	91
PULLX-5	3	2.2

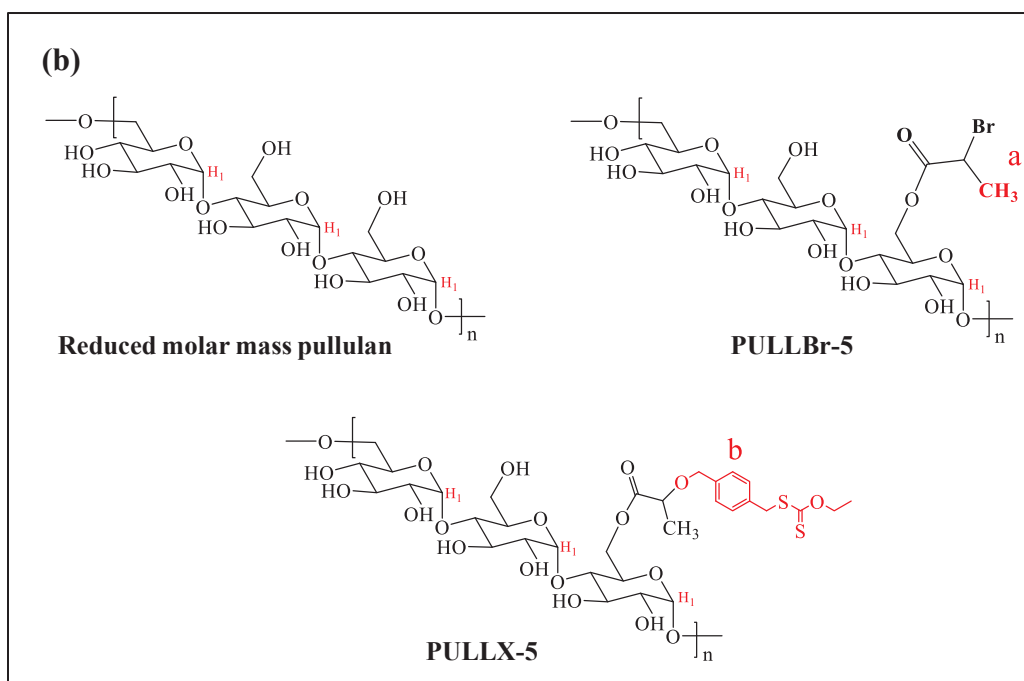
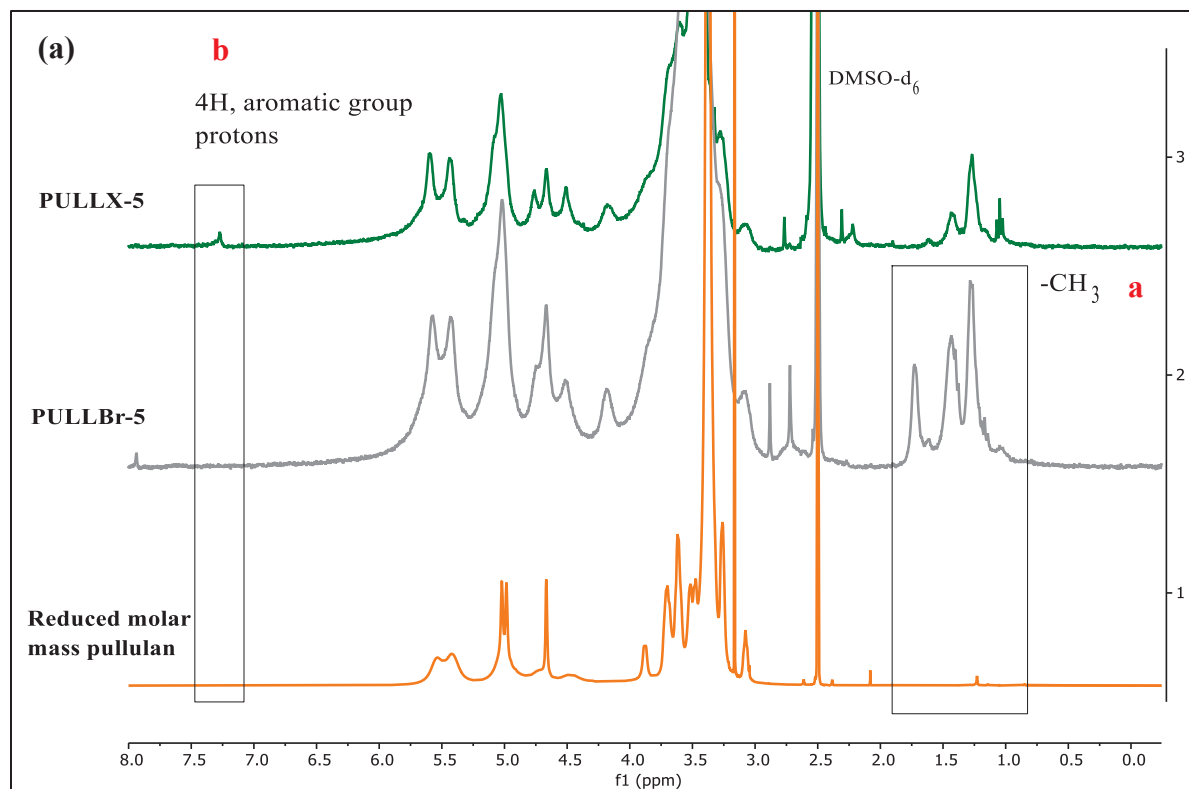


Figure 5.2 ^1H NMR spectra of reduced molar mass pullulan, PULLBr-5, and PULLX-5 in DMSO- d_6

5.2.2 PULL-g-PAA Characterization

5.2.2.1 ^1H NMR Analysis

Figure 5.3 presents the ^1H NMR spectra of PULL-g-PAA (150) and PULL-g-PAA (300) samples recorded in D_2O . The spectra display the characteristic peaks of poly (acrylic acid) at 1.30–2.13 ppm (2H, $-\text{CH}_2-$) and 2.21–2.65 ppm (1H, $-\text{CH}-$), along with the typical signals corresponding to the maltotriose units of pullulan in both samples. Notably, a higher integral intensity of the PAA resonances was observed when the AA concentration in the feed was increased. We initially hypothesized that this was due to the formation of longer PAA chains in the PULL-g-PAA (300) sample. However, as AA is very reactive in polymerization, we were concerned that homopolymerization towards PAA might be a preferred reaction pathway rather than grafting from pullulan through RAFT/MADIX polymerization (Figure 5.4).

The ^1H NMR analysis revealed that the PAA proton signals for PULL-g-PAA (300) exhibited an integration value of 5.647 (2.21–2.65 ppm, b), whereas PULL-g-PAA (150) showed 0.789 (2.21–2.65 ppm, b). These integrations were calculated using the anomeric proton resonances of pullulan as integral reference peaks. It should be noted that the 2.21–2.65 ppm region was selected for integration-based quantification because it is not overlapped by signals from other structural components of the PULLX-5 backbone.

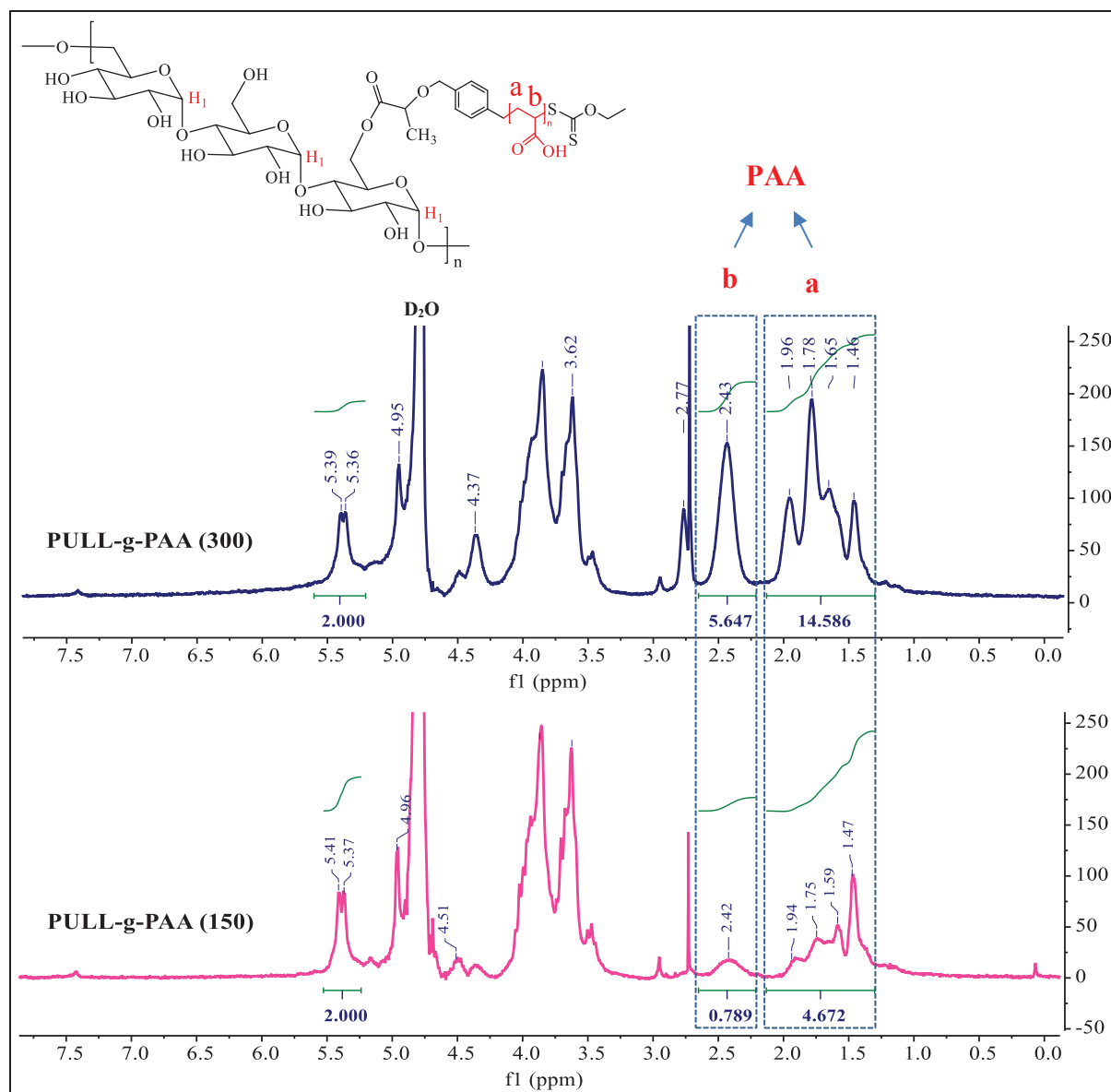


Figure 5.3 ^1H NMR spectra of PULL-g-PAA (150) and PULL-g-PAA (300) in D_2O ; Hydrogen 'b' lies closer to electron-withdrawing groups and is therefore deshielded, giving a peak at a higher ppm

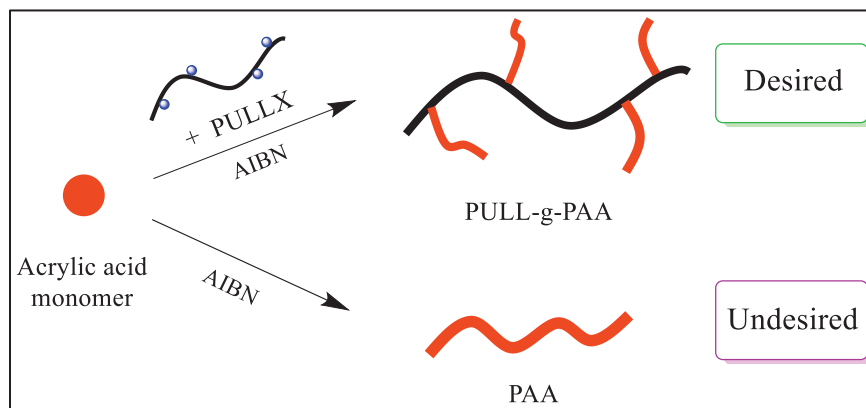


Figure 5.4 Proposed reaction pathways for graft polymerization of acrylic acid using PULLX

5.2.2.2 GPC Analysis

GPC analyses of PULLX-5 and PULL-g-PAA (150) were performed to further assess acrylic acid polymerization from the pullulan backbone. Figure 5.5 shows the GPC chromatograms of PULLX-5 and PULL-g-PAA (150) in DMF/LiBr. The PULL-g-PAA (300) sample didn't dissolve in the solvent for GPC (DMF/LiBr) analysis; consequently, it could not be analyzed by GPC under these conditions. Figure 5.5 (a) indicates that the GPC chromatogram for PULL-g-PAA (150) shifted to a lower retention time relative to PULLX-5. Moreover, Figure 5.5 (b) shows the deconvolution of this chromatogram and demonstrates two populations for this sample. In addition, the corresponding molar masses and dispersities for PULLX-5 and PULL-g-PAA (150) are summarized in Table 5.2. Although the number average and weight average molar masses of PULL-g-PAA (150) exceed those of PULLX-5, the non-unimodal, asymmetric chromatogram of PULL-g-PAA (150), and its deconvolution, suggested the possible presence of PAA homopolymer in addition to grafted PAA on the pullulan backbone.

Table 5.2 Molar masses ($\overline{M}_n$ and $\overline{M}_w$) and dispersity for PULLX-5, and PULL-g-PAA (150) in DMF/LiBr (0.1% v/v)

Samples	$\overline{M}_n$ (kg/mol)	$\overline{M}_w$ (kg/mol)	$\overline{D}$
PULLX-5	20	45	2.2
PULL-g-PAA (150)	32	71	2.2

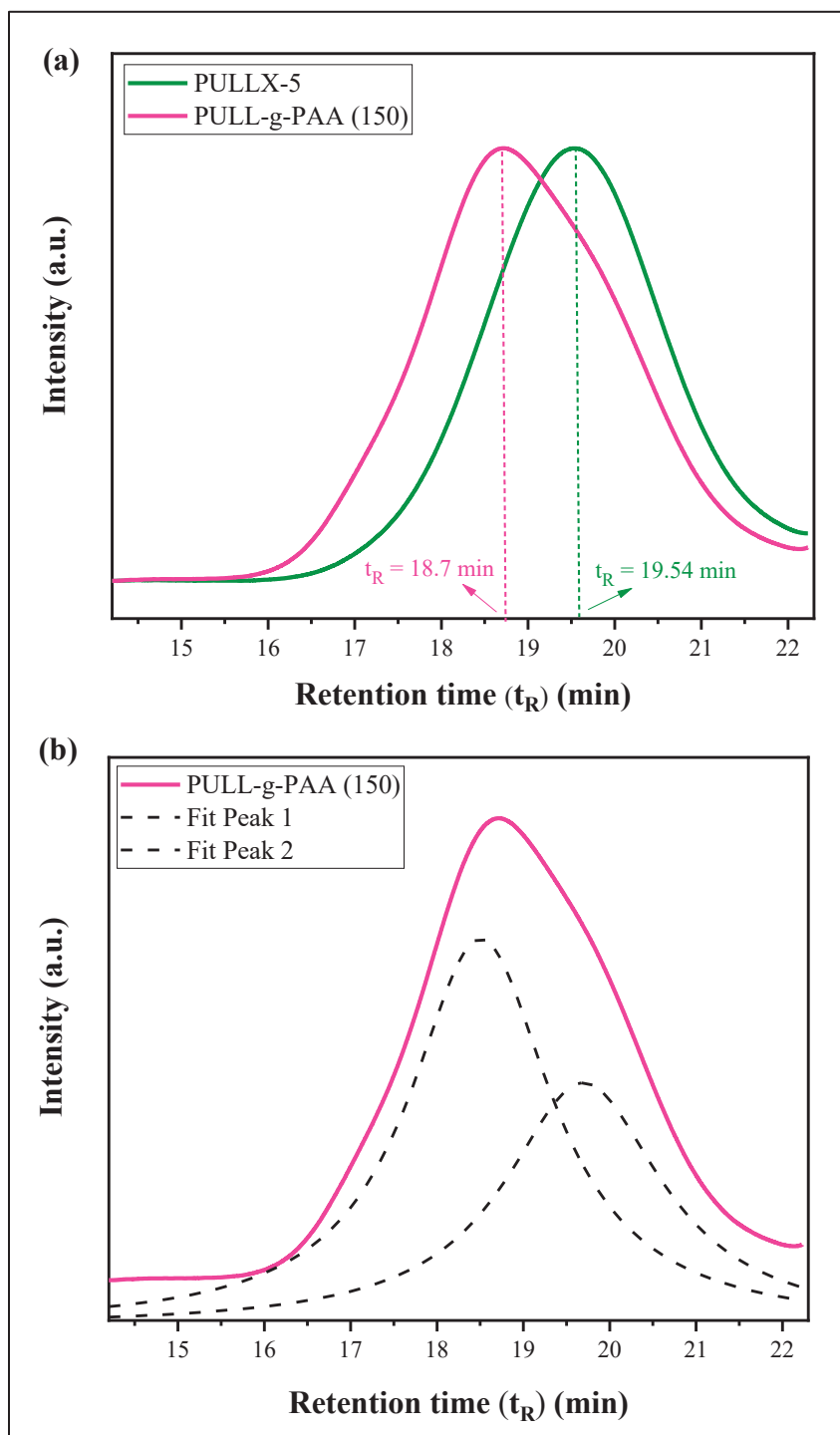


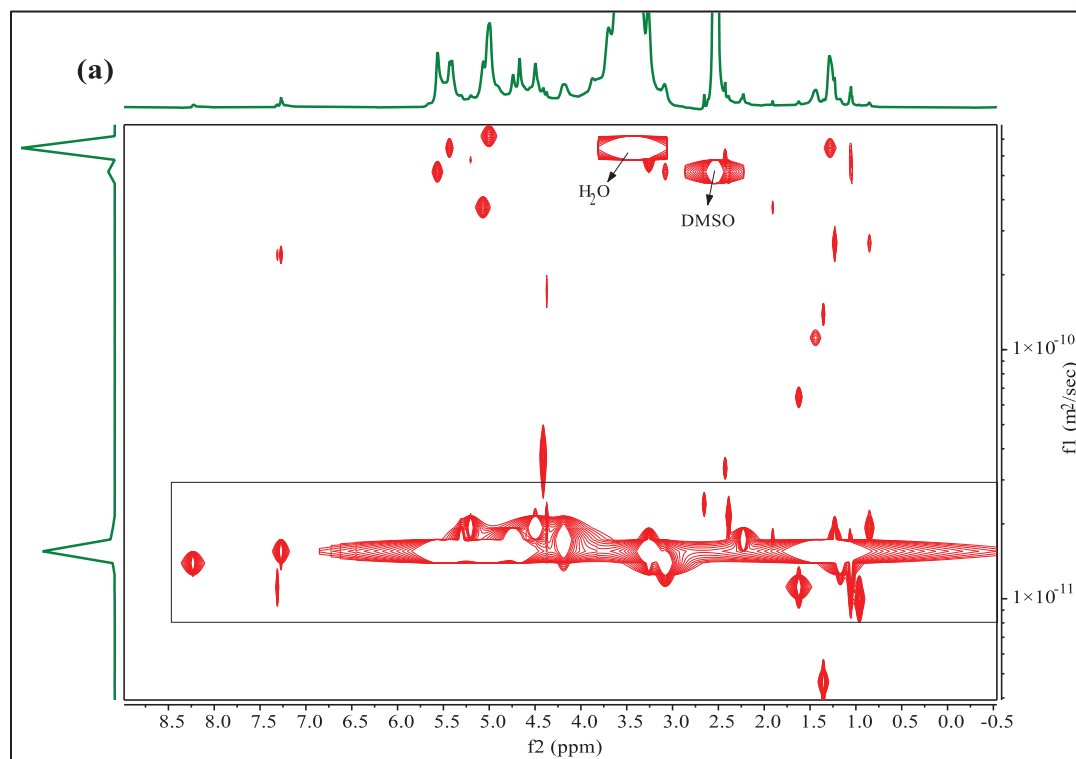
Figure 5.5 (a) GPC chromatograms of PULLX-5 and PULL-g-PAA (150) in DMF/LiBr (b) deconvolution of the PULL-g-PAA (150) chromatogram

5.2.2.3 DOSY NMR Analysis

A DOSY NMR analysis was conducted to verify the successful formation of the PULL-g-PAA copolymer and to elucidate whether the PAA chains were covalently grafted from the PULLX-5 precursor or present as homopolymers. Figure 5.6 displays the DOSY spectra of (a) PULLX-5, (b) PULL-g-PAA (150), and (c) PULL-g-PAA (300).

First, the PULLX-5 was analyzed, and the DOSY spectra revealed that the diffusion coefficient of the signals attributed to the CTA moiety aligned with that of the pullulan backbone, indicating that both components are part of the same molecule. This further confirms the successful functionalization of pullulan by the CTA onto the pullulan backbone. The sample was purified as much as possible without greatly reducing yield, yet a small fraction of unreacted CTA-OH remained after multiple rounds of purification. Both PULL-g-PAA (150) and PULL-g-PAA (300) samples (Figures 5.6 (b) and (c)) contained ungrafted and grafted PAA to the pullulan backbone. This was determined by the alignment of the diffusion coefficients of the PAA and pullulan signals, specifying that both components are part of the same copolymer structure. Due to the presence of homopolymerization products, we could not verify the growth of longer PAA side chains in the PULL-g-PAA (300) sample that was hypothesized in the previous section. This competing undesired reaction, shown in Figure 5.4, decreased the grafting efficiency, and dialysis after polymerization (14 kDa MW cutoff) did not purify the ungrafted PAA species.

In this context, signals exhibiting higher or lower diffusion coefficients than those of pullulan suggest the presence of free, ungrafted PAA chains in the samples. Figure 5.6 (b) presents that the ungrafted PAA chains exhibit higher diffusion coefficients, corresponding to shorter polymer chains, compared to the PULL-g-PAA (150) sample. Meanwhile, Figure 5.6 (c) displays lower diffusion coefficients for the ungrafted PAA, indicating the presence of longer PAA chains compared to the PULL-g-PAA (300) sample.



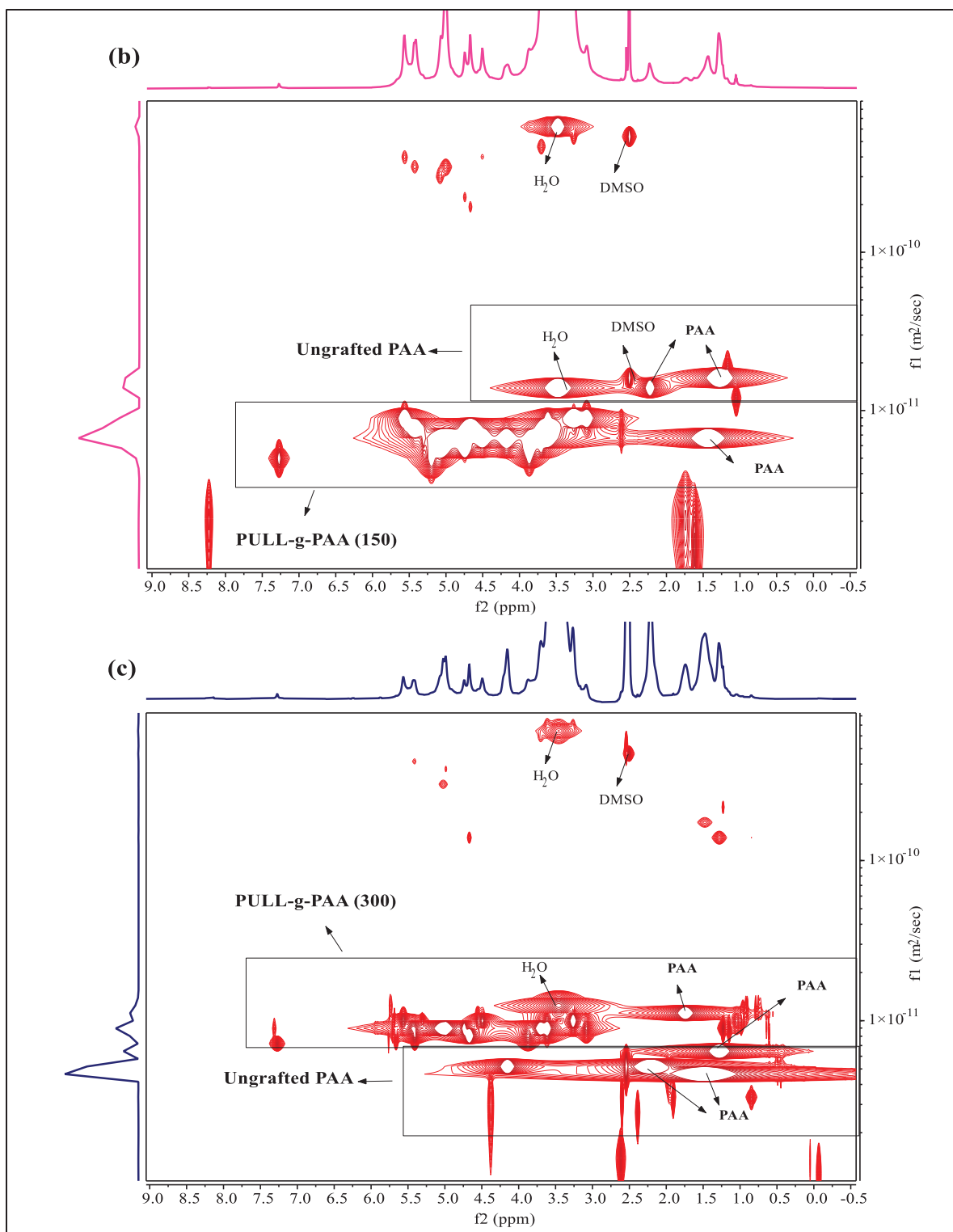


Figure 5.6 DOSY spectra of (a) PULLX-5, (b) PULL-g-PAA (150), and (c) PULL-g-PAA (300) in DMSO- d_6

5.2.2.4 Cleavage of PAA From PULL-g-PAA

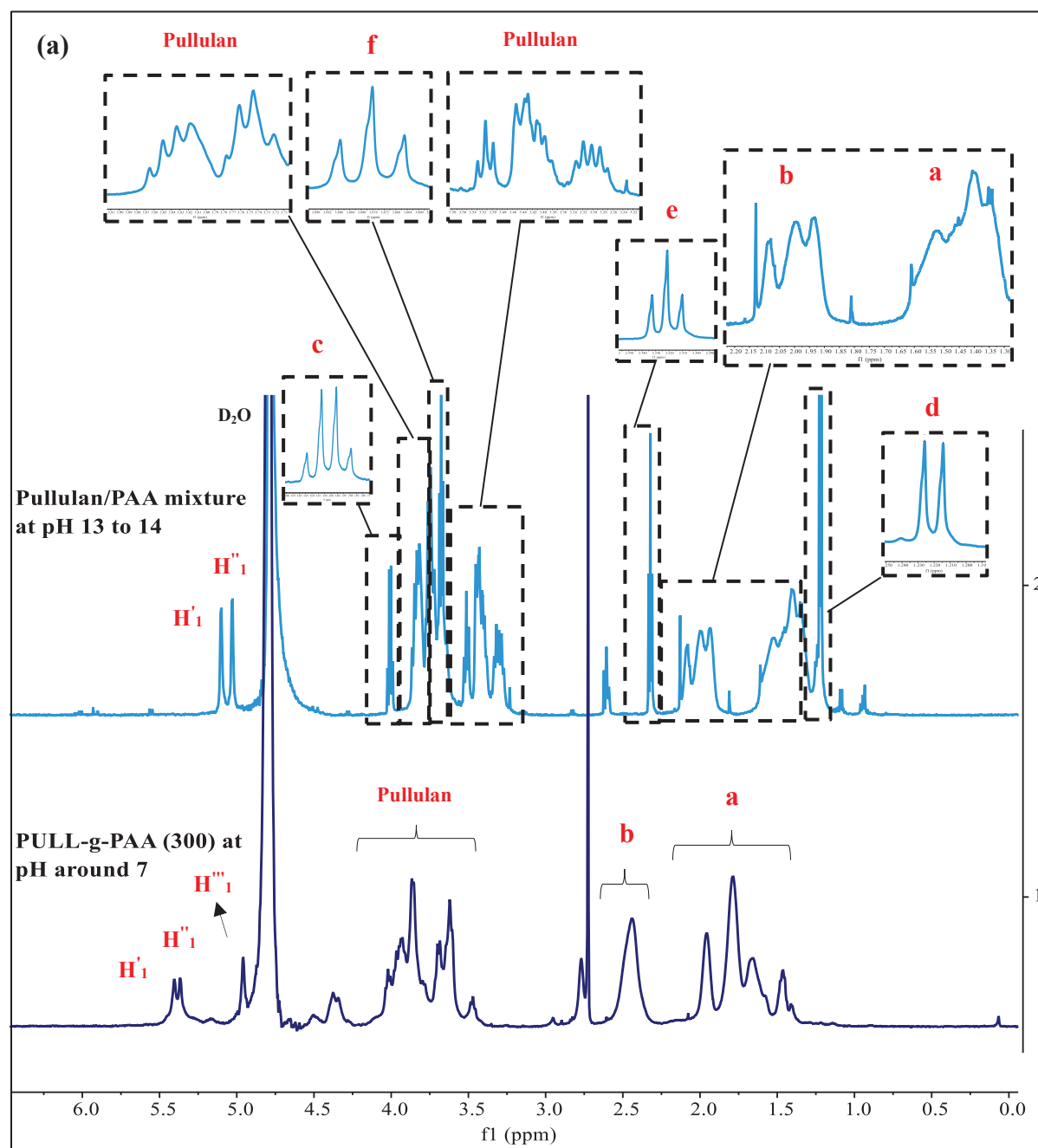
To further investigate the covalent attachment of PAA chains onto the Pullulan backbone, a cleavage experiment was conducted by dissolving PULL-g-PAA (300) in D₂O and adjusting pH to 13-14. This base-catalyzed ester hydrolysis cleaves the ester bond introduced in the first step (functionalization with BPB), recovering non-functionalized pullulan and releasing free PAA chains. The sample was analyzed using ¹H NMR and DOSY. Figure 5.7 (a) shows the ¹H NMR spectra for PULL-g-PAA (300) in D₂O at pH around 7 and PULL-g-PAA (300) in D₂O at pH around 13-14, which indicates a pullulan/PAA mixture. Also, the chemical structures of molecules expected in the PULL-g-PAA (300) sample at both pH levels are shown in Figure 5.7 (b).

For clarity, the key findings are summarized below, as bullet points:

- Comparing the two spectra of PULL-g-PAA (300) showed that the pullulan resonances at pH 13–14 are noticeably sharper than at pH ~ 7 (pullulan peaks outlined in Figure 5.7 (a)). This sharpening is consistent with greater structural uniformity of pullulan after de-functionalization at high pH. In contrast, a more heterogeneous local environment in the functionalized pullulan at neutral pH broadens its resonances. This is attributed to the detachment of the bromopropionyl units and PAA side chains from the PULL-g-PAA (300) sample under strongly basic conditions.
- At pH ~ 7, electron-withdrawing acyl functional groups on the pullulan backbone in PULL-g-PAA (300) cause a downfield (to higher ppm) shift of pullulan resonances compared to respective resonances in the spectrum at pH 13–14 (for example, anomeric protons: 5.06 ppm (pH 13-14) → 5.38 ppm (pH 7)).
- At pH 13–14, the PAA resonances in the pullulan/PAA mixture shift upfield (to lower ppm) relative to those at pH 7. This upfield displacement can be due to the formation

of the carboxylate ($-\text{COO}^-$), which increases shielding (backbone $-\text{CH}$ of PAA, b: 2.43 ppm (pH 7) $\rightarrow$ 2.00 ppm (pH 13-14)).

- Peaks attributable to side-products from bromopropionyl units that were not substituted by CTA-OH are evident and present in relatively significant quantities. The resulting signals are consistent with lactic acid and 3-hydroxypropionic acid (Chemama, Pumas, Tragoolpua, & Thongwai, 2020; Fernández-García, Grefenstette, & Powner, 2017; Kim, Park, Lee, & Son, 2024; Rahmayetty, Sukirno, Prasetya, & Gozan, 2017; Xu et al., 2019). Lactic acid arises from bimolecular nucleophilic substitution (S_{N}^2) displacement of bromide by water, whereas 3-hydroxypropionic acid forms via elimination followed by Michael-type addition reactions. Although these side reactions may occur during other stages of the synthesis, they are more likely to happen under the strongly basic conditions used for side-chain cleavage. The corresponding pathways leading to lactic acid and 3-hydroxypropionic acid at high pH are summarized in Figure 5.8, and these side-reactions that may happen during earlier steps of the synthesis are presented in APPENDIX II (Figure-A II-5). From this perspective, loss of bromide sites may help explain the lower DS achieved for CTA incorporation relative to the number of bromopropionyl groups in both Chapters 4 and 5 (Table 5.1).



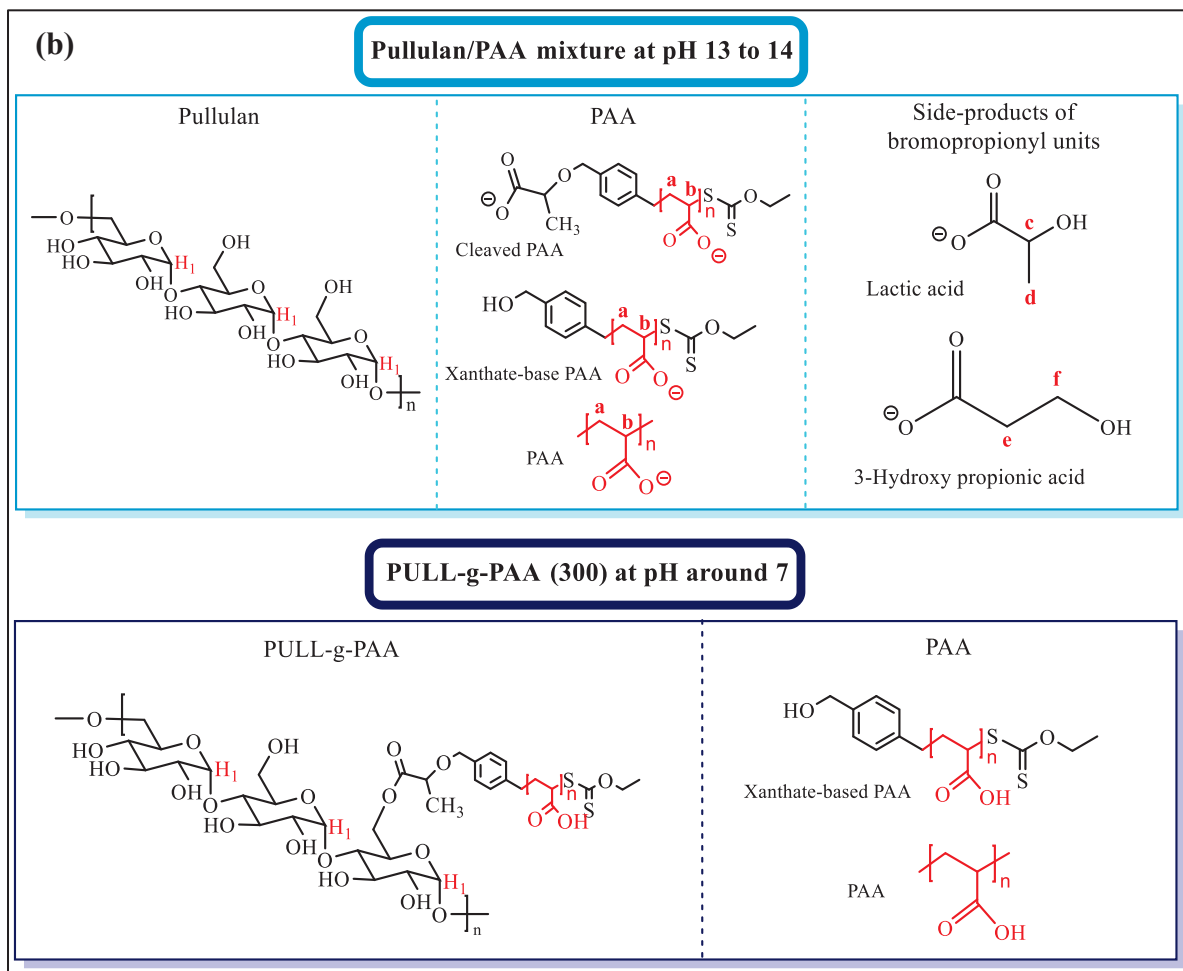


Figure 5.7 (a) ^1H NMR spectra for PULL-g-PAA (300) in D_2O at pH around 7 and pullulan/PAA mixture in D_2O at pH 13-14 (b) Chemical structures of components expected under both pH conditions

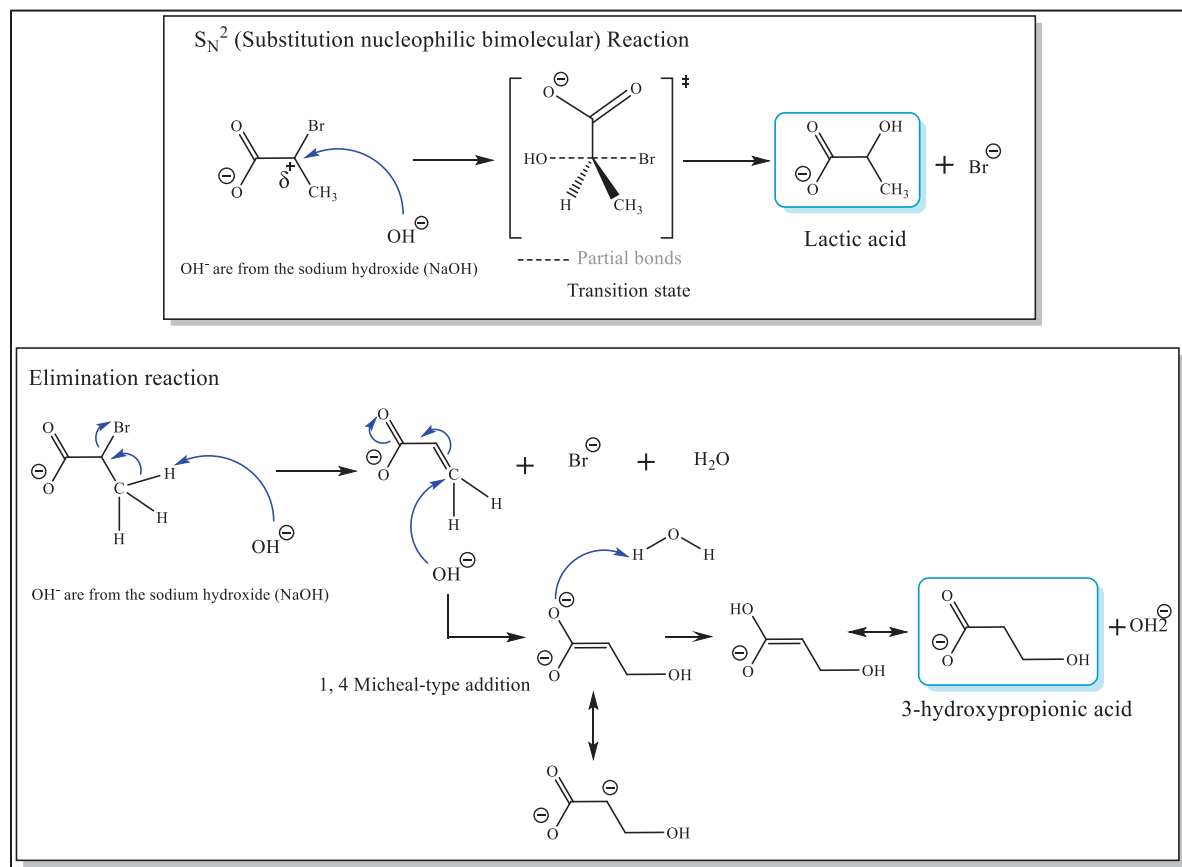


Figure 5.8 Br removal mechanisms to form lactic acid and 3-hydroxypropionic acid at high pH during the side-chain cleavage process

Figure 5.9 (a) and (b) present the DOSY spectra of PULL-g-PAA (300) in D₂O at pH around 7 and pullulan/PAA mixture in D₂O at pH 13-14, respectively. Figure 5.9 (a), similar to Figure 5.6 (c), displays the alignment of diffusion coefficients for PAA and pullulan peaks. This overlap in diffusion coefficients supports the presence of PAA segments grafted from the pullulan backbone. Additionally, signals with diffusion coefficients lower than that of pullulan are consistent with free, ungrafted PAA. As shown in Figure 5.9 (b), cleavage of PAA is confirmed by the absence of signals related to PAA aligned with pullulan. The appearance of PAA resonances at both lower and higher diffusion coefficients than pullulan confirms the separation of the side chains from the grafted copolymer. Therefore, the results confirm that acrylic acid polymerization was partially controlled by PULLX-5, yielding some PAA chains grafted from the pullulan backbone.

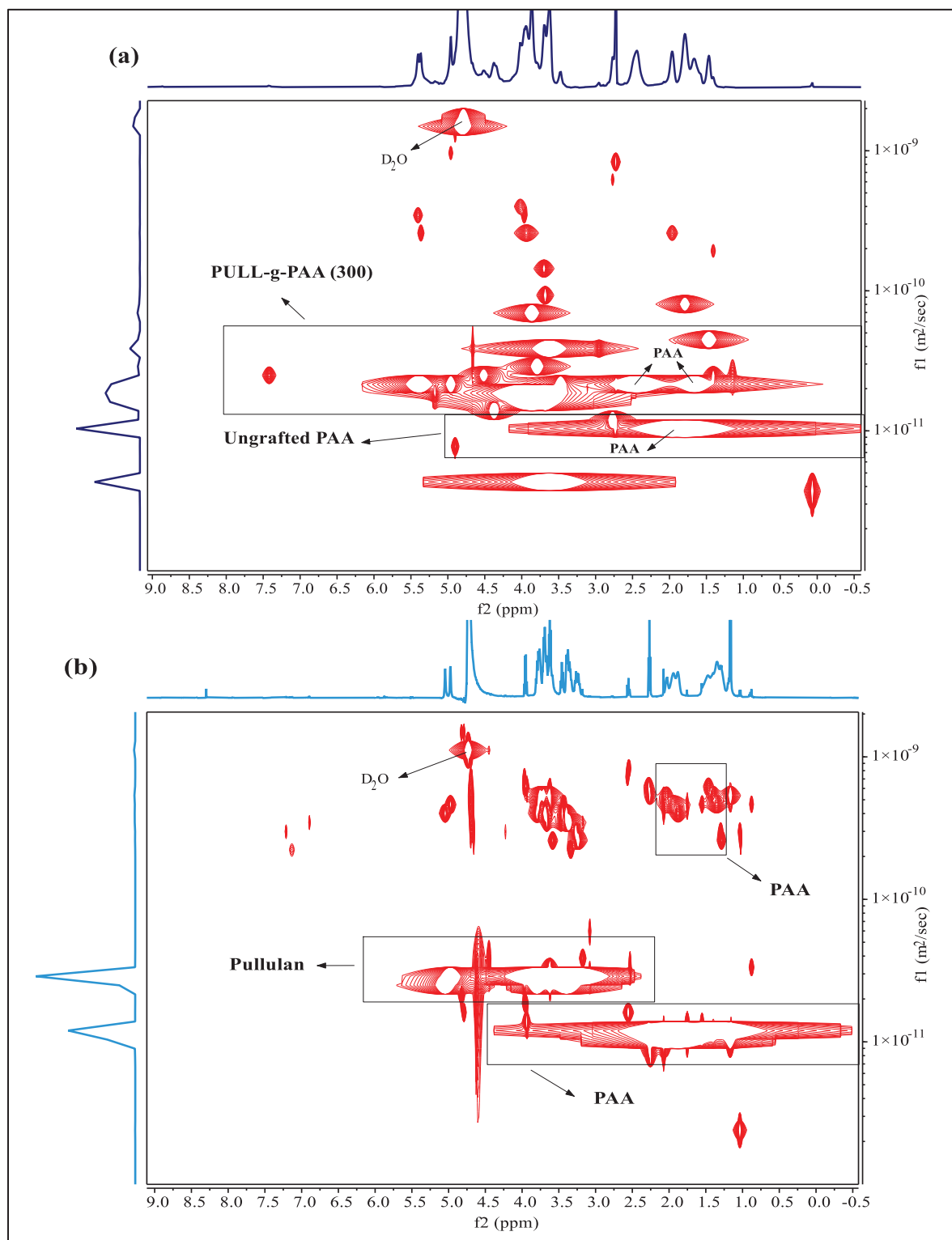


Figure 5.9 DOSY spectra of (a) PULL-g-PAA (300) in D₂O at pH around 7 (b) Pullulan/PAA mixture in D₂O at pH 13-14

5.3 Conclusion

This chapter extended the grafting from RAFT/MADIX platform to acrylic acid (AA) by employing a pullulan-xanthate macro-chain transfer agent (PULLX) and examined to what extent grafting could be achieved. Convergent evidence from ^1H NMR/DOSY, base-catalyzed cleavage, and GPC demonstrated that acrylic acid polymerization from PULLX results in grafted chains along with a significant amount of free poly(acrylic acid) (PAA) chains. In this context, DOSY showed co-diffusion of some PAA and pullulan signals prior to cleavage, and loss of co-diffusion thereafter. Also, GPC of PULL-g-PAA (150) displayed a shift to lower retention time with a relatively bimodal distribution. Collectively, these findings indicate that while PULLX mediates some grafting of acrylic acid, competing homopolymerization represents a major side reaction that greatly reduces the appeal of xanthate-based strategies toward PAA grafting. Given the high reactivity of AA, a fraction of monomer may have polymerized by conventional free radical polymerization before all chain transfer agents (CTAs) attached to the pullulan backbone were fully activated, as RAFT systems typically exhibit an initial activation period.

At the same time, the data revealed some additional limitations. Side products attributable to bromopropionyl units (e.g., lactic acid and 3-hydroxypropionic acid) were detected, highlighting the low levels of CTA substitution and the need for further optimization of the reaction conditions. Therefore, the 2-bromopropionyl bromide (BPB) may not be the most suitable reagent for this synthetic sequence. To mitigate side-products, a reagent that avoids the presence of a proton on neighboring carbons would be preferable. For example, bromoacetyl bromide (Figure 5.10) is a plausible alternative and may also perform better in the subsequent CTA-coupling step due to reducing steric hindrance after generating bromide-bearing pullulan.

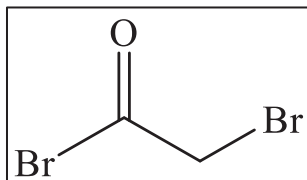


Figure 5.10 Bromoacetyl bromide structure

However, to isolate intrinsic performance, it would be essential to compare the properties of this sample with a pure PULL-g-PAA (300) (without free PAA homopolymers), by replacing the xanthate-based CTA with a trithiocarbonate-based one (Ji, Jia, Yan, & Bangal, 2010; Krivorotova, Jonikaite-Svegziene, Radzevicius, & Makuska, 2014; Ladavière et al., 2001; Llauro et al., 2004; Loiseau et al., 2003).

Overall, the findings of this chapter expose the limits of the xanthate-based platform for polymerizing acidic monomers from a polysaccharide backbone, and further use for the preparation of well-controlled functional PAA-grafted polymers is not recommended.

CHAPTER 6

PULLULAN-ACETATE FIBROUS MEMBRANES: ELECTROSPINNABILITY AND WATER STABILITY

6.1 Introduction

This chapter addresses the third objective of this thesis, which is to conduct a preliminary study aimed at developing water-stable fibrous membranes based on chemically modified pullulan. To this end, pullulan was acetylated using acetic anhydride to obtain pullulan acetate with different degrees of substitution, and the chemical structure of the resulting derivatives was characterized using Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (^1H NMR) spectroscopy.

Electrospinning, which enables the fabrication of micro- to nanoscale fibers with tunable morphology, was selected as the fabrication method. The broad applicability of these fibers in biomedical (drug delivery, wound dressings) and environmental fields (air filtration, water treatment) stems from their distinctive features, notably their high surface area and porosity (Ahmadi Bonakdar & Rodrigue, 2024; Al-Abduljabbar & Farooq, 2023; Borban, Gohain, Yadav, Karki, & Ingole, 2023; Elfawal, Šišková, & Andicsová, 2025; Lim, 2017). In this context, establishing formulations and processing windows that enable the generation of uniform, defect-poor pullulan acetate fibers is a key step toward the practical use of pullulan-based electrospun membranes.

Accordingly, the electrospinnability of pullulan acetate solutions was investigated as a function of solvent composition and processing parameters, with the objective of identifying conditions capable of producing continuous, low-defect fibers. Finally, the water stability and morphological evolution of the electrospun pullulan acetate membranes after contact with water were assessed to determine whether the chemical modification effectively reduces the rapid dissolution typically observed for native pullulan fibers.

On this basis, we hypothesize that the degree of substitution (DS) governs the affinity of pullulan acetate for water, which in turn directly impacts both its processability by electrospinning and the stability of the resulting membranes in aqueous media. A lower DS is expected to preserve a higher polymer-water affinity, leading to swelling and/or partial dissolution of the membranes, whereas increasing DS should reduce water affinity and improve membrane stability. In parallel, it is anticipated that pullulan acetate samples with different DS will require distinct solvent compositions and operating conditions for electrospinning. Furthermore, it is expected that pullulan acetate will not electrospin under the same conditions as native pullulan.

6.2 Results and Discussion

6.2.1 Pullulan-Acetate Characterization

The modification of pullulan with acetic anhydride, as described in Chapter 3 (Methodology), led to the formation of pullulan acetate, hereafter referred to as PULLAc. Acetylation was carried out using three different molar feed ratios of acetic anhydride to one monomer unit of pullulan: 5, 9, and 15. The resulting materials are denoted PULLAc-5, PULLAc-9, and PULLAc-15, respectively. The success of the chemical modification was first assessed by Fourier transform infrared (FTIR) spectroscopy, and the corresponding spectra are shown in Figure 6.1.

Relative to pullulan, all PULLAc samples exhibit a new absorption band at 1739 cm^{-1} , assigned to the carbonyl ($\text{C}=\text{O}$) stretching vibration of the acetyl groups, confirming the formation of acetate functionalities along the pullulan backbone. Additional spectral changes characteristic of acetylation are observed, in particular in the regions around 1370 cm^{-1} (CH_3 bending vibrations), and 1212 cm^{-1} (due to $\text{O}-\text{C}-\text{O}$ stretching vibrations). These observations are consistent with previous works (Hernandez-Tenorio & Giraldo-Estrada, 2022; Jung, Jeong, Kim, & Kim, 2004; Na, Jeong, & Lee, 1997; Niu, Shao, Chen, & Sun, 2019; Teramoto & Shibata, 2006a; Zhang et al., 2009). Another decent indicator is the marked decrease in intensity of the broad hydroxyl ($\text{O}-\text{H}$) stretching band ($\approx 3200\text{--}3600\text{ cm}^{-1}$) in the PULLAc

samples relative to pullulan. Although FTIR was not employed to quantitatively determine the degree of substitution, it provided qualitative evidence of successful acetylation of pullulan.

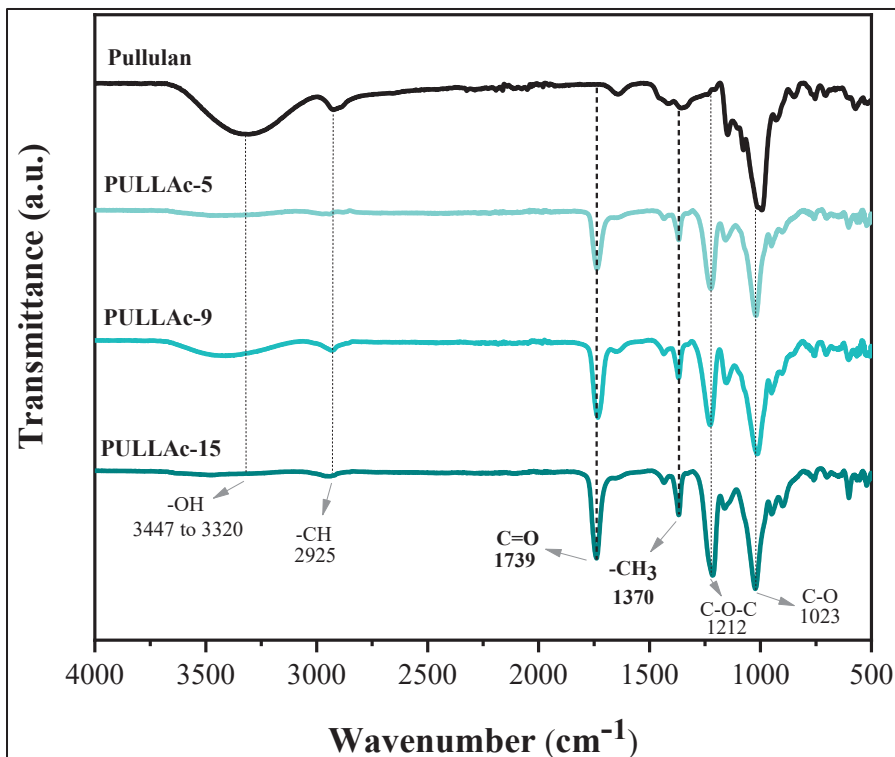


Figure 6.1 FTIR spectra of pullulan and PULLAc-5, PULLAc-9, and PULLAc-15

In order to quantify the degree of substitution (DS) of hydroxyl groups in PULLAc samples, the ^1H NMR analysis was performed. Comparative ^1H NMR spectra of pullulan and PULLAc samples in DMSO-d_6 are shown in Figure 6.2. The peaks arising from the ring protons and hydroxyl groups of pullulan appeared between 3.44 and 6.00 ppm in PULLAc spectra. Furthermore, all PULLAc samples exhibited additional signals between 1.75 and 2.20 ppm, which are attributed to the methyl ($-\text{CH}_3$) protons of the acetate groups (Figure 6.3). The appearance of these methyl resonances, together with the persistence of the polysaccharide backbone signals, confirms the successful introduction of acetyl substituents along the pullulan chain, in line with previous reports (Enomoto-Rogers, Iio, Takemura, & Iwata, 2015b; Hernandez-Tenorio & Giraldo-Estrada, 2022; Niu et al., 2019; Teramoto & Shibata, 2006a; H. Zhang et al., 2009). Additional peaks at approximately 2.54 ppm and 3.38 ppm arise from residual DMSO and water, respectively.

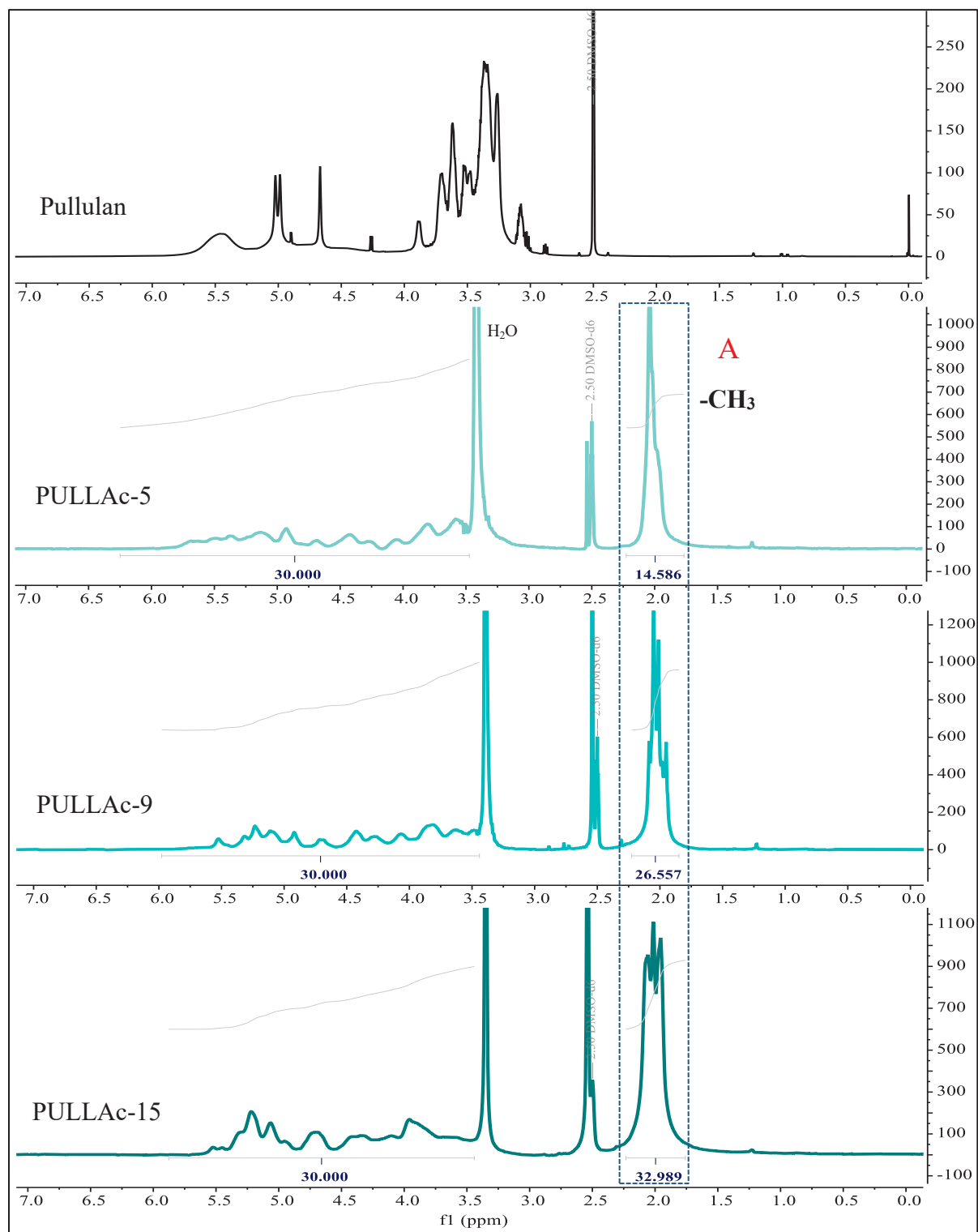


Figure 6.2 ^1H NMR spectra of pullulan and PULLAc-5, PULLAc-9, and PULLAc-15 in DMSO- d_6

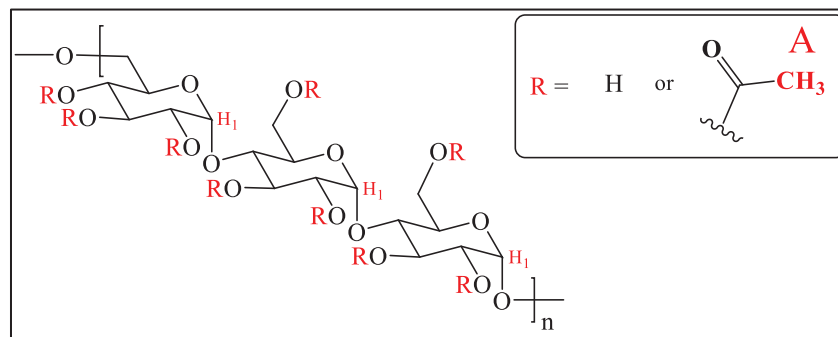


Figure 6.3 Chemical structure of PULLAc

The DS of hydroxyl groups in PULLAc samples was determined from the ^1H NMR spectra using Equation 6.1, adapted from previous reports, with the modification that the total protons of the pullulan monomer unit were considered rather than those of a single glucose unit (Hernandez-Tenorio & Giraldo-Estrada, 2022; Teramoto & Shibata, 2006a; Zhang et al., 2009). In this equation, “A” denotes the integral value of the acetate methyl protons (signals between ~ 1.75 and ~ 2.20 ppm), which is shown as the outlined region in Figure 6.2. Because each acetyl group contributes three protons, “A” corresponds to $(3 \times \text{DS})$ protons per pullulan repeat unit. The factor 30 represents the total number of protons in the native pullulan monomer unit, and acetylation replaces protons; therefore, the remaining number of pullulan protons is $(30 - \text{DS})$, which leads to the proton-balance relationship used in Equation 6.1. All the integral values used for A are indicated in Figure 6.2.

$$\frac{A}{3 \times \text{DS}} = \frac{30}{30 - \text{DS}} \quad (6.1)$$

The DS values of hydroxyl groups in PULLAc samples obtained are summarized in Table 6.1. The measured DS increases with increasing acetic anhydride feed ratio, confirming that higher reagent ratios lead to more acetylation of the pullulan backbone. However, even at the highest molar feed ratio investigated (15 molar ratio of acetic anhydride per pullulan monomer unit), full substitution of all hydroxyl groups was not achieved. This incomplete acetylation is likely related to the non-uniform reactivity of hydroxyl groups along the pullulan backbone, arising

from differences in their local microenvironment (e.g., participation in intramolecular hydrogen bonding). Furthermore, differences in nucleophilicity and steric environment between primary and secondary hydroxyl groups are expected to contribute to their distinct reactivity.

Table 6.1 ^1H NMR results showing the A parameters from Equation 6.1 (integral values) and the calculated DS for each PULLAc sample

Sample	Equation 6.1	~ DS per one pullulan monomer
	A	
PULLAc-5	14.58	4
PULLAc-9	26.55	7
PULLAc-15	32.99	8

Other studies, such as by Teramoto et al. (2006), also synthesized pullulan acetate using comparable initial molar ratios of acetic anhydride to pullulan monomer unit (4.5, 7.5, 10.5, and 12), obtaining degrees of substitution of 3.0, 5.1, 7.2, and 9.0, respectively (Teramoto & Shibata, 2006a). The DS values measured in the present work are in good agreement with the literature data, which supports the reliability of the NMR-based methodology employed here.

6.2.2 Electrospinning

6.2.2.1 Electrospinnability of Pullulan

In this study, electrospinning was used to obtain fibrous materials. The electrospinnability of pullulan was first examined to establish a reference point before investigating pullulan acetate. Although electrospinning of pullulan has been reported, the optimal process parameters strongly depend on the specific pullulan batch (molar mass, polydispersity, etc.) (Ai et al., 2024; Guerrini et al., 2021; Li et al., 2017; Ponrasu, Chen, Chou, Wu, & Cheng, 2021; Román et al., 2019). Polymer concentration is one of the key parameters governing electrospinning behavior and final morphology, as it directly affects the solution's ability to form continuous fibers or particles (Al-Abduljabbar & Farooq, 2023; Deitzel, Kleinmeyer, Harris, & Tan, 2001;

Ponrasu et al., 2021). Therefore, identifying the concentration that produces stable jets and well-defined fibers from pullulan was an essential step, providing a baseline for the electrospinning conditions used in subsequent experiments with pullulan acetate. Therefore, a series of aqueous pullulan solutions with concentrations ranging from 1 to 24 wt% were prepared and electrospun using the setup and procedure described in Chapter 3 (Methodology). The electrospinning parameters, which were kept constant for all concentrations, are summarized in Table 6.2.

Table 6.2 Electrospinning parameters for pullulan solutions at different concentrations

Parameters		Values
Solution	Concentrations %wt *	1, 2, 4, 8, 12, 15, 18, 22, 24
Process	Applied voltage (kV)	Adjusted to stabilize the Taylor cone (Varied from ~14 to 22)
	Flow rate (mL/h)	1.5
	Needle to collector distance (cm)	18
Environment	Temperature (°C)	23 ± 1
	Humidity (%)	27 ± 1

*%wt relative to the weight of the solution

The evolution of particle or fiber diameters as a function of polymer concentration, together with the corresponding SEM micrographs, is presented in Figure 6.4 and Figure 6.5. At low pullulan concentrations (1, 2, and 4 wt%), the number of polymer chains in solution is insufficient to generate the intermolecular entanglements required for stable jet formation. Under these conditions, the electrospinning jet breaks up into droplets, leading to the formation of particles rather than fibers (Ahmadi Bonakdar & Rodrigue, 2024; Jacobs, Anandjiwala, & Maaza, 2010). At intermediate concentrations of 8, 12, and 15 wt%, fiber formation begins to occur, but the resulting structures consist of continuous fibers decorated with beads, often described as “beads-on-string” morphologies. This beaded regime represents an intermediate state between particle and fully developed fiber formation, where increasing pullulan concentration enhances chain entanglement and, as expected, leads to an increase in fiber diameter (Al-Abduljabbar & Farooq, 2023). In the present work, 18 wt% was identified as the lowest pullulan concentration that produced uniform fibers with very few defects (beads

considered as defects), and was therefore selected for subsequent experiments. The average diameter of the fibers obtained at 18 wt% was $(67 \pm 7) \times 10$ nm.

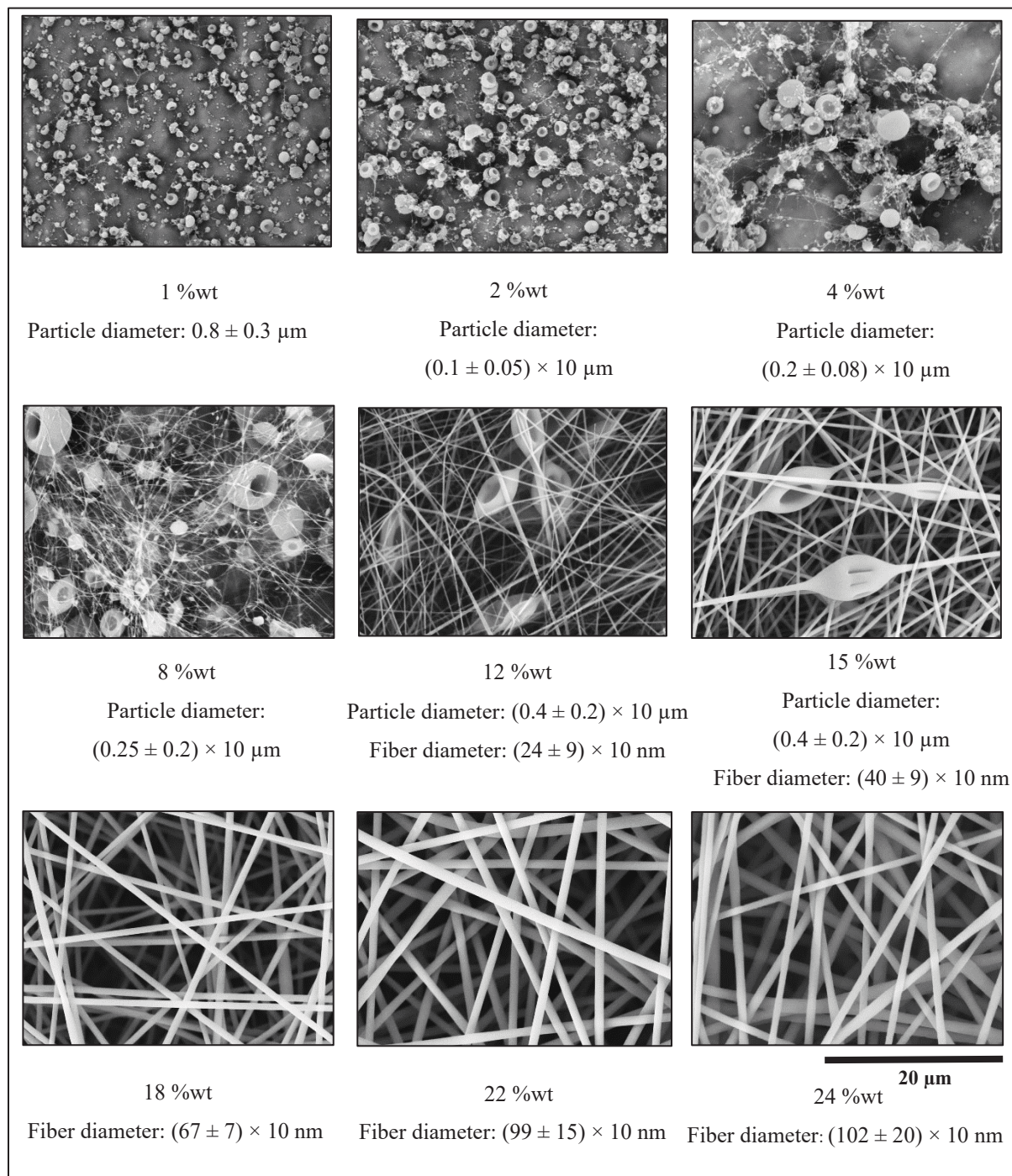


Figure 6.4 SEM micrographs and corresponding diameter of particles or fibers obtained from pullulan solutions at different concentrations

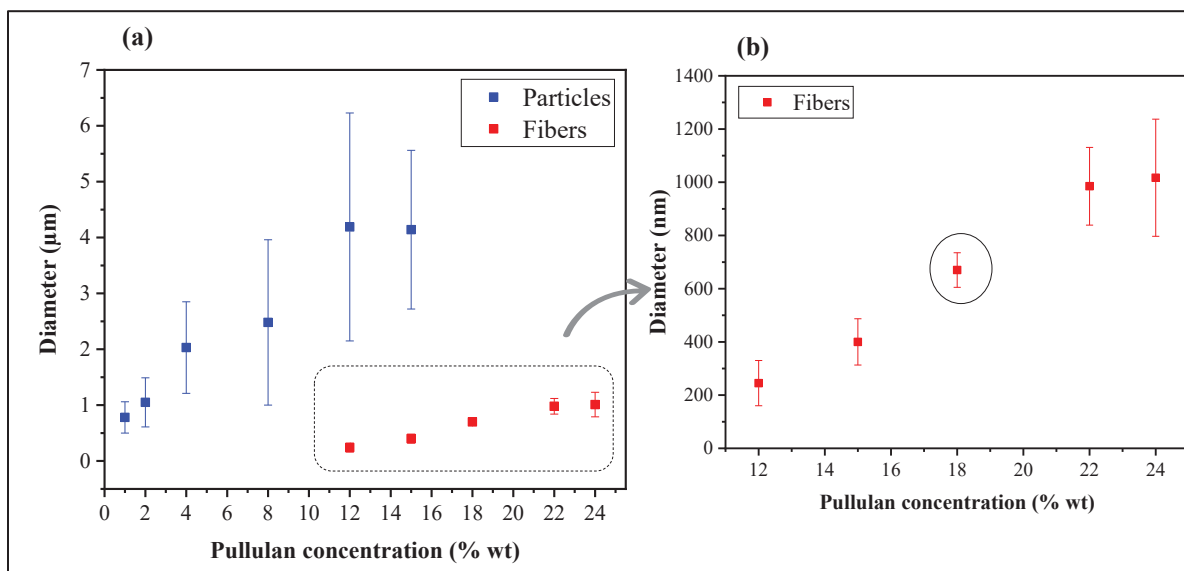


Figure 6.5 (a) Average diameter of particles or fibers (μm) as a function of pullulan concentration, and (b) average fiber diameter at higher magnification (nm)

6.2.2.2 Electropinnability of Pullulan-Acetate

To electrospin PULLAc materials with different DS, each sample required optimization of its own set of processing parameters, in agreement with the hypothesis stated in section 6.1. Acetylation is expected to reduce intermolecular hydrogen bonding, thereby potentially altering polymer-polymer and polymer-solvent interactions. As a result, achieving sufficient chain entanglement and stable jet formation required an organic solvent system.

Based on the electropinnability study of pullulan (Section 6.2.2.1), a polymer concentration of 18 wt% was selected and kept constant for all PULLAc solutions. To identify suitable electrospinning conditions for each PULLAc sample, we varied (i) the solvent composition (acetone and acetone/DMSO mixtures of 70/30, 50/50, and 30/70 % v/v), (ii) the flow rate (0.5 and 1.5 mL/h), and (iii) the needle-to-collector distance (18 and 46 cm, the maximum achievable with the electrospinning setup). The applied voltage was also adjusted as needed to stabilize the Taylor cone and process. DMSO and acetone were selected here as both have been

reported to dissolve acetylated pullulan (Ganeshkumar, Ponrasu, Subamekala, Janani, & Suguna, 2016; Jung, Jeong, & Kim, 2003a; Pavaloiu et al., 2021; Teramoto & Shibata, 2006a).

The SEM micrographs of the obtained materials corresponding to the different processing conditions are presented in Figure 6.6. These micrographs highlight several challenges observed during electrospinning, including film formation, needle clogging, and poor fiber deposition on the collector. As shown in Figure 6.5 (a), electrospinning of PULLAc-5 from an acetone/DMSO (30/70 %v/v) system yielded very thin fibers that were largely embedded within a continuous film. In contrast, when acetone was used as the sole solvent (Figure 6.5 (b)), its high volatility promoted rapid solvent loss at the needle tip, resulting in frequent needle clogging. Under these conditions, the collected fibers exhibited a ribbon-like (flattened) morphology, consistent with rapid skin formation followed by collapse of a still-solvated core during evaporation. Although PULLAc-9 is shown as a representative example, similar clogging behavior was observed for the other PULLAc samples when acetone was used alone. Finally, increasing the needle-to-collector distance to 46 cm (Figure 6.5 (c)) led to inefficient collection, characterized by substantial off-target deposition and limited fiber accumulation on the collector; PULLAc-15 is shown as an example, but the same trend was observed across the other samples.

Additional SEM micrographs obtained under other conditions, illustrating defects such as bead-on-string morphologies and film formation, are provided in APPENDIX III (Figure-A III-1).

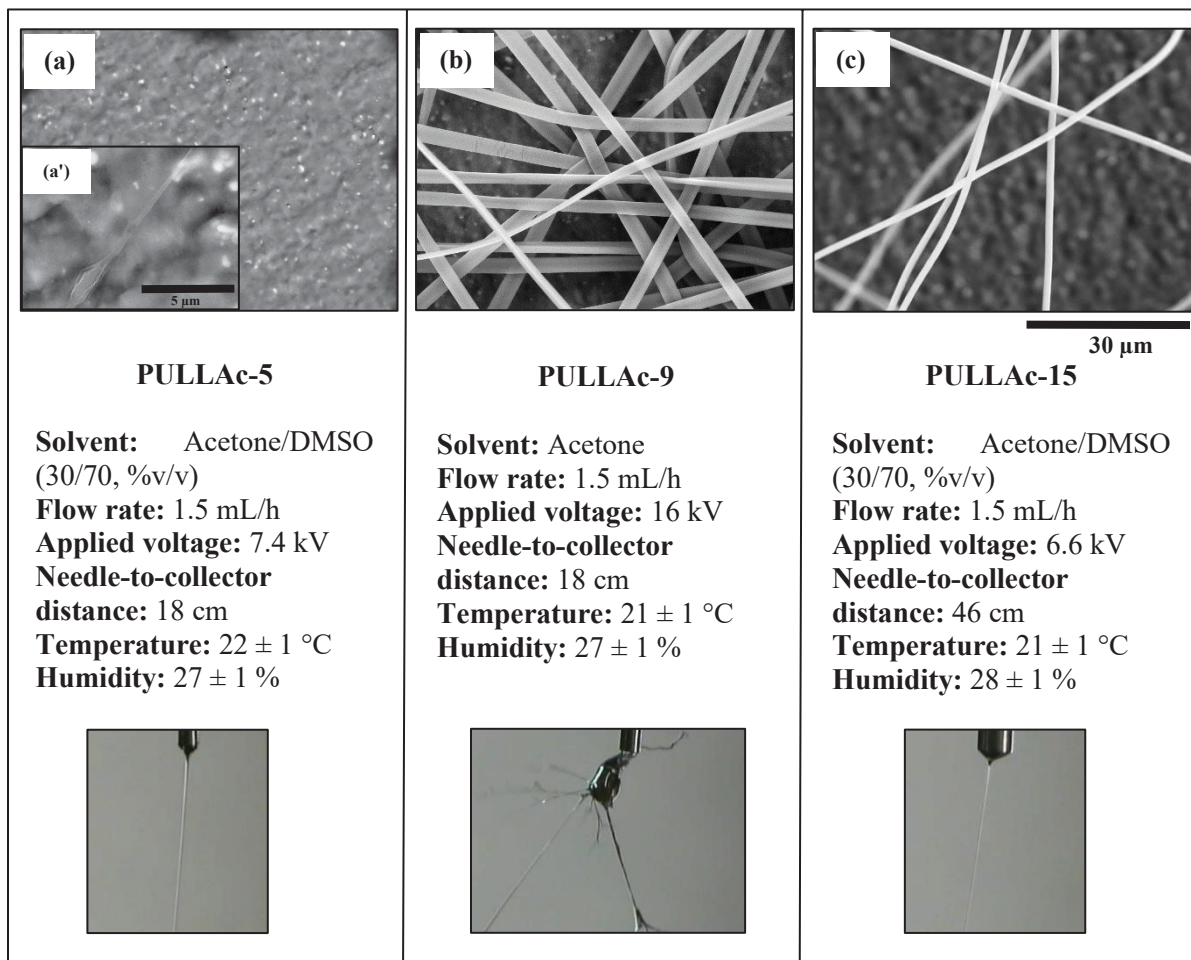


Figure 6.6 SEM micrographs of electrospun PULLAc membranes obtained under selected processing conditions, together with photographs of the corresponding Taylor cone during electrospinning: (a) and (a') PULLAc-5 electrospun from acetone/DMSO (30/70, %v/v) at a flow rate of $1.5 \text{ mL} \cdot \text{h}^{-1}$ and a needle-to-collector distance of 18 cm in different magnifications; (b) PULLAc-9 electrospun from acetone at a flow rate of $1.5 \text{ mL} \cdot \text{h}^{-1}$ and a needle-to-collector distance of 18 cm; and (c) PULLAc-15 electrospun from acetone/DMSO (30/70, %v/v) at a flow rate of $1.5 \text{ mL} \cdot \text{h}^{-1}$ and a needle-to-collector distance of 46 cm. Bright white spots are attributed to the aluminum-foil substrate artifacts (e.g., local charging and/or topographic contrast) and do not correspond to features of the fibrous network

The optimal electrospinning parameters to obtain the most continuous, low-defect PULLAc fibrous membranes are summarized in Table 6.3. Representative electrospun membranes and their corresponding SEM micrographs, used to assess fiber morphology, are shown in Figure 6.7.

Table 6.3 Optimized electrospinning parameters for 18 wt% PULLAc solutions

Parameters		PULLAc-5	PULLAc-9	PULLAc-15
Solution	Solvent	Acetone/DMSO (70/30 %v/v)	Acetone/DMSO (30/70 %v/v)	Acetone/DMSO (30/70 %v/v)
Process	Applied voltage (kV) (Adjusted to stabilize the Taylor cone)	~ 8.0	~ 6.6	~ 10.1
	Flow rate (mL/h)	1.5		
	Needle to collector distance (cm)	18		
Environment	Temperature (°C)	22 ± 1		
	Humidity (%)	27 ± 1		

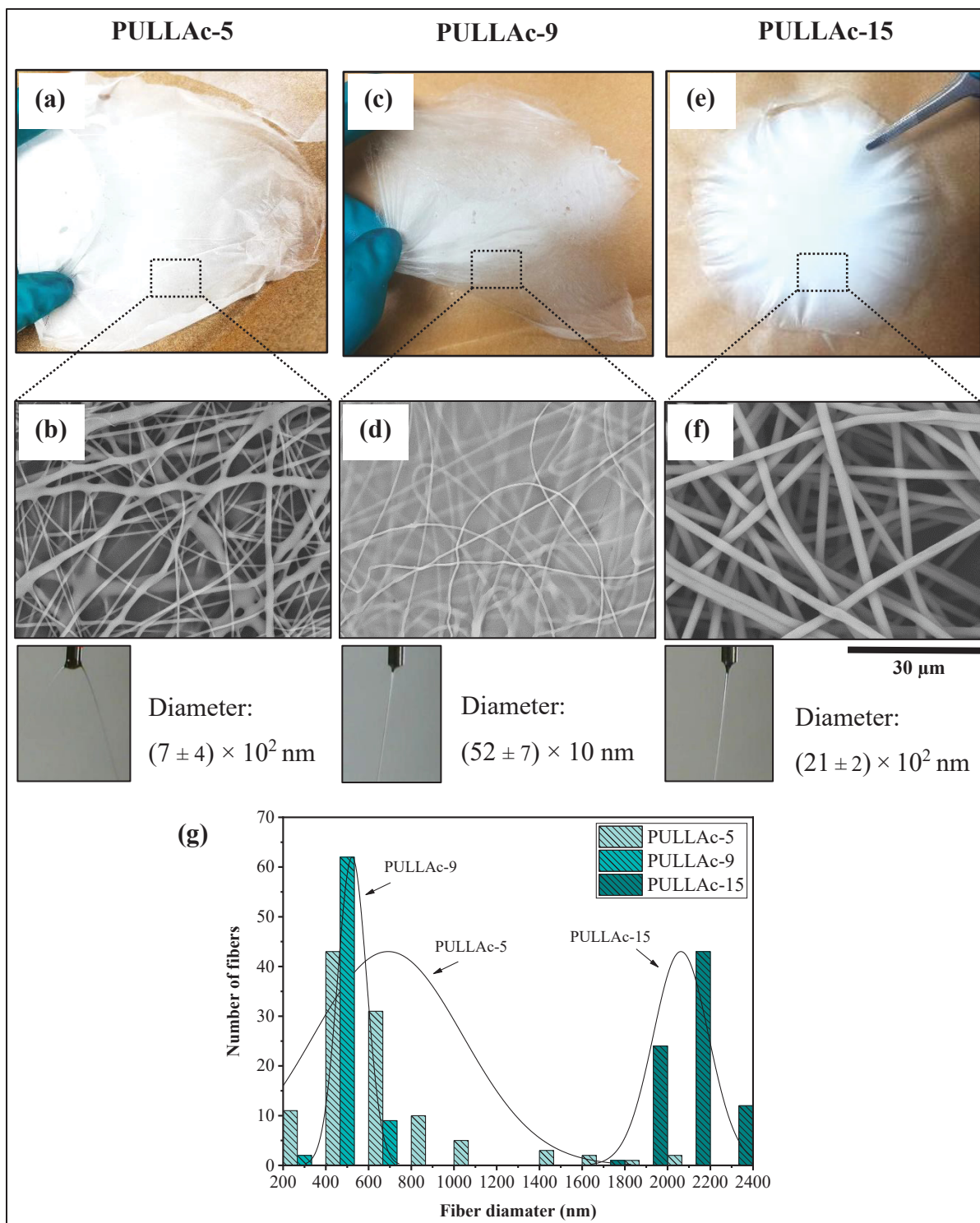


Figure 6.7 Examples of electrospun PULLAc fibrous membranes and their corresponding SEM micrographs for (a, b) PULLAc-5, (c, d) PULLAc-9, and (e, f) PULLAc-15, along with (g) the corresponding histograms of fiber diameter distributions. Membrane specimens ($\sim 1 \text{ cm} \times 1 \text{ cm}$) were cut and mounted on double-sided tape prior to SEM analysis

As shown in Figure 6.6, the average fiber diameters and their distributions highlight the combined influence of acetylation degree and solvent composition on the electrospinning behavior of PULLAc solutions. PULLAc-5 ($DS \approx 4$) electrospun from acetone/DMSO (70/30 %v/v) yielded fibers with an average diameter of $(7 \pm 4) \times 10^2$ nm, indicating a relatively broad and heterogeneous diameter distribution. In contrast, PULLAc-9 ($DS \approx 7$) and PULLAc-15 ($DS \approx 8$), both processed from acetone/DMSO (30/70 %v/v), produced more monodisperse fiber populations with average diameters of $(52 \pm 7) \times 10$ nm and $(21 \pm 2) \times 10^2$ nm, respectively. For highly substituted PULLAc derivatives (PULLAc-9 and PULLAc-15), the polymer becomes more hydrophobic. This observation is consistent with previous reports indicating that pullulan acetate with $DS \geq 5.1$ (corresponding to ~ 1.7 acetyl groups per glucose unit) becomes insoluble in water and soluble in DMSO, DMF, acetone, and pyridine, except the sample with $DS = 9$ (Ganeshkumar et al., 2016; Jung, Jeong, & Kim, 2003b; Pavaloiu et al., 2021; Teramoto & Shibata, 2006a).

For PULLAc-5, the SEM images reveal well-defined cylindrical fibers, but with a mixture of very thin and comparatively thick strands, consistent with the large standard deviation in diameter. The high acetone content likely leads to fast solvent evaporation at the needle tip and along the jet, promoting tip drying and temporal fluctuations in solution viscosity. These instabilities in the Taylor cone and jet stretching can cause alternating segments of over-stretched (thin) and under-stretched (thick) fibers, resulting in a broad diameter distribution (Deitzel et al., 2001).

For PULLAc-9, the SEM micrographs show a film-like background with flattened, ribbon-like fibers and welded junctions. This morphology suggests that, owing to the high boiling point and slow evaporation rate of DMSO, the jet reaches the collector while still containing residual DMSO (Ahmadi Bonakdar & Rodrigue, 2024; Deitzel et al., 2001; Kong & Ziegler, 2014; Kotomin, Malkin, & Skvortsov, 2020). On impact, the incomplete solidification allows fibers to deform, lose their circular cross-section, and locally coalesce.

Despite being electrospun from the same solvent system as PULLAc-9, PULLAc-15 displayed a markedly different morphology, characterized by thicker and more cylindrical fibers, with minimal evidence of film formation or welding. This may arise from the difference in intermolecular hydrogen bonding, which can change the chain entanglements, thereby influencing the deposited fiber diameter, consistent with the hypothesis stated at the beginning of this section.

Given the use of acetone/DMSO solvent systems during electrospinning, membranes obtained from all PULLAc-5, PULLAc-9, and PULLAc-15 were immersed in D₂O to qualitatively evaluate their solubility and to assess the presence of residual solvent. In the resulting ¹H NMR spectra (APPENDIX III, Figure-A III-2), signals attributable to DMSO and acetone were detected, indicating that some solvents remain trapped within the as-spun fibers. Furthermore, under the measurement conditions and within the detection limits of NMR at room temperature, no characteristic PULLAc signals were observed in the D₂O phase, suggesting that the membranes exhibit low solubility in water at ambient temperature.

6.2.3 Water Stability Analysis

To assess the influence of acetylation degree on the water resistance of PULLAc fibers, the as-spun membranes were immersed in deionized water for over 24 hours at room temperature (25 ± 2 °C), followed by drying. Figure 6.8 presents SEM micrographs of the membrane microstructure before and after water exposure. The representative photographs of the membranes during water immersion and after drying are also shown in APPENDIX III (Figure-A III-3).

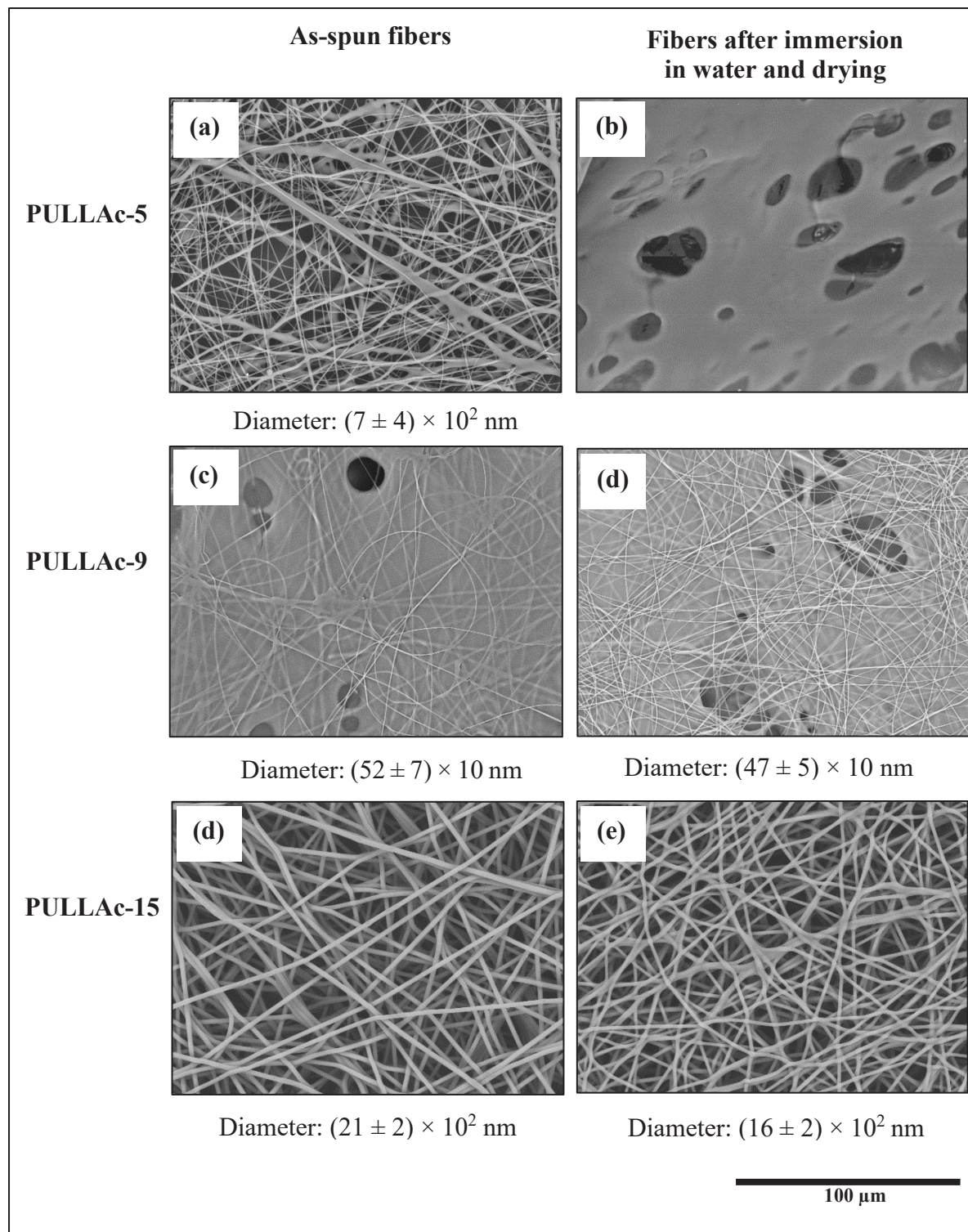


Figure 6.8 SEM micrographs of PULLAc membranes in the as-spun state and after immersing in deionized water over 24 hours, followed by drying, for (a, b) PULLAc-5, (c, d) PULLAc-9, and (e, f) PULLAc-15. Drying was performed at 40 °C under vacuum

The membrane prepared from PULLAc-5 showed pronounced loss of fibrous morphology after immersion in water and subsequent drying: the initially well-defined fibrous network collapsed into a compact, film-like structure, with no discernible individual fibers. This behavior can be consistent with the higher hydrophilicity and lower DS of PULLAc-5, which promotes extensive water uptake, leading to coalescence of fibers and loss of the original electrospun morphology (Mahdian-Dehkordi, Sarrafzadeh-Rezaei, Razi, & Mahmoudian, 2021; Qin, Jia, Liu, Kong, & Wang, 2020). Although within the method's detection limits, no characteristic PULLAc resonances were detected in the D₂O phase by ¹H NMR, the observed post-immersion morphological changes indicate that the PULLAc-5 membrane nonetheless undergoes some level of water uptake and/or partial dissolution.

In contrast, the membranes prepared with more highly substituted PULLAc-9 and PULLAc-15 retained their fibrous architecture after water immersion and drying. Individual fibers remained clearly distinguishable, indicating that the increased hydrophobic character was sufficient to prevent fiber coalescence. The average fiber diameters of PULLAc-9 and PULLAc-15 after water treatment were $(47 \pm 5) \times 10 \text{ nm}$ and $(16 \pm 2) \times 10^2 \text{ nm}$, respectively, both lower than those of the corresponding as-spun fibers ($(52 \pm 7) \times 10 \text{ nm}$ and $(21 \pm 2) \times 10^2 \text{ nm}$, respectively, discussed in Figure 6.6 and section 6.2.2.2). This effect can be attributed to the extraction of residual DMSO and acetone from the DMSO/acetone spinning solvent and the consequent densification of the polymer matrix upon drying (Kotomin et al., 2020). To examine solvent removal upon water exposure, membranes obtained from all PULLAc-5, PULLAc-9, and PULLAc-15 after water immersion, then drying, were subsequently immersed in D₂O for ¹H NMR analysis (APPENDIX III, Figure-A III-4). The absence of resonances attributable to DMSO and acetone after this treatment indicates that the water immersion step effectively extracted the residual spinning solvents from the electrospun membranes. In this context, the post-immersion SEM micrograph of the PULLAc-9 membrane exhibits a less pronounced film-like background and fewer welded junctions between adjacent fibers, suggesting that removal of residual solvent reduced fiber coalescence.

Additional SEM micrographs of the water-immersed and dried fibrous membranes, acquired at different magnifications, are provided in APPENDIX III (Figure-A III-5).

6.3 Conclusion

The preliminary results presented in this chapter highlight the influence of acetylation degree on the properties and performance of pullulan acetate (PULLAc) fibrous membranes. The degree of acetylation was directly affected by the initial molar ratio of acetic anhydride, which in turn affected the ability to form electrospun fibers and the water resistance of the resulting membranes.

The preliminary study on the electrospinning experiments indicated that both the degree of substitution (DS) and the solvent composition influence the formation and morphology of PULLAc fibrous membranes. Water-stability studies further revealed that acetylation effectively extends the applicability of pullulan-based fibers in aqueous environments. While the lower-DS PULLAc membrane lost its fibrous architecture and transformed into a film upon immersion in water, higher-DS membranes preserved a porous fibrous scaffold. Moreover, post-electrospinning treatment through water immersion removes residual solvents and should therefore be considered an essential step for applications where solvent removal is critical.

Overall, this work establishes acetylation as a simple yet efficient route to engineer the electrospinnability and aqueous stability of pullulan-based materials. By tuning the DS and solvent system, it is possible to design pullulan acetate membranes that can be useful in different applications, including wound dressings, drug-loaded scaffolds, barrier or coating layers, and filtration or separation membranes based on renewable, polysaccharide-derived materials. Importantly, these PULLAc membranes may remain potentially biodegradable through enzymatic degradation.

These preliminary results suggest that PULLAc-5 may still be useful as films or coatings, whereas PULLAc-9 and PULLAc-15 appear more promising for applications requiring preservation of a porous, fibrous architecture in aqueous environments, such as wound dressings or filtration membranes (Borban et al., 2023; Enomoto-Rogers et al., 2015; Hernandez-Tenorio & Giraldo-Estrada, 2022; Niu et al., 2019).

In future work, a more detailed investigation of solution properties (such as viscosity, conductivity, and surface tension), together with scale-up of the pullulan acetate electrospinning process and evaluation of membrane mechanical properties, would be valuable to support translation toward larger-scale and application-oriented use.

CONCLUSION

This thesis sets out to evaluate and develop pullulan-based functional materials by tailoring graft copolymerization routes and fabricating electrospun membranes, with an emphasis on establishing structure-property relationships across these approaches.

A first major contribution of this work is the establishment of a pullulan-xanthate RAFT/MADIX platform enabling the synthesis of pullulan-g-poly(N-vinyl caprolactam) (PULL-g-PNVCL) copolymers. The approach follows a three-step sequence: partial functionalization of pullulan with a brominated reagent, preparing a pullulan-xanthate-based macro-chain transfer agent (macro-CTA), and controlled N-vinyl caprolactam (NVCL) polymerization from the resulting macro-CTA agent. Within this framework, grafted architectures were obtained with dispersities similar to the pullulan-macro-CTA, consistent with effective mediation of NVCL polymerization by the xanthate CTA. Grafting also introduced changes in thermal behavior and particle characteristics. In particular, longer PNVCL chains produced larger particles and shifted the transition temperature window, illustrating a structure-property lever for tailoring temperature-responsiveness in water.

The second contribution is a rigorous feasibility study extending the same macro-CTA platform to acrylic acid, targeting pullulan-g-poly(acrylic acid) (PULL-g-PAA). Convergent evidence from complementary analyses demonstrated that acrylic acid polymerization from the pullulan-xanthate macro-CTA produced some grafted PAA chains but also a significant fraction of free PAA, indicating that competing homopolymerization is a dominant side reaction under the investigated conditions. This outcome constrains the ability to establish clean structure-property relationships for pH-responsive behavior when ungrafted PAA is present in substantial quantities. Overall, while the pullulan-xanthate macro-CTA can mediate acrylic acid polymerization to some extent, xanthate-based strategies are not recommended for preparing well-controlled PAA-grafted pullulan in the present architecture and conditions.

Complementing the molecular engineering route, the preliminary study demonstrated that acetylation provides a practical handle to tune pullulan processability and water resistance. Increasing the degree of substitution (DS) shifted solubility away from water and enabled solution formulation in organic solvent systems relevant to electrospinning. Electrospinning experiments showed that solvent composition and DS jointly governed fiber formation and morphology. Water-immersion testing indicated that the lower-DS membrane lost its fibrous architecture, while the higher-DS membranes retained a porous fibrous scaffold. Furthermore, these studies highlighted the practical importance of post-processing water immersion as a means to remove residual solvents retained in the as-spun fibers.

Taken together, the natural and renewable origin of pullulan highlights its value as a sustainable platform for designing polymeric materials. A key novelty of this thesis is the development of a pullulan-based grafting-from RAFT/MADIX platform, together with its extension to electrospun pullulan acetate membranes. The main limitations are the preliminary nature of the electrospinning study and the formation of a significant fraction of free PAA in the PULL-g-PAA system. These points are addressed in the recommendation section, where future work is outlined to further optimize the developed materials. Thus, building on the foundation established in this thesis, future studies may facilitate the transition of pullulan-based functional materials from laboratory-scale demonstrations toward industrially relevant, application-oriented products.

RECOMMENDATIONS

The research presented in this thesis establishes strategies toward the development of pullulan-based functional materials. Concurrently, the outcomes open multiple directions for future work. The recommendations below are designed to extend the scientific understanding developed herein and to address the principal experimental and translational challenges encountered during the work. They are organized according to the thesis objectives.

Objective A – RAFT/MADIX-based grafting platform for stimuli-responsive pullulan copolymers

- **Implement reaction-kinetics monitoring**

Quantifying monomer conversion as a function of time (e.g., timed aliquots by ^1H NMR integration) and correlating conversion with molar-mass evolution and dispersity.

- **Adopt controlled monomer feeding strategies**

Evaluating stepwise monomer addition over several smaller portions at time intervals to potentially improve control of grafted chain growth and uniformity.

- **Replace xanthate with an acrylic-acid-compatible chain transfer agent (CTA) for PAA grafting**

Performing a direct comparison using a CTA known to better control acrylic acid polymerization (e.g., trithiocarbonate-based systems), with the specific goal of minimizing free PAA and enabling clean property evaluation of PULL-g-PAA.

- **Redesign the bromoacylation step to suppress side-product formation and increase CTA substitution efficiency**

Evaluating less side-reaction-prone reagents to minimize side-products associated with bromopropionyl units and to improve CTA substitution efficiency.

- **Evaluate “grafting-onto” via click coupling as an alternative architecture route**

Considering attaching pre-formed, well-characterized side chains of controlled molar mass to functionalized pullulan using click-type coupling reactions.

Objective B – Development of pullulan-based electrospun membranes

- **Expand the electrospinning processing window of pullulan acetate using a structured experimental design**

Applying a design-of-experiments (DoE) framework across pullulan acetate samples with different degrees of substitution, systematically varying electrospinning parameters (e.g., solution concentration).

- **Add solution-property and membrane-performance characterization**

Measuring solution viscosity (and, where feasible, conductivity and surface tension) and relating these properties to electrospinnability and fiber morphology, as well as evaluating membrane mechanical performance.

- **Explore scale-up pathways in membrane manufacturing**

Investigating electrospinning scale-up strategies to enable the fabrication of larger membranes.

APPENDIX I

Supplementary information for Chapter 4

Synthesis of Bromide-Bearing Pullulan

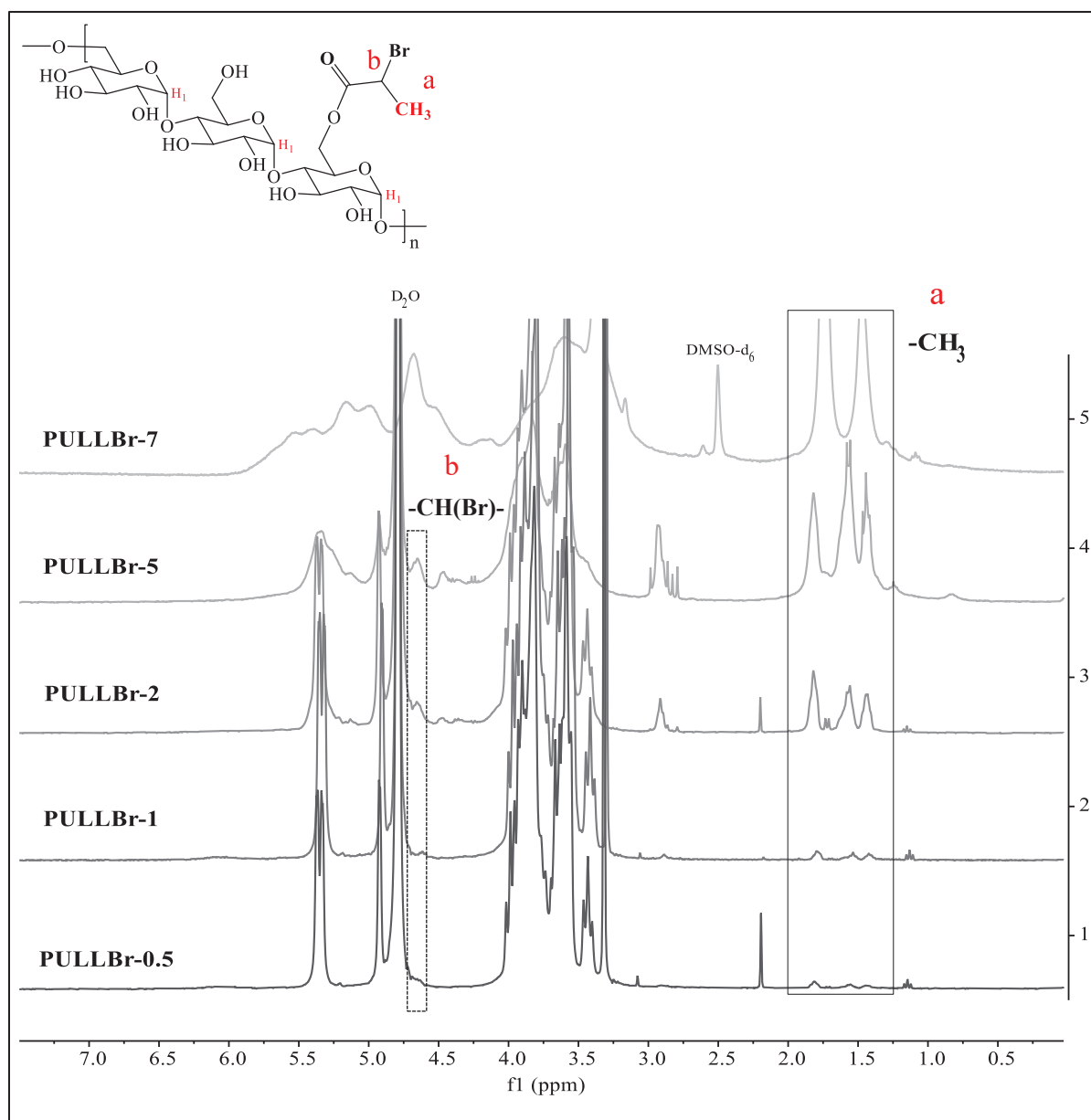


Figure-A I-1 ^1H NMR spectra of all PULLBr samples

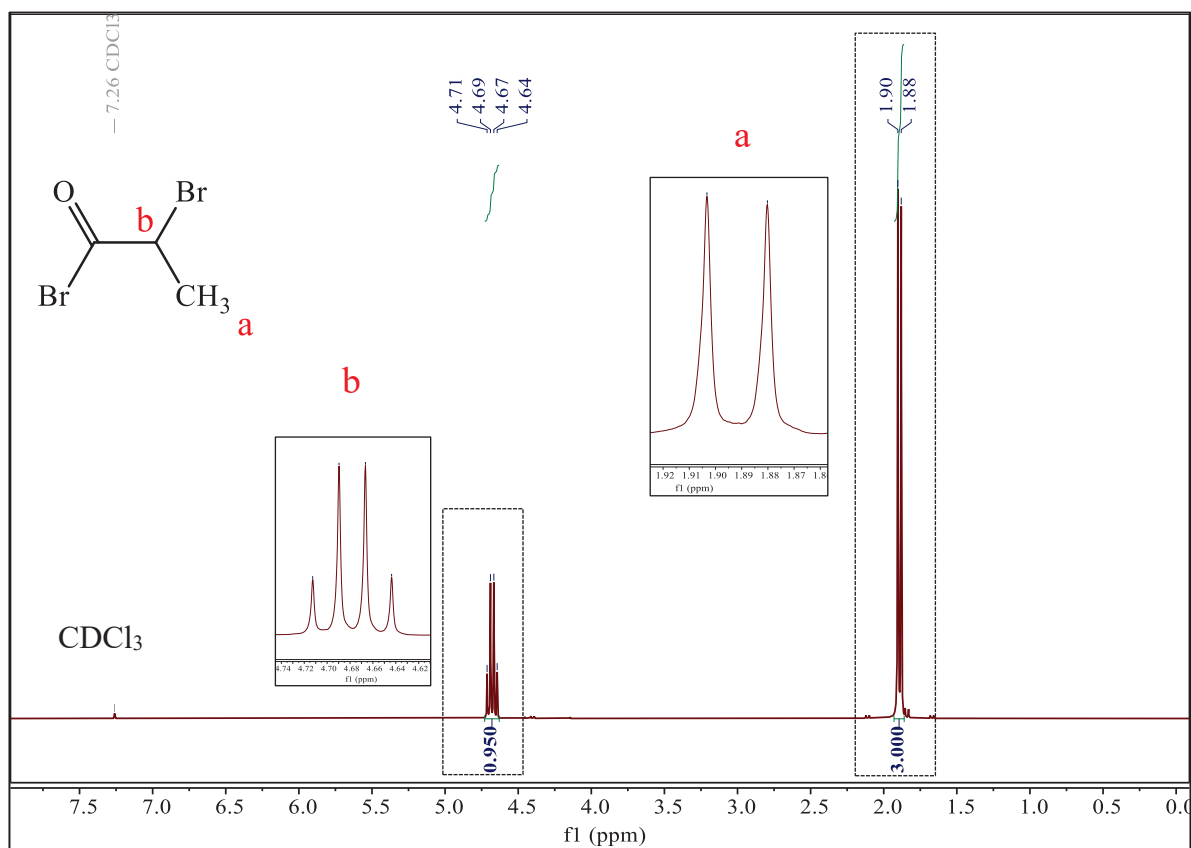


Figure-A I-2 ^1H NMR spectrum of 2-bromopropionyl bromide in CDCl_3

Calculations for the Degree of Substitution on Hydroxyl Groups of Pullulan by the Bromopropionyl Group

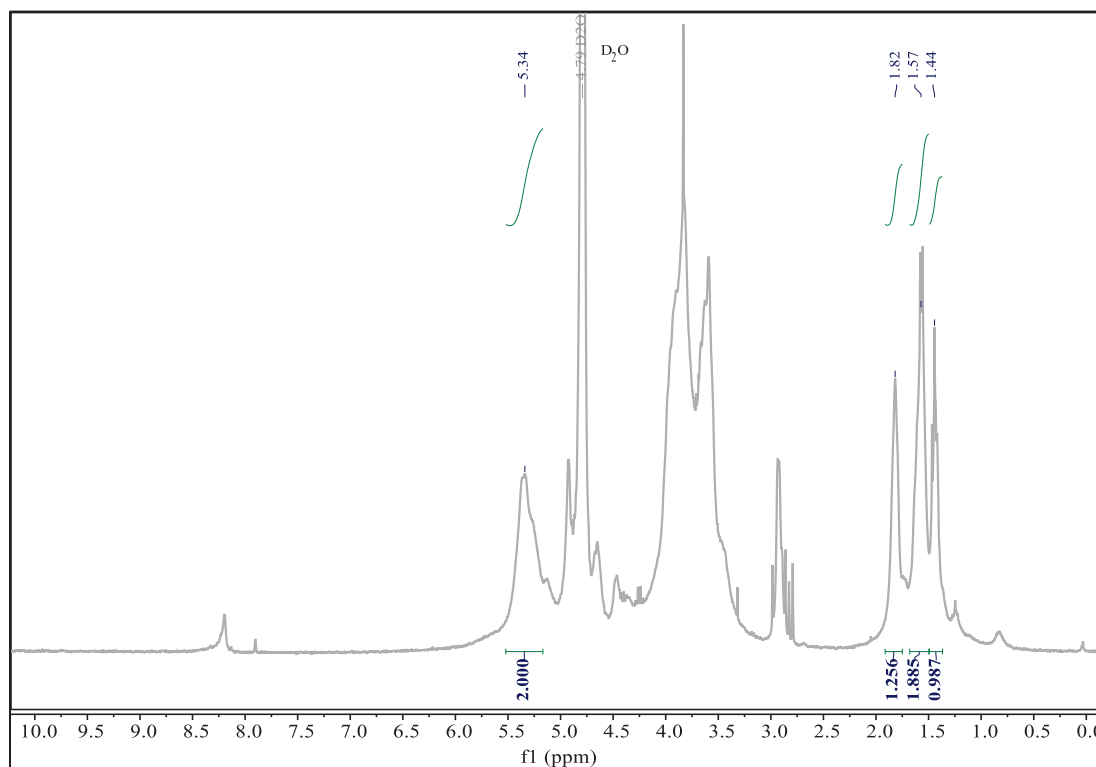


Figure-A I-3 ¹H NMR spectra of PULLBr-5 in D₂O

The degree of substitution (DS) was calculated using the formula:

$$DS = \frac{\text{Sum of the integral values of the } -CH_3 \text{ corresponding to the bromopropionyl group}}{27} \times 100$$

In this equation, the value of 27 represents the expected maximum integral value observed for 100% substitution of the hydroxyl groups by the bromopropionyl group. This is based on the assumption that the normalized integral value for the two protons at the C₁ positions of the α-(1→4) linkages in pullulan is set to 2. For example, Figure-A I-3 shows a DS of 15 bromide groups in 100 hydroxyl groups for PULLBr-5, calculated according to this method. Because each pullulan repeat unit bears nine hydroxyl groups, 100 hydroxyl groups correspond to 100/9 ≈ 11.1 repeat units (≈ 11 monomer units). Accordingly, the number of

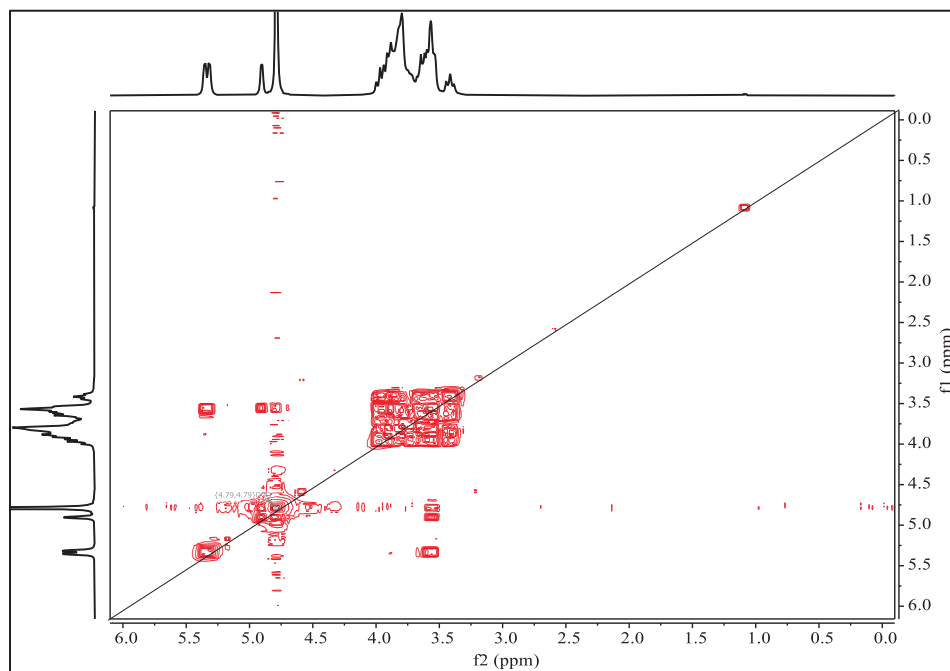
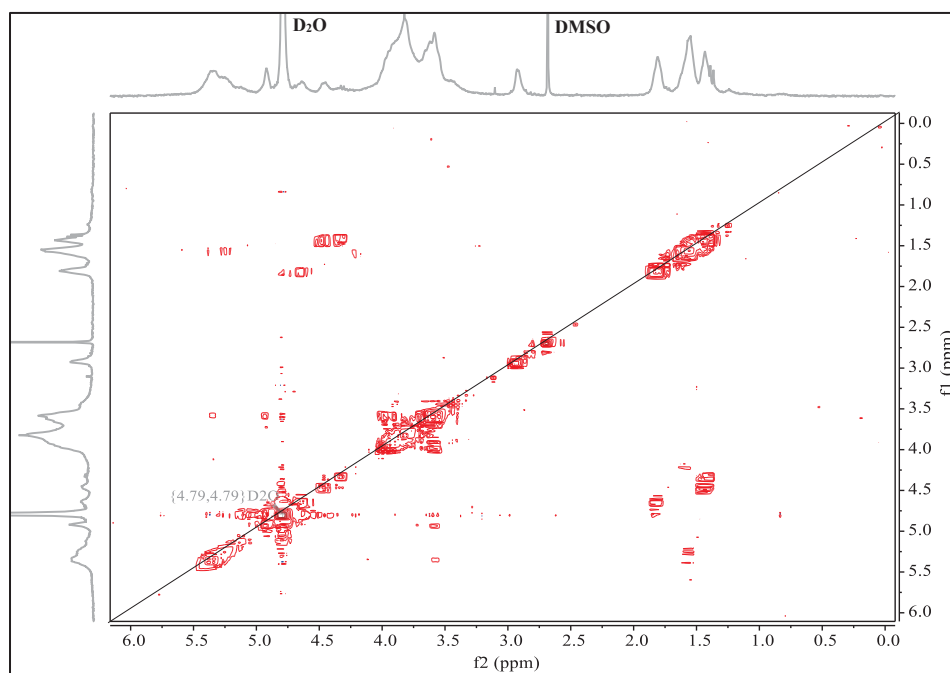
bromide groups per 100 monomer units was obtained by converting to the equivalent value per ~11 monomer units and subsequently scaling to 100 monomer units, as shown in the table 4.1 in the main text.

Table-A I-1 The degree of substitution and the experimental conditions for the PULLBr-5 and PULLBr-7 samples on a larger scale

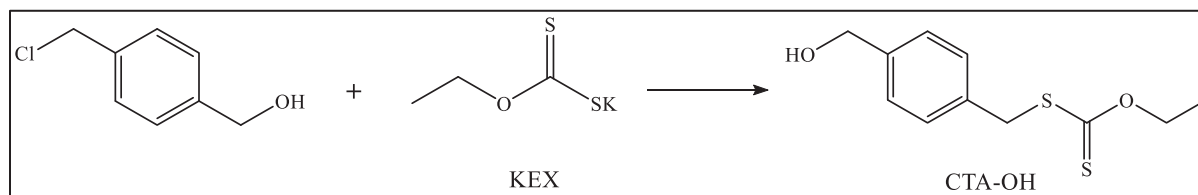
Sample	Solvent for NMR	Mass of reduced molar mass pullulan (mol of monomer units)	mmol of BPB	Bromide groups in 100 hydroxyl groups (DS)	Bromide groups in 100 monomers (DS)
PULLBr-5^a	D ₂ O	2 g (4.11 mmol)	20.5	9	82
PULLBr-7^{a*}	DMSO-d ₆	2 g (4.11 mmol)	28.80	-----	-----

* The ¹HNMR analysis couldn't be determined because of solubility issues and because excess unreacted BPB could not be separated from the product in a satisfactory manner.

^a Larger-scale DS

^1H - ^1H Correlation Spectroscopy (COSY) spectra of Pullulan and PULLBr-5Figure-A I-4 COSY spectra of PULL in D_2O Figure-A I-5 COSY spectra of PULLBr-5 in D_2O

Synthesis of the O-Ethyl S-4-(Hydroxymethyl) Benzyl Carbonodithioate Chain Transfer Agent (CTA-OH)



Scheme-A I-1 Synthesis route of CTA-OH

^1H NMR (300 MHz, ppm, DMSO- d_6), δ : 1.36 (3H, CH_3), 4.38 (2H, SCH_2), 4.47 (2H, CH_2OH), 4.62 (2H, CH_3CH_2), 5.20 (1H, OH), 7.26–7.34 (4H, Ph).

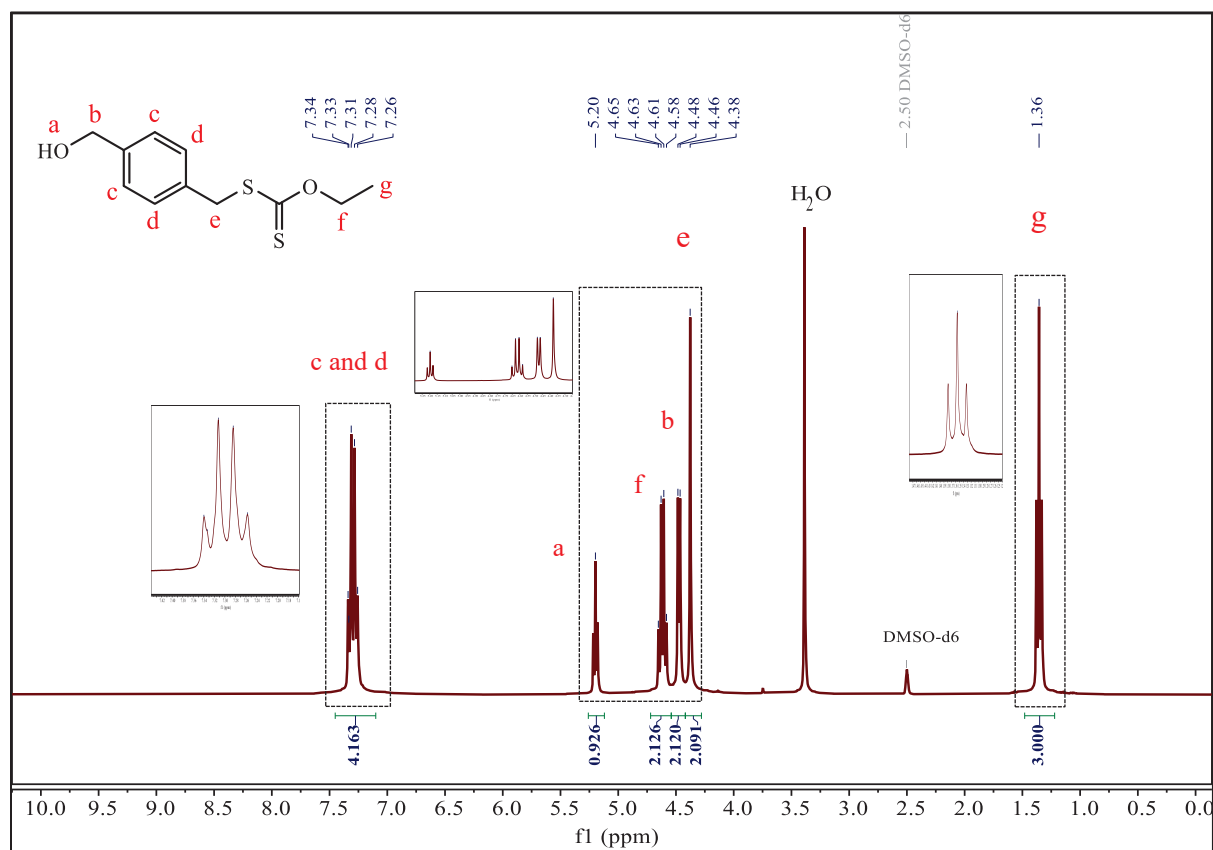


Figure-A I-6 ^1H NMR spectra of CTA-OH in DMSO- d_6

Synthesis of PULL-Xanthate-Based Macro-Chain Transfer Agent

Based on the results observed in the synthesis of PULLBr, we selected PULLBr-5 and PULLBr-7 as the best candidates for the continuation of the synthetic sequence. In the main text, the synthetic route exploiting PULLBr-5 was reported in detail. We also pushed forward PULLBr-7 through grafted polymerization, and the resulting findings are only depicted in the APPENDIX.

To optimize the synthesis of PULLX, two samples in total were prepared: PULLX-5 using PULLBr-5 (result reported in the main text) and PULLX-7 using PULLBr-7 (reported below).

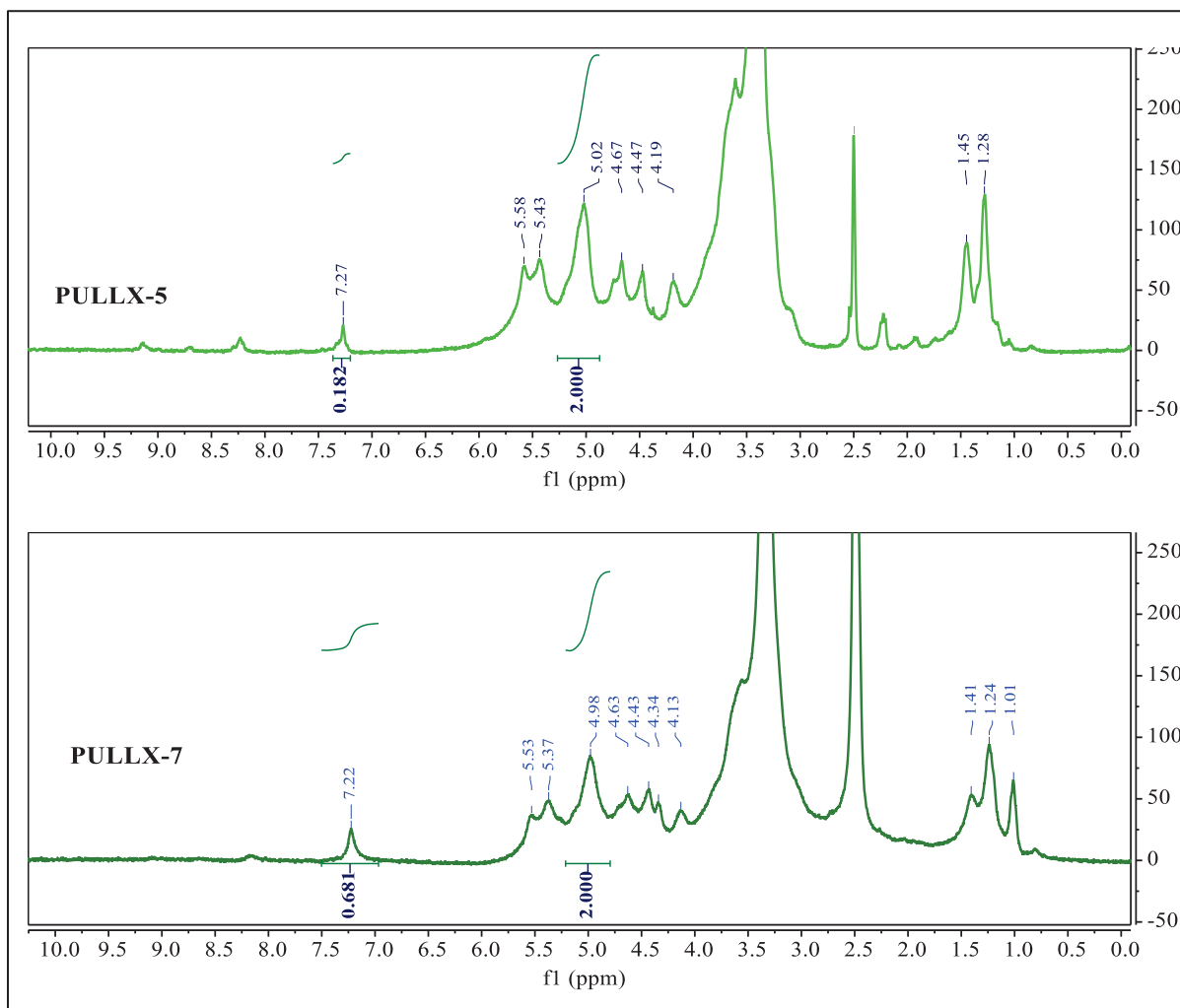


Figure-A I-7 ^1H NMR spectra of PULLX-5 and PULLX-7 in DMSO-d_6

Below is an example of how we calculated the degree of substitution of the bromopropionyl group by CTA-OH. In this case, the calculation for PULLX-5 is shown.

The degree of substitution (DS) of the bromopropionyl group by CTA-OH on PULLX-5 was calculated using the formula:

$$DS = \left(\frac{\text{Integral value of the aromatic protons of the CTA}}{4} \right) \times 100$$

This DS calculation reflects the number of CTA molecules per 100 monomer units. For example, PULLX-5 shows a DS of 4.5 in 100 monomers, calculated according to this method.

The same method can be used to calculate the DS on bromopropionyl group by CTA molecules in PULLX-7, as well as the larger-scale samples (Table-A I-2).

Table-A I-2 The DS of CTA molecules of PULLX samples

Samples	CTA groups in 100 monomers (DS)
PULLX-5	≈ 4.5
PULLX-7	≈ 17
PULLX-5^a	≈ 3
PULLX-7^{a*}	≈ 2.7

* The ¹HNMR analysis for this sample could be performed because of enhanced solubility compared to the PULLBr-7^a

^a Larger-scale DS

Table-A I-3 The experimental conditions for the PULLX-5 and PULLX-7 samples on a larger scale

Sample	Mass of PULLBr	Mmol of CTA-OH
PULLX-5^a	1.33 g	2.20
PULLX-7^a	1.93 g	12.50

^a Larger-scale

Synthesis of PULL-g-PNVCL

To optimize the synthesis of PULL-g-PNVCL, the polymerization was carried out using PULLX-5^a (synthetic methods and results reported in the main text), and PULLX-7^a. Here we report only the results for the synthesis of PULL-g-PNVCL-7 using PULLX-7^a. To synthesize PULL-g-PNVCL-7, [CTA]:[AIBN]:[NVCL] feed molar ratio of 1:0.5:150 (PULL-g-PNVCL-7 (150)) and 1:0.5:300 (PULL-g-PNVCL-7 (300)) were used. The characterizations of PULL-g-PNVCL-7 (150) and PULL-g-PNVCL-7 (300) samples are shown below.

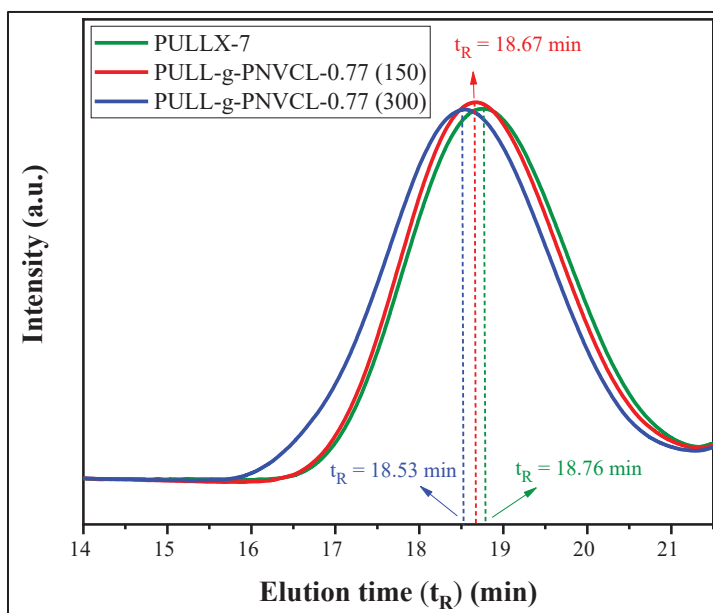


Figure-A I-8 GPC chromatograms of PULLX-7, PULL-g-PNVCL-7 (150), and PULL-g-PNVCL-7 (300) in DMF/LiBr

Table-A I-4 Molar masses ($\overline{M}_n$ and $\overline{M}_w$), dispersity, and percentage of PNVCCL in the copolymer for PULLBr-7, PULLX-7, and PULL-g-PNVCL-7 (150) and PULL-g-PNVCL-7 (300) in DMF/LiBr

Samples	$\overline{M}_n$ (kg/mol) ^a	$\overline{M}_w$ (kg/mol) ^a	$\overline{D}$ ^a	PNVCL in copolymer (%) ^b
PULLBr-7	20	43	2.21	-----
PULLX-7	21	38	1.78	-----
PULL-g-PNVCL-7 (150)	23	40	1.71	12
PULL-g-PNVCL-7 (300)	26	47	1.83	22

^a Determined by GPC in DMF/LiBr (0.1% v/v) at 60 °C, ^b PNVCL in copolymer (%) = $\left(\frac{\overline{M}_n(\text{PULL-g-PNVCL}) - \overline{M}_n(\text{PULLX})}{\overline{M}_n(\text{PULLX})} \right) \times 100$ (Gürdağ & Sarmad, 2013)

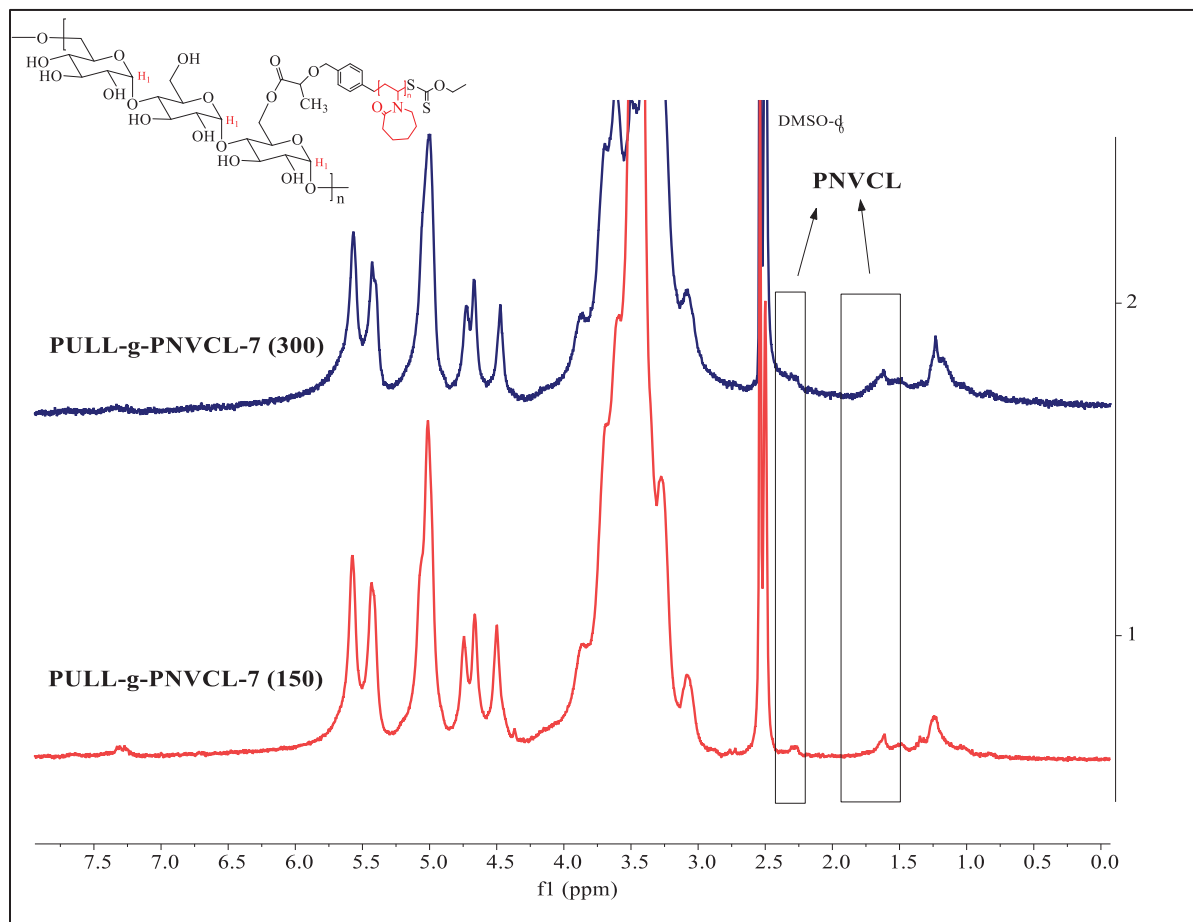


Figure-A I-9 ¹H NMR spectra of PULL-g-PNVCL-7 (150), and PULL-g-PNVCL-7 (300) in DMSO-d₆

Isolated Yield for All Reactions

Although the yield can be higher, only the isolated yield, which is after purification, is reported here.

Table-A I-5 Isolated yield of reduced molar mass pullulan, PULLBr, PULLX, and PULL-g-PNVCL

Sample		Isolated yield (%)	
Reduced molar mass PULL		92	
PULLBr-5 ^a	PULLBr-7 ^a	58	50
PULLX-5 ^a	PULLX-7 ^a	73	76
PULL-g-PNVCL (150)	PULL-g-PNVCL-7 (150)	38	61
PULL-g-PNVCL (300)	PULL-g-PNVCL-7 (300)	37	52

^a Larger scale

Cooling Thermograms for DSC Analysis

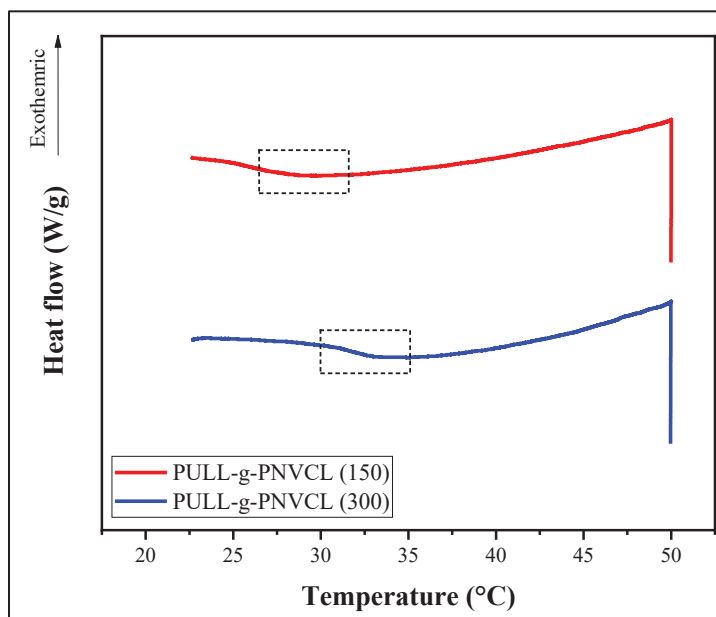


Figure-A I-10 Cooling DSC thermograms of PULL-g-PNVCL (150) and PULL-g-PNVCL (300) aqueous dispersion

APPENDIX II

Supplementary information for Chapter 5

Calculations for the Degree of Substitution on Hydroxyl Groups of Pullulan by the Bromopropionyl Group

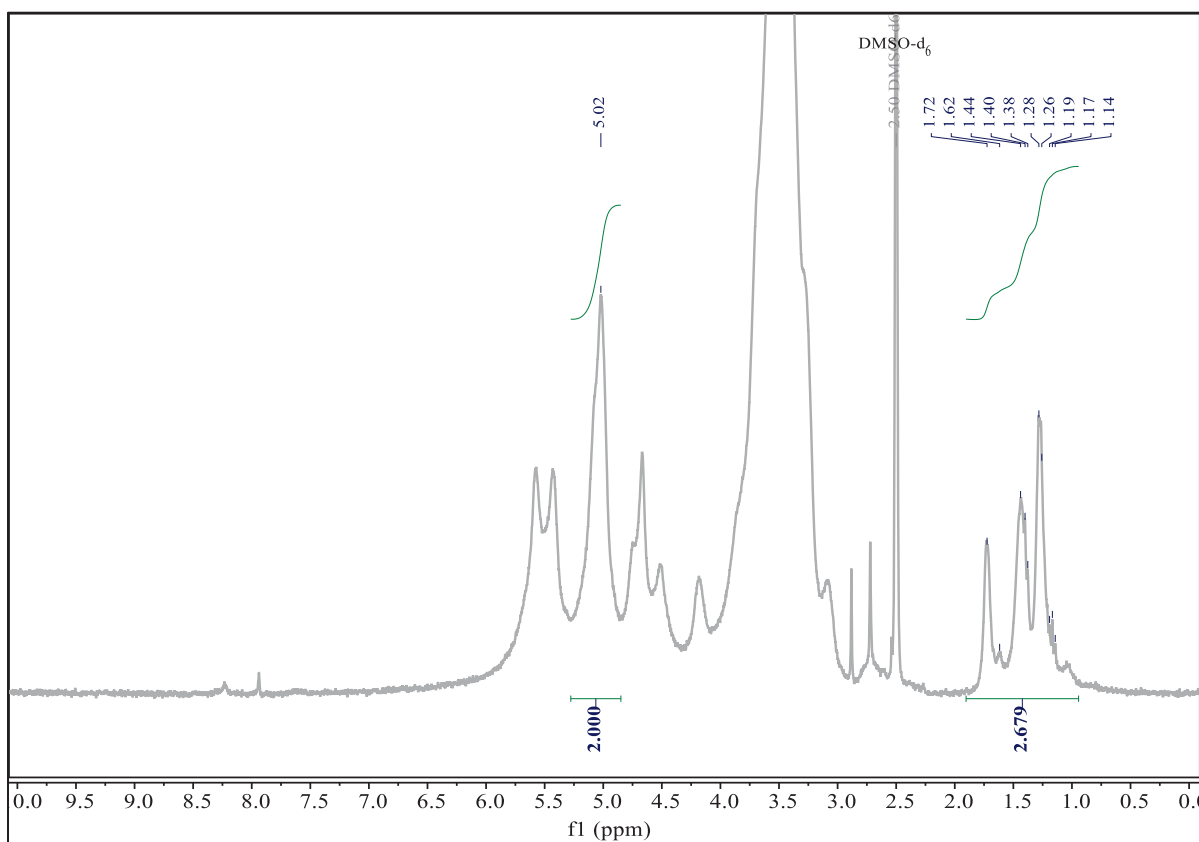


Figure-A II-1 ^1H NMR spectra of PULLBr-5 in DMSO-d_6

The degree of substitution (DS) was calculated using the formula:

$$\text{DS} = \frac{\text{Sum of the integral values of the } -\text{CH}_3 \text{ corresponding to the bromopropionyl group}}{27} \times 100$$

Figure-A II-1 shows a DS of 10 bromide groups in 100 hydroxyl groups and 91 per 100 monomer units of PULLBr-5, calculated according to this method.

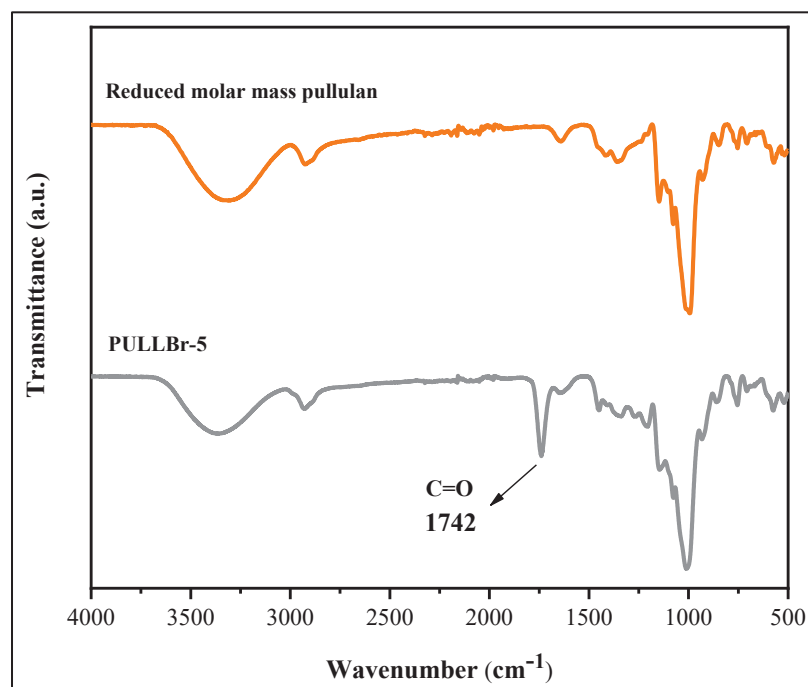
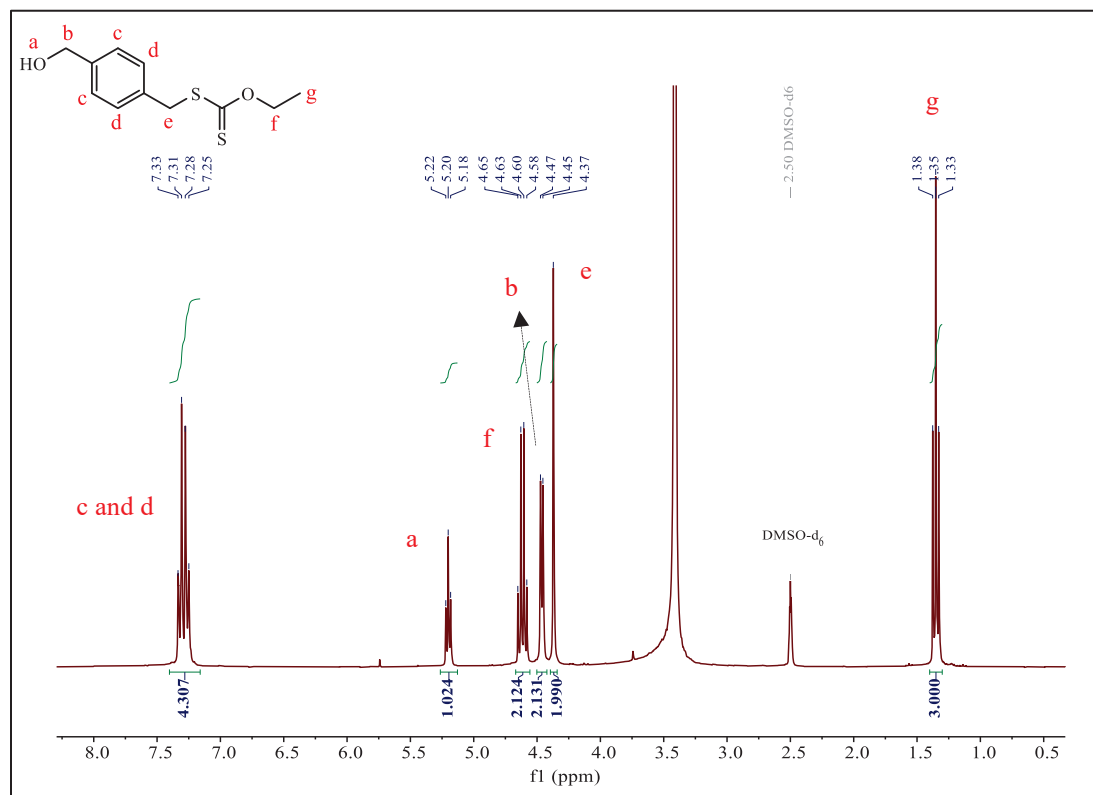


Figure-A II-2 ATR-FTIR spectra of reduced molar mass pullulan and PULLBr-5

Figure-A II-3 ^1H NMR spectra of CTA-OH in DMSO-d_6

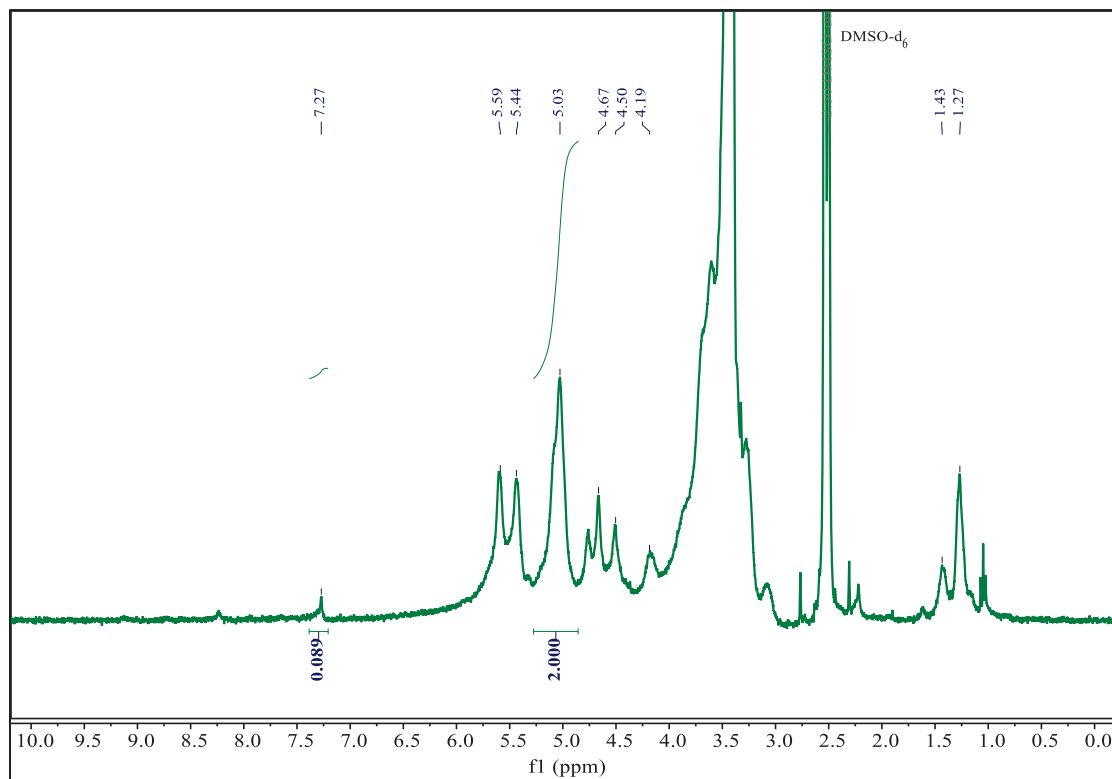


Figure-A II-4 ^1H NMR spectra of PULLX-5 in DMSO-d_6

The degree of substitution (DS) of the bromopropionyl group by CTA-OH on PULLX-5 was calculated using the formula:

$$\text{DS} = \left(\frac{\text{Integral value of the aromatic protons of the CTA}}{4} \right) \times 100$$

This DS calculation reflects the number of CTA molecules per 100 monomer units. PULLX-5 shows a DS of 2.22 in 100 monomers, calculated according to this method.

Side Reaction Mechanisms Result in Br Removal

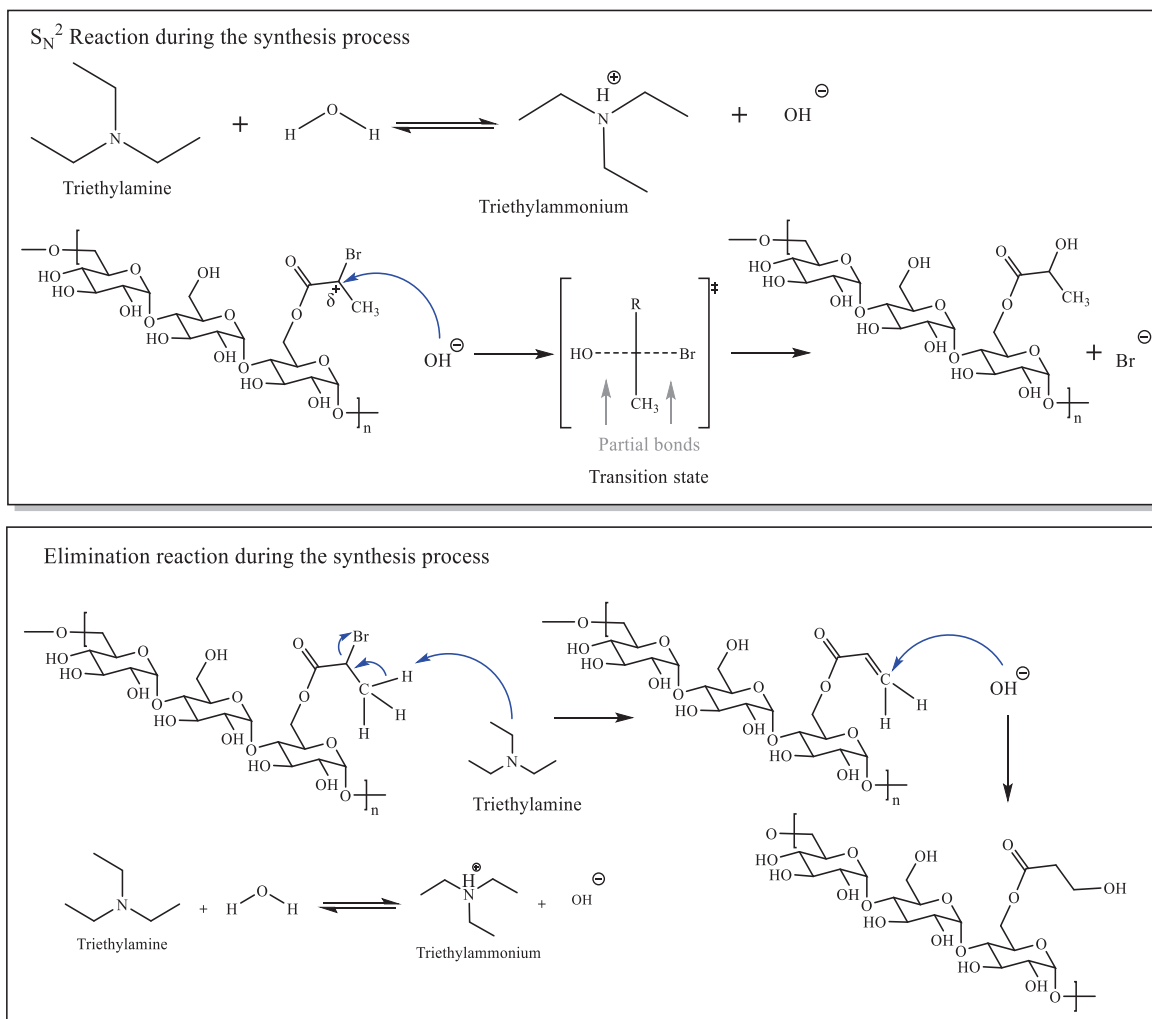


Figure-A II-5 Br removal mechanisms to form lactic acid and 3-hydroxypropionic acid may happen during other reaction steps

APPENDIX III

Supplementary information for Chapter 6

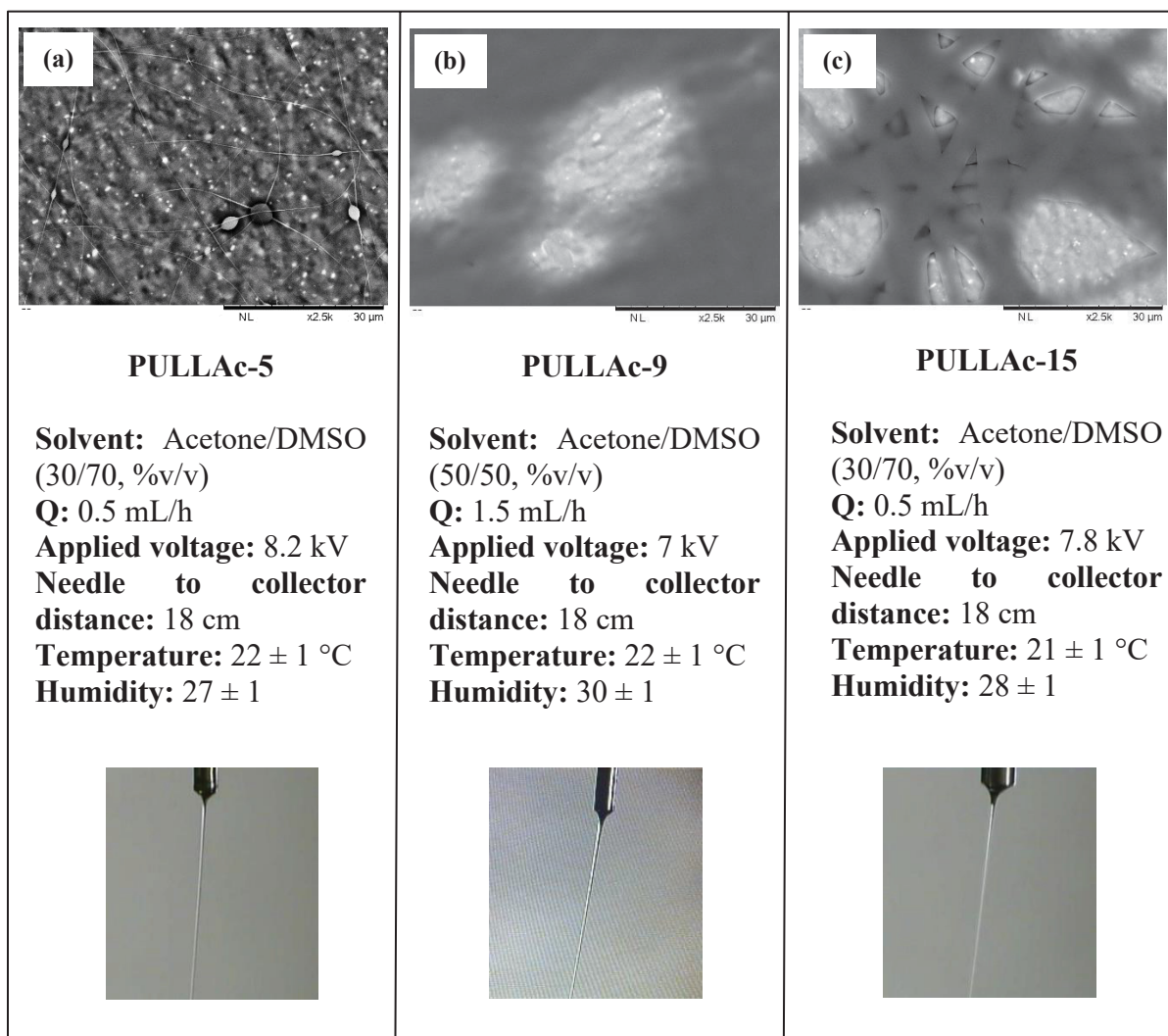


Figure-A III-1 SEM micrographs of electrospun PULLAc membranes obtained under selected processing conditions: (a) PULLAc-5 electrospun from acetone/DMSO (30/70, %v/v) at a flow rate of $0.5 \text{ mL} \cdot \text{h}^{-1}$ and a needle-to-collector distance of 18 cm, showing excessively thin fibers with pronounced bead formation (bead-on-string morphology); (b) PULLAc-9 electrospun from acetone/DMSO (50/50, %v/v) at a flow rate of $1.5 \text{ mL} \cdot \text{h}^{-1}$ and a needle-to-collector distance of 18 cm, exhibiting film formation; and (c) PULLAc-15 electrospun from acetone/DMSO (30/70, %v/v) at a flow rate of $0.5 \text{ mL} \cdot \text{h}^{-1}$ and a needle-to-collector distance of 18 cm, showing fiber coalescence. Bright white spots arise from the aluminum-foil substrate (e.g., local charging and/or topographic contrast) and do not correspond to features of the fibrous network.

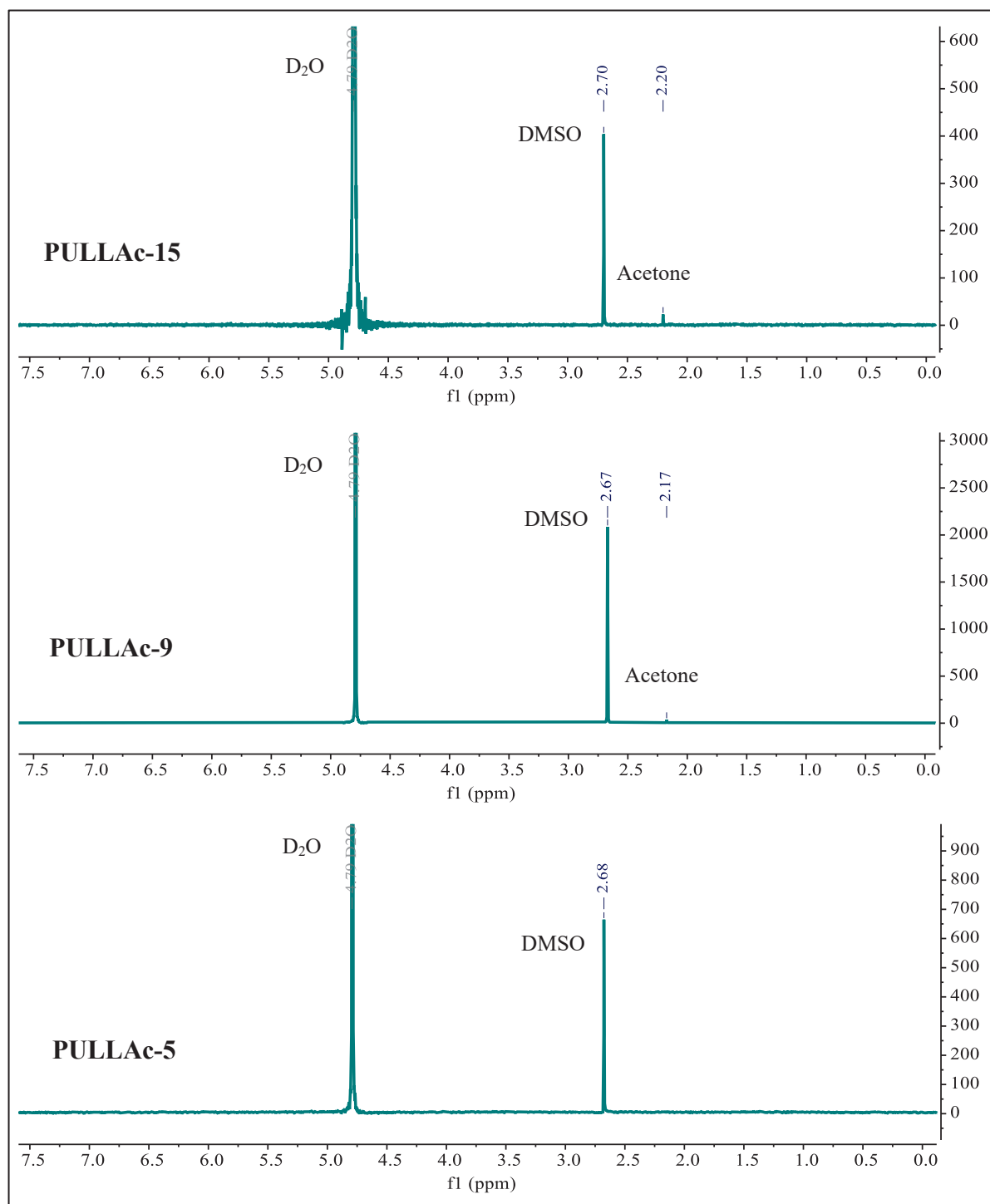


Figure-A III-2 ^1H NMR spectrum of PULLAc-5, PULLAc-9 and PULLAc-15 as-spun membrane in D_2O

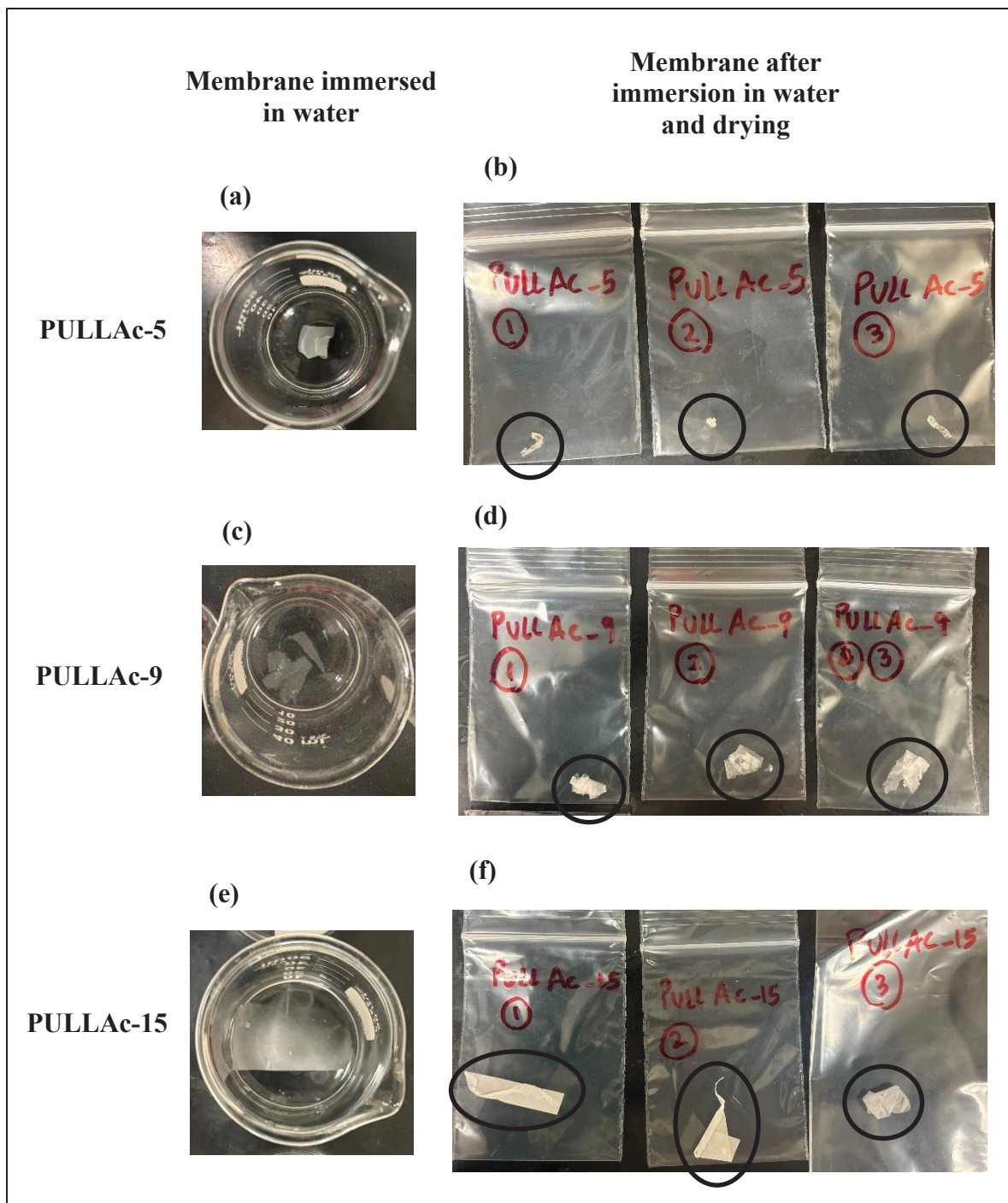


Figure-A III-3 Representative photographs of the membranes during water immersion and after drying for (a, b) PULLAc-5, (c, d) PULLAc-9, and (e, f) PULLAc-15. The dried membranes are indicated by a black circle

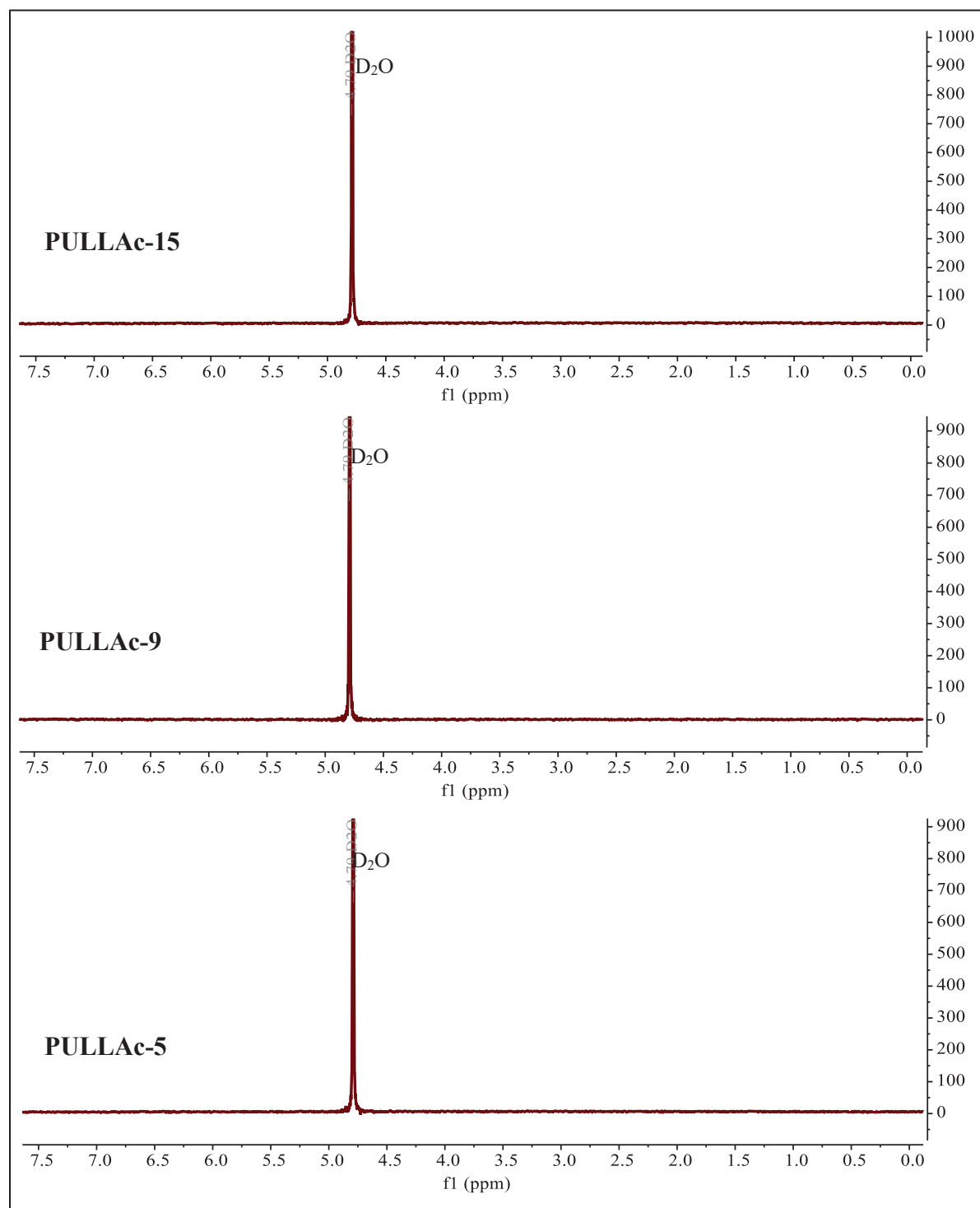


Figure-A III-4 ^1H NMR spectrum of PULLAc-5, PULLAc-9 and PULLAc-15 membranes after immersion in water and drying in D_2O

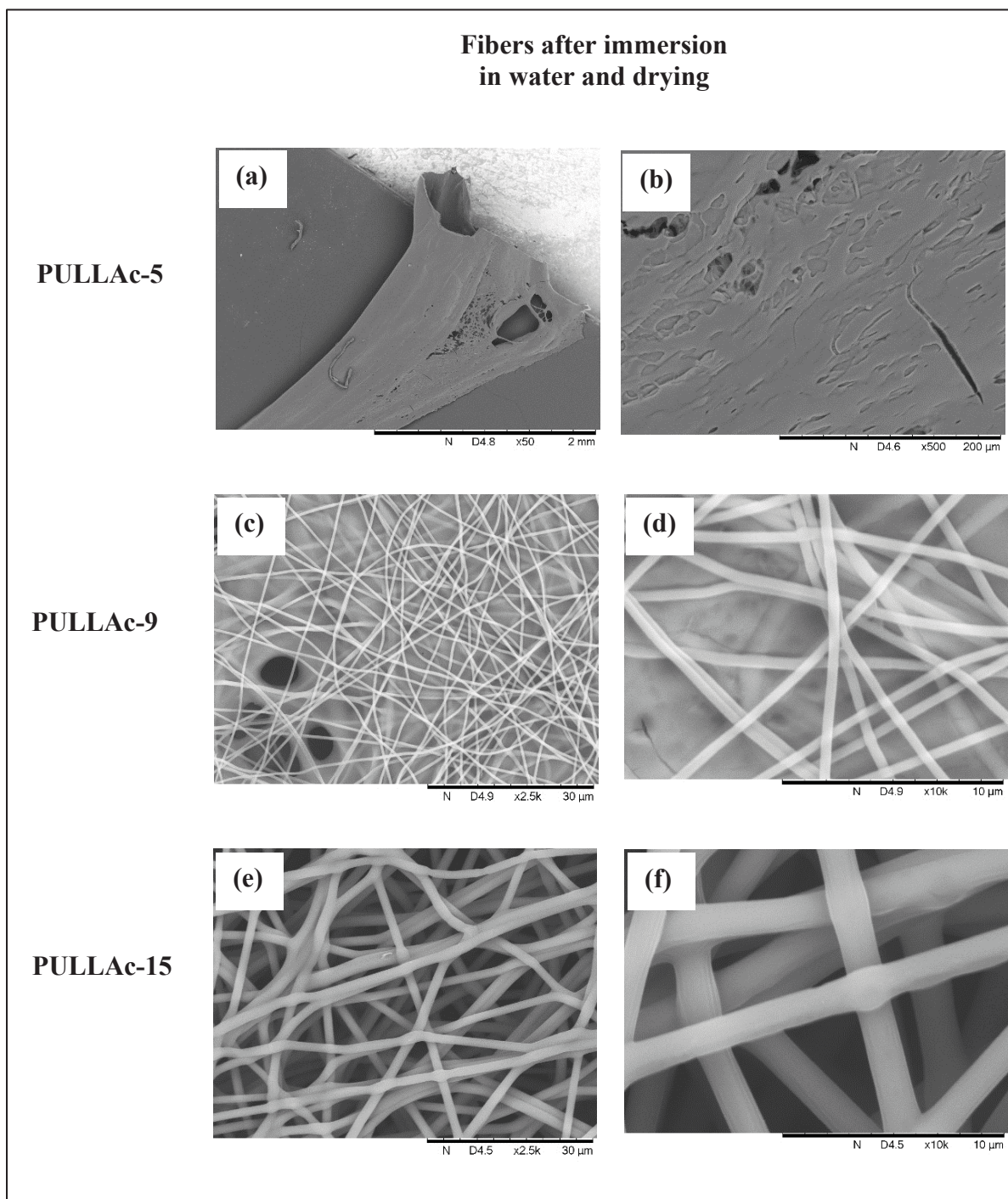


Figure-A III-5 SEM micrographs of PULLAc membranes after immersing in water for 24 hours and subsequent drying, at different magnifications: PULLAc-5: (a) $\times 50$ and (b) $\times 500$; PULLAc-9: (c) $\times 2500$ and (d) $\times 10000$; PULLAc-15: (e) $\times 2500$ and (f) $\times 10000$. Drying was performed at 40 °C under vacuum.

APPENDIX IV

VITA

EDUCATION

- *Doctor of Philosophy (Ph. D.)* – École de Technologie Supérieure (ÉTS). From September 2021 to March 2026, Montréal, QC, Canada.
- *Master of Applied Science (M.A.Sc.)* in Polymer Engineering – University of Tehran. From September 2017 to September 2020, Tehran, Iran.
- *Bachelor of Engineering (B.Eng.)* in Polymer Engineering – University of Tehran. From September 2013 to September 2017, Tehran, Iran.

AWARDS AND SCHOLARSHIPS

- Graduate Study Scholarships (Doctorate) – École de technologie supérieure (ÉTS), Montréal, Canada, 2021–2025.
- Conference Travel Award – Society of Plastics Engineers (SPE), 2025, CAD 1,000 to support attendance at the European Polymer Congress.
- Conference Travel Award (International Mobility Grant) – École de technologie supérieure (ÉTS), 2025, CAD 500 to support attendance at the Americas Regional Polymer Processing Society (PPS) Conference.
- Conference Travel Award (Research Outreach Grant) – École de technologie supérieure (ÉTS), 2025, CAD 1,000 to support attendance at the Americas Regional Polymer Processing Society (PPS) Conference.
- Mitacs Globalink Research Award (GRA) – École de technologie supérieure (ÉTS), 2023, CAD 6,000 to support conducting a research project as an internship in Brazil.
- Study Scholarships (Bachelor's and Master's) – University of Tehran, Tehran, Iran, 2013–2020.

JOURNAL PUBLICATIONS

- Sadeghi Lari, A., Silva, G. A. B., Alves, G. M., Brlek, M.B., Santos, A.M.D., & Demarquette, N. R. Synthesis and characterization of Pullulan-graft-Poly (N-vinyl caprolactam) via RAFT/MADIX polymerization. *Industrial Crops and Products* (Submitted, under review, October 2025).
- Sadeghi Lari, A., Zahedi, P., Ghourchian, H., Khatibi, A. Microfluidic-based synthesized carboxymethyl chitosan nanoparticles containing metformin for diabetes therapy: in vitro and in vivo assessments. *Carbohydrate polymers*, 2021, 261, 117889. <https://doi.org/10.1016/j.carbpol.2021.117889>.
- Sadeghi Lari, A., Khatibi, A., Zahedi, P., Ghourchian, H. Microfluidic-assisted production of poly (ϵ -caprolactone) and cellulose acetate nanoparticles: Effects of polymers, surfactants, and flow rate ratios. *Polymer Bulletin*, 2021, 78, 5449-5466. <https://doi.org/10.1007/s00289-020-03367-1>.
- Khatibi, A., Zahedi, P., Ghourchian, H., Sadeghi Lari, A. Development of microfluidic-based cellulose acetate phthalate nanoparticles containing omeprazole for antiulcer activity: in vitro and in vivo evaluations. *European Polymer Journal*, 2021, 147, 110294. <https://doi.org/10.1016/j.eurpolymj.2021.110294>.

CONFERENCE PRESENTATIONS

- 2025 – Americas Regional Conference of the Polymer Processing Society (PPS), Guelph, Canada. Oral presentation: Synthesis of Pullulan-g-Poly (N-vinyl caprolactam) via RAFT Polymerization.
- 2025 – European Polymer Congress (EPF), Groningen, Netherlands. Oral presentation: Synthesis of Pullulan-g-Poly (N-vinyl caprolactam).
- 2025 – CREPEC Student Colloquium (Centre for Research on High-Performance Polymer and Composite Systems), Université de Montréal, Canada. Poster presentation: Modification of Pullulan to Temperature Sensitivity, Towards Drug Delivery Systems.

- 2024 – CREPEC Student Colloquium (Centre for Research on High-Performance Polymer and Composite Systems), Concordia University, Canada. Poster presentation: Modification of Pullulan to Temperature Sensitivity, Towards Drug Delivery Systems.
- 2023 – 17th Polymer Week Conference, Professor Eloisa Mano Institute of Macromolecules, Federal University of Rio de Janeiro, Brazil. Poster presentation: Investigating the Effect of Electrospinning Parameters on the Pullulan Nanofibers.

LIST OF BIBLIOGRAPHICAL REFERENCES

- Agrawal, S., Budhwani, D., Gurjar, P., Telange, D., & Lambole, V. (2022). Pullulan based derivatives: Synthesis, enhanced physicochemical properties, and applications. *Drug delivery*, 29(1), 3328-3339.
- Ahmadi Bonakdar, M., & Rodrigue, D. (2024). Electrospinning: Processes, structures, and materials. *Macromol*, 4(1), 58-103.
- Ai, C., Liu, S., Zhao, Y., Huang, X., Teng, H., Li, S., & Chen, L. (2024). Hybrid electrospinning of pullulan and citrus pectin to load astaxanthin: Preparation, characterization, and functional evaluation. *Food Chemistry: X*, 24, 101990.
- Akiyoshi, K., Kang, E.-C., Kurumada, S., Sunamoto, J., Principi, T., & Winnik, F. M. (2000). Controlled Association of Amphiphilic Polymers in Water: Thermosensitive Nanoparticles Formed by Self-Assembly of Hydrophobically Modified Pullulans and Poly(N-isopropylacrylamides). *Macromolecules*, 33(9), 3244-3249. <https://doi.org/10.1021/ma991798d>
- Al-Abduljabbar, A., & Farooq, I. (2023). Electrospun Polymer Nanofibers: Processing, Properties, and Applications. *Polymers*, 15(1). <https://doi.org/10.3390/polym15010065>
- Alban, S., Schauerte, A., & Franz, G. (2002). Anticoagulant sulfated polysaccharides: Part I. Synthesis and structure–activity relationships of new pullulan sulfates. *Carbohydrate Polymers*, 47(3), 267-276. [https://doi.org/https://doi.org/10.1016/S0144-8617\(01\)00178-3](https://doi.org/https://doi.org/10.1016/S0144-8617(01)00178-3)
- Amjadi, S., Gholizadeh, S., Ebrahimi, A., Almasi, H., Hamishehkar, H., & Taheri, R. A. (2022). Development and characterization of the carvone-loaded zein/pullulan hybrid electrospun nanofibers for food and medical applications. *Industrial Crops and Products*, 183, 114964. <https://doi.org/https://doi.org/10.1016/j.indcrop.2022.114964>
- Arkaban, H., Barani, M., Akbarizadeh, M. R., Pal Singh Chauhan, N., Jadoun, S., Dehghani Soltani, M., & Zarrintaj, P. (2022). Polyacrylic acid nanoplatfoms: antimicrobial, tissue engineering, and cancer theranostic applications. *Polymers*, 14(6), 1259.
- Basu, S., Agrawal, A. K., & Jassal, M. (2011). Concept of minimum electrospinning voltage in electrospinning of polyacrylonitrile N, N-dimethylformamide system. *Journal of Applied Polymer Science*, 122(2), 856-866.

- Beaudoin, É. (2025). Extensional rheology and electric field control: strategies for electrospinning scale-up.
- Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: A fascinating fiber fabrication technique. *Biotechnology Advances*, 28(3), 325-347. <https://doi.org/https://doi.org/10.1016/j.biotechadv.2010.01.004>
- Bhattacharya, A., & Misra, B. N. (2004). Grafting: a versatile means to modify polymers: Techniques, factors and applications. *Progress in Polymer Science*, 29(8), 767-814. <https://doi.org/https://doi.org/10.1016/j.progpolymsci.2004.05.002>
- Binauld, S., Delafresnaye, L., Charleux, B., D'Agosto, F., & Lansalot, M. (2014). Emulsion Polymerization of Vinyl Acetate in the Presence of Different Hydrophilic Polymers Obtained by RAFT/MADIX. *Macromolecules*, 47(10), 3461-3472. <https://doi.org/10.1021/ma402549x>
- Bontempo, D., Masci, G., De Leonardis, P., Mannina, L., Capitani, D., & Crescenzi, V. (2006). Versatile Grafting of Polysaccharides in Homogeneous Mild Conditions by Using Atom Transfer Radical Polymerization. *Biomacromolecules*, 7(7), 2154-2161. <https://doi.org/10.1021/bm0601373>
- Borban, B., Gohain, M. B., Yadav, D., Karki, S., & Ingole, P. G. (2023). Nano-electrospun membranes: Green solutions for diverse industrial needs. *Journal of Hazardous Materials Advances*, 12, 100373.
- Bucătariu, S., Stoica, I., & Fundueanu, G. (2017). Smart nanoparticles based on pullulan-g-poly(N-isopropylacrylamide) for controlled delivery of indomethacin. *International Journal of Biological Macromolecules*, 94, 698-708. <https://doi.org/10.1016/j.ijbiomac.2016.10.064>
- Carvalho, L. T., Moraes, R. M., Alves, G. M., Lacerda, T. M., Santos, J. C., Santos, A. M., & Medeiros, S. F. (2020). Synthesis of amphiphilic pullulan-graft-poly(ϵ -caprolactone) via click chemistry. *International Journal of Biological Macromolecules*, 145, 701-711. <https://doi.org/10.1016/j.ijbiomac.2019.12.207>
- Carvalho, L. T., Teixeira, A. J. R. M., Moraes, R. M., Barbosa, R. F. S., Queiroz, R. C., Tada, D. B., ... Medeiros, S. F. (2022). Preparation and characterization of cationic pullulan-based polymers with hydrophilic or amphiphilic characteristics for drug delivery. *Reactive and Functional Polymers*, 181, 105441. <https://doi.org/10.1016/j.reactfunctpolym.2022.105441>

- Chemama, T., Pumas, C., Tragoolpua, Y., & Thongwai, N. (2020). Feasibility study for D-lactic acid production from thai rice by *Leuconostoc pseudomesenteroides* TC49 and D-lactic acid purification. *Chiang Mai Journal of Science*, 47(3), 403-417.
- Cheng, K.-C., Demirci, A., & Catchmark, J. M. (2011). Pullulan: biosynthesis, production, and applications. *Applied microbiology and biotechnology*, 92(1), 29-44.
- Chiefari, J., Chong, Y. K. (Bill), Ercole, F., Krstina, J., Jeffery, J., Le, T. P. T., ... Thang, S. H. (1998). Living Free-Radical Polymerization by Reversible Addition–Fragmentation Chain Transfer: The RAFT Process. *Macromolecules*, 31(16), 5559-5562. <https://doi.org/10.1021/ma9804951>
- Cho, Y., Baek, J. W., Sagong, M., Ahn, S., Nam, J. S., & Kim, I. (2025). Electrospinning and nanofiber technology: fundamentals, innovations, and applications. *Advanced Materials*, 37(28), 2500162.
- Coltelli, M.-B., Danti, S., De Clerck, K., Lazzeri, A., & Morganti, P. (2020). Pullulan for advanced sustainable body-and skin-contact applications. *Journal of Functional Biomaterials*, 11(1), 20.
- Constantin, M., Cosman, B., Bercea, M., Ailiesei, G.-L., & Fundueanu, G. (2021). Thermosensitive poloxamer-graft-carboxymethyl pullulan: A potential injectable hydrogel for drug delivery. *Polymers*, 13(18), 3025.
- Constantin, M., Mihalcea, I., Oanea, I., Harabagiu, V., & Fundueanu, G. (2011). Studies on graft copolymerization of 3-acrylamidopropyl trimethylammonium chloride on pullulan. *Carbohydrate Polymers*, 84(3), 926-932. <https://doi.org/10.1016/j.carbpol.2010.12.043>
- Corrigan, N., Jung, K., Moad, G., Hawker, C. J., Matyjaszewski, K., & Boyer, C. (2020). Reversible-deactivation radical polymerization (Controlled/living radical polymerization): From discovery to materials design and applications. *Progress in Polymer Science*, 111, 101311. <https://doi.org/https://doi.org/10.1016/j.progpolymsci.2020.101311>
- Cortez-Lemus, N. A., & Licea-Claverie, A. (2016). Poly(N-vinylcaprolactam), a comprehensive review on a thermoresponsive polymer becoming popular. *Progress in Polymer Science*, 53, 1-51. <https://doi.org/https://doi.org/10.1016/j.progpolymsci.2015.08.001>

- Cortez-Lemus, N. A., & Licea-Claverie, A. (2016). Synthesis and characterization of “Living” star-shaped poly(*N*-vinylcaprolactam) with four arms and carboxylic acid end groups. *Journal of Polymer Science Part A: Polymer Chemistry*, 54(14), 2156-2165. <https://doi.org/10.1002/pola.28086>
- Dalei, G., & Das, S. (2022). Polyacrylic acid-based drug delivery systems: A comprehensive review on the state-of-art. *Journal of Drug Delivery Science and Technology*, 78, 103988. <https://doi.org/10.1016/j.jddst.2022.103988>
- De Vrieze, S., Van Camp, T., Nelvig, A., Hagström, B., Westbroek, P., & De Clerck, K. (2009). The effect of temperature and humidity on electrospinning. *Journal of materials science*, 44(5), 1357-1362.
- Deitzel, J. M., Kleinmeyer, J., Harris, D., & Tan, N. B. (2001). The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, 42(1), 261-272.
- de Souza, C. K., Ghosh, T., Lukhmana, N., Tahiliani, S., Priyadarshi, R., Hoffmann, T. G., ... Han, S. S. (2023). Pullulan as a sustainable biopolymer for versatile applications: A review. *Materials Today Communications*, 36, 106477.
- Destarac, M. (2011). On the critical role of RAFT agent design in reversible addition-fragmentation chain transfer (RAFT) polymerization. *Polymer Reviews*, 51(2), 163-187.
- Diab, C., Akiyama, Y., Kataoka, K., & Winnik, F. M. (2004). Microcalorimetric Study of the Temperature-Induced Phase Separation in Aqueous Solutions of Poly(2-isopropyl-2-oxazolines). *Macromolecules*, 37(7), 2556-2562. <https://doi.org/10.1021/ma0358733>
- Ding, Y., Jiang, F., Chen, L., Lyu, W., Chi, Z., Liu, C., & Chi, Z. (2020). An Alternative Hard Capsule Prepared with the High Molecular Weight Pullulan and Gellan: Processing, Characterization, and In Vitro Drug Release. *Carbohydrate Polymers*, 237, 116172. <https://doi.org/https://doi.org/10.1016/j.carbpol.2020.116172>
- Dionísio, M., Braz, L., Corvo, M., Lourenço, J. P., Grenha, A., & Costa, A. M. R. da. (2016). Charged pullulan derivatives for the development of nanocarriers by polyelectrolyte complexation. *International Journal of Biological Macromolecules*, 86, 129-138. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2016.01.054>
- Dionísio, Marita, Cordeiro, C., Remuñán-López, C., Seijo, B., Costa, A. M. R. da, & Grenha, A. (2013). Pullulan-based nanoparticles as carriers for transmucosal protein delivery.

- European Journal of Pharmaceutical Sciences*, 50(1), 102-113. <https://doi.org/https://doi.org/10.1016/j.ejps.2013.04.018>
- Dong, Q., Luo, C., Li, N., Chi, J., & Zhang, Q. (2018). Temperature and Recognition Dual Responsive Poly(N-Isopropylacrylamide) and Poly(N,N-Dimethylacrylamide) with Adamantyl Side Group. *Materials*, 11(4), 473. <https://doi.org/10.3390/ma11040473>
- El Ghoul, Y., Ammar, C., & Alsultan, R. M. (2025). Polysaccharide Chemical Functionalization: Innovative Advances, Techniques, Overview and Biological Applications. *Egyptian Journal of Chemistry*.
- Elangwe, C. N., Uspenskaya, M. V., & Olekhovich, R. O. (2025). Tunable hydrogel properties: effect of polymer ratio in chitosan/oxidized pullulan adhesive networks. *Macromolecular Research*, 1-16.
- Elfawal, G. F., Šišková, A. O., & Andicsová, A. E. (2025). Electrospinning: A game-changer in fiber production and practical applications. *Fibers and Polymers*, 1-28.
- Enomoto-Rogers, Y., Iio, N., Takemura, A., & Iwata, T. (2015a). Synthesis and characterization of pullulan alkyl esters. *European Polymer Journal*, 66, 470-477. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2015.03.007>
- Erceg, T., Aćimović, M., Šovljanski, O., Lončar, B., Tomić, A., Pavlović, M., ... Hadnadev, M. (2024). Preparation and characterization of carboxymethylated pullulan/butyric acid-modified chitosan active sustainable bi-layer coatings intended for packaging of cheese slices. *International Journal of Biological Macromolecules*, 277, 134053. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2024.134053>
- Etchenausia, L., Khoukh, A., Deniau Lejeune, E., & Save, M. (2017). RAFT/MADIX emulsion copolymerization of vinyl acetate and N-vinylcaprolactam: towards waterborne physically crosslinked thermoresponsive particles. *Polym. Chem.*, 8(14), 2244-2256. <https://doi.org/10.1039/C7PY00221A>
- Fernández-García, C., Grefenstette, N. M., & Powner, M. W. (2017). Prebiotic synthesis of aminooxazoline-5'-phosphates in water by oxidative phosphorylation. *Chem. Commun.*, 53(36), 4919-4921. <https://doi.org/10.1039/C7CC02183F>
- Fernández-Quiroz, D., González-Gómez, Á., Lizardi-Mendoza, J., Vázquez-Lasa, B., Goycoolea, F. M., Román, J. S., & Argüelles-Monal, W. M. (2015). Effect of the molecular architecture on the thermosensitive properties of chitosan-g-poly(N-

- vinylcaprolactam). *Carbohydrate Polymers*, 134, 92-101.
<https://doi.org/https://doi.org/10.1016/j.carbpol.2015.07.069>
- Fleet, R., McLeary, J. B., Grumel, V., Weber, W. G., Matahwa, H., & Sanderson, R. D. (2008). RAFT mediated polysaccharide copolymers. *European Polymer Journal*, 44(9), 2899-2911. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2008.06.042>
- Ganeshkumar, M., Ponrasu, T., Subamekala, M. K., Janani, M., & Suguna, L. (2016). Curcumin loaded on pullulan acetate nanoparticles protects the liver from damage induced by DEN. *RSC advances*, 6(7), 5599-5610.
- Ganie, S. A., Rather, L. J., Assiri, M. A., & Li, Q. (2024). Recent innovations (2020–2023) in the approaches for the chemical functionalization of curdlan and pullulan: A mini-review. *International journal of biological macromolecules*, 260, 129412.
- González, E., & Frey, M. W. (2017). Synthesis, characterization and electrospinning of poly(vinyl caprolactam-co-hydroxymethyl acrylamide) to create stimuli-responsive nanofibers. *Polymer*, 108, 154-162.
<https://doi.org/https://doi.org/10.1016/j.polymer.2016.11.053>
- Gopinath, V., Saravanan, S., Al-Maleki, A., Ramesh, M., & Vadivelu, J. (2018). A review of natural polysaccharides for drug delivery applications: Special focus on cellulose, starch and glycogen. *Biomedicine & Pharmacotherapy*, 107, 96-108.
- Grunlan, J. C., Liu, L., & Kim, Y. S. (2006). Tunable single-walled carbon nanotube microstructure in the liquid and solid states using poly (acrylic acid). *Nano letters*, 6(5), 911-915.
- Guerrini, L. M., Oliveira, M. P., Stapait, C. C., Maric, M., Santos, A. M., & Demarquette, N. R. (2021a). Evaluation of different solvents and solubility parameters on the morphology and diameter of electrospun pullulan nanofibers for curcumin entrapment. *Carbohydrate Polymers*, 251, 117127.
<https://doi.org/https://doi.org/10.1016/j.carbpol.2020.117127>
- Gupta, C., & Washburn, N. R. (2014). Polymer-grafted lignin surfactants prepared via reversible addition–fragmentation chain-transfer polymerization. *Langmuir*, 30(31), 9303-9312.
- Gupta, S., Singh, A., & Matsumi, N. (2019). Controlled Phase Behavior of Thermally Sensitive Poly(N-isopropylacrylamide/ionic liquid) with Embedded Au Nanoparticles. *ACS Omega*, 4(25), 20923-20930. <https://doi.org/10.1021/acsomega.9b01826>

- Gürdağ, G., & Sarmad, S. (2013). Cellulose Graft Copolymers: Synthesis, Properties, and Applications. Dans S. Kalia & M. W. Sabaa (Éds), *Polysaccharide Based Graft Copolymers* (pp. 15-57). Berlin, Heidelberg : Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-36566-9_2
- Haider, A., Haider, S., & Kang, I.-K. (2018). A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arabian Journal of Chemistry*, 11(8), 1165-1188. <https://doi.org/https://doi.org/10.1016/j.arabjc.2015.11.015>
- Hawthorne, D. G., Moad, G., Rizzardo, E., & Thang, S. H. (1999). Living radical polymerization with reversible addition– fragmentation chain transfer (RAFT): Direct ESR observation of intermediate radicals. *Macromolecules*, 32(16), 5457-5459.
- Hernández-Guerrero, M., Davis, T. P., Barner-Kowollik, C., & Stenzel, M. H. (2005). Polystyrene comb polymers built on cellulose or poly(styrene-co-2-hydroxyethylmethacrylate) backbones as substrates for the preparation of structured honeycomb films. *European Polymer Journal*, 41(10), 2264-2277. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2005.04.040>
- Hernandez-Tenorio, F., & Giraldo-Estrada, C. (2022). Characterization and chemical modification of pullulan produced from a submerged culture of *Aureobasidium pullulans* ATCC 15233. *Polymer Testing*, 114, 107686.
- Hernandez-Tenorio, F., Saez, A. A., Palacio, D. A., Galeano, E., Marin-Palacio, L. D., & Giraldo-Estrada, C. (2024). Formulations based on pullulan and a derivative as coating material for the food sector. *Carbohydrate Polymers*, 342, 122393. <https://doi.org/https://doi.org/10.1016/j.carbpol.2024.122393>
- Hezarkhani, M., Ustürk, S., Özbilenler, C., & Yilmaz, E. (2024). pH-Responsive Pullulan Based Adsorbent Functionalized by Poly (N-vinylimidazole): Synthesis, Characterization and Dye Removal Application. *Journal of Water Chemistry and Technology*, 46(5), 531-542.
- Hezarkhani, M., & Yilmaz, E. (2019). Pullulan modification via poly(N-vinylimidazole) grafting. *International Journal of Biological Macromolecules*, 123, 149-156. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2018.11.022>
- Hou, L., & Wu, P. (2015). Comparison of LCST-transitions of homopolymer mixture, diblock and statistical copolymers of NIPAM and VCL in water. *Soft Matter*, 11(14), 2771-2781. <https://doi.org/10.1039/C5SM00026B>

- Hua, D., Tang, J., Cheng, J., Deng, W., & Zhu, X. (2008). A novel method of controlled grafting modification of chitosan via RAFT polymerization using chitosan-RAFT agent. *Carbohydrate Polymers*, 73(1), 98-104. <https://doi.org/https://doi.org/10.1016/j.carbpol.2007.11.008>
- Ibrahim, H. M., & Klingner, A. (2020). A review on electrospun polymeric nanofibers: Production parameters and potential applications. *Polymer Testing*, 90, 106647.
- İÇOĞLU, H. İ., & OĞULATA, R. T. (2013). EFFECT OF AMBIENT PARAMETERS ON MORPHOLOGY OF ELECTROSPUN POLYETHERIMIDE (PEI) FIBERS. *Journal of Textile & Apparel/Tekstil ve Konfeksiyon*, 23(4).
- Ikkene, D., Arteni, A. A., Song, H., Laroui, H., Six, J.-L., & Ferji, K. (2020). Synthesis of dextran-based chain transfer agent for RAFT-mediated polymerization and glyco-nanoobjects formulation. *Carbohydrate Polymers*, 234, 115943. <https://doi.org/https://doi.org/10.1016/j.carbpol.2020.115943>
- Ilić, Lj., Jeremić, K., & Jovanović, S. (1991). Kinetics of pullulan depolymerization in hydrochloric acid. *European Polymer Journal*, 27(11), 1227-1229. [https://doi.org/10.1016/0014-3057\(91\)90059-W](https://doi.org/10.1016/0014-3057(91)90059-W)
- Jacobs, V., Anandjiwala, R. D., & Maaza, M. (2010). The influence of electrospinning parameters on the structural morphology and diameter of electrospun nanofibers. *Journal of applied polymer science*, 115(5), 3130-3136.
- Ji, J., Jia, L., Yan, L., & Bangal, P. R. (2010). Efficient synthesis of poly (acrylic acid) in aqueous solution via a RAFT process. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 47(5), 445-451.
- Jiang, B., Zhang, L., Yan, J., Huang, Q., Liao, B., & Pang, H. (2014). Effects of graft length and density of well-defined graft polymers on the thermoresponsive behavior and self-assembly morphology. *Journal of Polymer Science Part A: Polymer Chemistry*, 52(17), 2442-2453. <https://doi.org/10.1002/pola.27256>
- Jiang, X., Li, Y., Lu, G., & Huang, X. (2013). A novel poly(N-vinylcaprolactam)-based well-defined amphiphilic graft copolymer synthesized by successive RAFT and ATRP. *Polym. Chem.*, 4(5), 1402-1411. <https://doi.org/10.1039/C2PY20933K>
- Jiao, Y., Fu, Y., & Jiang, Z. (2004). The synthesis and characterization of poly(ethylene glycol) grafted on pullulan. *Journal of Applied Polymer Science*, 91(2), 1217-1221. <https://doi.org/10.1002/app.13238>

- Jung, S.-W., Jeong, Y., Kim, Y.-H., & Kim, S.-H. (2004). Self-assembled polymeric nanoparticles of poly (ethylene glycol) grafted pullulan acetate as a novel drug carrier. *Archives of pharmacal research*, 27(5), 562-569.
- Jung, S.-W., Jeong, Y.-I., & Kim, S.-H. (2003a). Characterization of hydrophobized pullulan with various hydrophobicities. *International Journal of Pharmaceutics*, 254(2), 109-121. [https://doi.org/https://doi.org/10.1016/S0378-5173\(03\)00006-1](https://doi.org/https://doi.org/10.1016/S0378-5173(03)00006-1)
- Kang, H., Liu, W., He, B., Shen, D., Ma, L., & Huang, Y. (2006). Synthesis of amphiphilic ethyl cellulose grafting poly(acrylic acid) copolymers and their self-assembly morphologies in water. *Polymer*, 47(23), 7927-7934. <https://doi.org/https://doi.org/10.1016/j.polymer.2006.09.017>
- Karim, M. R., Lee, H. W., Kim, R., Ji, B. C., Cho, J. W., Son, T. W., ... Yeum, J. H. (2009). Preparation and characterization of electrospun pullulan/montmorillonite nanofiber mats in aqueous solution. *Carbohydrate Polymers*, 78(2), 336-342. <https://doi.org/https://doi.org/10.1016/j.carbpol.2009.04.024>
- Karmakar, P. D., & Pal, S. (2021). Dextran based amphiphilic self-assembled biopolymeric macromolecule synthesized via RAFT polymerization as indomethacin carrier. *International Journal of Biological Macromolecules*, 183, 718-726. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2021.04.145>
- Kim, Y. K., Park, S. L., Lee, J. D., & Son, S. U. (2024). Hexagonal CeO₂ nanoplate@Au catalysts for the selective oxidation of 1,3-propanediol to a polymer platform, 3-hydroxypropionic acid at room temperature. *J. Mater. Chem. A*, 12(46), 31825-31832. <https://doi.org/10.1039/D4TA06419D>
- Ko, C.-H., Claude, K.-L., Niebuur, B.-J., Jung, F. A., Kang, J.-J., Schanzenbach, D., ... Papadakis, C. M. (2020). Temperature-Dependent Phase Behavior of the Thermoresponsive Polymer Poly(N-isopropylmethacrylamide) in an Aqueous Solution. *Macromolecules*, 53(16), 6816-6827. <https://doi.org/10.1021/acs.macromol.0c01256>
- Kong, L., & Ziegler, G. R. (2014). Rheological aspects in fabricating pullulan fibers by electro-wet-spinning. *Food Hydrocolloids*, 38, 220-226. <https://doi.org/https://doi.org/10.1016/j.foodhyd.2013.12.016>
- Kotomin, S., Malkin, A., & Skvortsov, I. (2020). Electrospinning and mechanotropic phenomena in polymer solutions (Vol. 389, p. 1900091). Communication présentée au Macromolecular Symposia, Wiley Online Library.

- Krivorotova, T., Jonikaite-Svegziene, J., Radzevicius, P., & Makuska, R. (2014). Synthesis by RAFT polymerization and properties of anionic cylindrical molecular brushes bearing poly (acrylic acid) side chains. *Reactive and Functional Polymers*, 76, 32-40.
- Ladavière, C., Dörr, N., & Claverie, J. P. (2001). Controlled Radical Polymerization of Acrylic Acid in Protic Media. *Macromolecules*, 34(16), 5370-5372. <https://doi.org/10.1021/ma010358v>
- Laukkanen, A., Valtola, L., Winnik, F. M., & Tenhu, H. (2005). Thermosensitive graft copolymers of an amphiphilic macromonomer and N-vinylcaprolactam: synthesis and solution properties in dilute aqueous solutions below and above the LCST. *Polymer*, 46(18), 7055-7065. <https://doi.org/https://doi.org/10.1016/j.polymer.2005.05.100>
- Legros, M., Cardinael, P., Dulong, V., Picton, L., & Le Cerf, D. (2008). Metastable amphiphilic hydrogels based on crosslinked carboxymethylpullulan. *Polymer journal*, 40(3), 233-240.
- Leonardis, P. D., Mannina, L., Diociaiuti, M., & Masci, G. (2010). Atom transfer radical polymerization synthesis and association properties of amphiphilic pullulan copolymers grafted with poly (methyl methacrylate). *Polymer international*, 59(6), 759-765.
- Li, R., Tomasula, P., De Sousa, A. M. M., Liu, S.-C., Tunick, M., Liu, K., & Liu, L. (2017). Electrospinning pullulan fibers from salt solutions. *Polymers*, 9(1), 32.
- Li, S., Kong, L., & Ziegler, G. R. (2021). Electrospinning of Octenylsuccinylated Starch-Pullulan Nanofibers from Aqueous Dispersions. *Carbohydrate Polymers*, 258, 116933. <https://doi.org/https://doi.org/10.1016/j.carbpol.2020.116933>
- Li, X., Liu, Y., Huang, Y., Wang, F., Feng, X., Zhu, B., ... Wang, Y. (2023). Preparation and characterization of edible pullulan/pectin nanofiber substrates and their digestion in simulated gastric and intestinal fluids. *Industrial Crops and Products*, 206, 117745. <https://doi.org/https://doi.org/10.1016/j.indcrop.2023.117745>
- Li, Y., Zheng, X., Zhu, H., Wu, K., & Lu, M. (2015). Synthesis and self-assembly of well-defined binary graft copolymer and its use in superhydrophobic cotton fabrics preparation. *RSC Adv.*, 5(57), 46132-46145. <https://doi.org/10.1039/C5RA06657C>
- Liang, Y., Zhao, X., Ma, P. X., Guo, B., Du, Y., & Han, X. (2019). pH-responsive injectable hydrogels with mucosal adhesiveness based on chitosan-grafted-dihydrocaffeic acid

- and oxidized pullulan for localized drug delivery. *Journal of Colloid and Interface Science*, 536, 224-234. <https://doi.org/https://doi.org/10.1016/j.jcis.2018.10.056>
- Lim, C. T. (2017). Nanofiber technology: current status and emerging developments. *Progress in polymer science*, 70, 1-17.
- Liu, J., Wang, Y., Fu, Q., Zhu, X., & Shi, W. (2008). Branched polymer via free radical polymerization of chain transfer monomer: A theoretical and experimental investigation. *Journal of Polymer Science Part A: Polymer Chemistry*, 46(4), 1449-1459. <https://doi.org/10.1002/pola.22484>
- Liu, R., Fraylich, M., & Saunders, B. R. (2009). Thermoresponsive copolymers: from fundamental studies to applications. *Colloid and Polymer Science*, 287(6), 627-643.
- Liu, X., Xu, Y., Yu, J., Li, S., Wang, J., Wang, C., & Chu, F. (2014). Integration of lignin and acrylic monomers towards grafted copolymers by free radical polymerization. *International Journal of Biological Macromolecules*, 67, 483-489. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2014.04.005>
- Llauro, M., Loiseau, J., Boisson, F., Delolme, F., Ladavière, C., & Claverie, J. (2004). Unexpected end-groups of poly (acrylic acid) prepared by RAFT polymerization. *Journal of Polymer Science Part A: Polymer Chemistry*, 42(21), 5439-5462.
- Loiseau, J., Doërr, N., Suau, J., Egraz, J., Llauro, M., Ladavière, C., & Claverie, J. (2003a). Synthesis and characterization of poly (acrylic acid) produced by RAFT polymerization. Application as a very efficient dispersant of CaCO₃, kaolin, and TiO₂. *Macromolecules*, 36(9), 3066-3077.
- Lu, D., Xiao, C., & Sun, F. (2012). Controlled grafting of poly(vinyl acetate) onto starch via RAFT polymerization. *Journal of Applied Polymer Science*, 124(4), 3450-3455. <https://doi.org/10.1002/app.35423>
- Lupo, C., Boulos, S., & Nyström, L. (2020). Influence of Partial Acid Hydrolysis on Size, Dispersity, Monosaccharide Composition, and Conformation of Linearly-Branched Water-Soluble Polysaccharides. *Molecules*, 25(13). <https://doi.org/10.3390/molecules25132982>
- Ma, Y., Tian, R., Song, Y., Yin, C., Huang, H., & Li, Y. (2025). Electrospun nanofiber membranes based on dialdehyde pullulan polysaccharide and schiff base crosslinked lysozyme for antibacterial packaging of blueberries. *Food Bioscience*, 67, 106348. <https://doi.org/https://doi.org/10.1016/j.fbio.2025.106348>

- Mahdian-Dehkordi, M., Sarrafzadeh-Rezaei, F., Razi, M., & Mahmoudian, M. (2021). Fabrication of chitosan-based electrospun nanofiber scaffold: Amplification of biomechanical properties, structural stability, and seeded cell viability (Vol. 12, p. 25). Communication présentée au Veterinary Research Forum.
- Mähner, C., Lechner, M. D., & Nordmeier, E. (2001). Synthesis and characterisation of dextran and pullulan sulphate. *Carbohydrate Research*, 331(2), 203-208. [https://doi.org/https://doi.org/10.1016/S0008-6215\(00\)00315-3](https://doi.org/https://doi.org/10.1016/S0008-6215(00)00315-3)
- Maia-Obi, L. P., & Bazito, R. C. (2025). Effect of Acetylation on the Behavior of Hyperbranched Polyglycerols in Supercritical CO₂. *Processes*, 13(8). <https://doi.org/10.3390/pr13082510>
- Matyjaszewski, K., & Davis, T. P. (2002). *Handbook of radical polymerization* (Vol. 922). (S.I.) : Wiley Online Library.
- Medeiros, S. F., Santos, A. M., Fessi, H., & Elaissari, A. (2011). Stimuli-responsive magnetic particles for biomedical applications. *International Journal of Pharmaceutics*, 403(1), 139-161. <https://doi.org/https://doi.org/10.1016/j.ijpharm.2010.10.011>
- Moad, G., Chiefari, J., Chong, Y. K., Krstina, J., Mayadunne, R. T. A., Postma, A., ... Thang, S. H. (2000). Living free radical polymerization with reversible addition–fragmentation chain transfer (the life of RAFT). *Polymer International*, 49(9), 993-1001.
- Moad, G., & Rizzardo, E. (2020). A 20th anniversary perspective on the life of RAFT (RAFT coming of age). *Polymer International*, 69(8), 658-661.
- Moad, G., Rizzardo, E., & Thang, S. H. (2005). Living radical polymerization by the RAFT process. *Australian journal of chemistry*, 58(6), 379-410.
- Moad, G., Rizzardo, E., & Thang, S. H. (2008a). Radical addition–fragmentation chemistry in polymer synthesis. *Polymer*, 49(5), 1079-1131.
- Moad, G., Rizzardo, E., & Thang, S. H. (2008b). Toward living radical polymerization. *Accounts of chemical research*, 41(9), 1133-1142.
- Moraes, R. M., Carvalho, L. T., Alves, G. M., Medeiros, S. F., Bourgeat-Lami, E., & Santos, A. M. (2020). Synthesis and Self-Assembly of Poly(N-Vinylcaprolactam)-b-Poly(ε-Caprolactone) Block Copolymers via the Combination of RAFT/MADIX and Ring-Opening Polymerizations. *Polymers*, 12(6). <https://doi.org/10.3390/polym12061252>

- Mori, H., Ookuma, H., Nakano, S., & Endo, T. (2006). Xanthate-Mediated Controlled Radical Polymerization of N-Vinylcarbazole. *Macromolecular Chemistry and Physics*, 207(12), 1005-1017.
- Morimoto, N., Qiu, X.-P., Winnik, F. M., & Akiyoshi, K. (2008). Dual stimuli-responsive nanogels by self-assembly of polysaccharides lightly grafted with thiol-terminated poly (N-isopropylacrylamide) chains. *Macromolecules*, 41(16), 5985-5987.
- Motozato, Y., Ihara, H., Tomoda, T., & Hirayama, C. (1986). Preparation and gel permeation chromatographic properties of pullulan spheres. *Journal of Chromatography A*, 355, 434-437.
- Na, K., Jeong, Y.-I., & Lee, K.-Y. (1997a). Release of indomethacin from pH-sensitive pullulan acetate microsphere. *Biotechnology and Bioprocess Engineering*, 2(1), 48-52.
- Naik, B., Kumar, V., Goyal, S., Dutt Tripathi, A., Mishra, S., Joakim Saris, P. E., ... Rustagi, S. (2023). Pullulanase: unleashing the power of enzyme with a promising future in the food industry. *Frontiers in Bioengineering and Biotechnology*, 11, 1139611.
- Nakabayashi, K., & Mori, H. (2013). Recent progress in controlled radical polymerization of N-vinyl monomers. *European Polymer Journal*, 49(10), 2808-2838. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2013.07.006>
- Niu, B., Shao, P., Chen, H., & Sun, P. (2019). Structural and physiochemical characterization of novel hydrophobic packaging films based on pullulan derivatives for fruits preservation. *Carbohydrate Polymers*, 208, 276-284.
- Ofridam, F., Tarhini, M., Lebaz, N., Gagnière, É., Mangin, D., & Elaissari, A. (2021a). pH - sensitive polymers: Classification and some fine potential applications. *Polymers for Advanced Technologies*, 32(4), 1455-1484. <https://doi.org/10.1002/pat.5230>
- Ofridam, F., Tarhini, M., Lebaz, N., Gagnière, É., Mangin, D., & Elaissari, A. (2021b). pH-sensitive polymers: Classification and some fine potential applications. *Polymers for Advanced Technologies*, 32(4), 1455-1484.
- Omar-Aziz, M., Yarmand, M. S., Khodaiyan, F., Mousavi, M., Gharaghani, M., Kennedy, J. F., & Hosseini, S. S. (2020a). Chemical modification of pullulan exopolysaccharide by octenyl succinic anhydride: Optimization, physicochemical, structural and functional properties. *International Journal of Biological Macromolecules*, 164, 3485-3495. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.08.158>

- Opiyo, G., & Jin, J. (2021). Recent progress in switchable RAFT agents: Design, synthesis and application. *European Polymer Journal*, 159, 110713. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2021.110713>
- Pal, A., Sarkar, A. N., Karmakar, P. D., & Pal, S. (2018). Amphiphilic graft copolymeric micelle using dextrin and poly (N-vinyl caprolactam) via RAFT polymerization: Development and application. *International Journal of Biological Macromolecules*, 119, 954-961. <https://doi.org/10.1016/j.ijbiomac.2018.07.198>
- Pasparakis, G., & Tsitsilianis, C. (2020). LCST polymers: Thermoresponsive nanostructured assemblies towards bioapplications. *Polymer*, 211, 123146.
- Pavaloiu, R.-D., Sha'at, F., Hlevca, C., Sha'at, M., Sevcenco, C., Petrescu, M., ... Moscovici, M. (2021). Preliminary Evaluation of Pullulan Nanoparticles Loaded with Valsartan. *Chemistry Proceedings*, 3(1). <https://doi.org/10.3390/ecsoc-24-08428>
- Pelipenko, J., Kristl, J., Janković, B., Baumgartner, S., & Kocbek, P. (2013). The impact of relative humidity during electrospinning on the morphology and mechanical properties of nanofibers. *International Journal of Pharmaceutics*, 456(1), 125-134. <https://doi.org/https://doi.org/10.1016/j.ijpharm.2013.07.078>
- Perrier, S. (2017). *50th Anniversary Perspective: RAFT Polymerization—A User Guide*. *Macromolecules*, 50(19), 7433-7447. <https://doi.org/10.1021/acs.macromol.7b00767>
- Ponce-Vargas, S. M., Cortez-Lemus, N. A., & Licea-Claveríe, A. (2013). Preparation of Poly(N-Vinylcaprolactam) (NVCL) and Statistical Copolymers of NVCL with Variable Cloud Point Temperature by Using A Trithiocarbonate RAFT Agent. *Macromolecular Symposia*, 325-326(1), 56-70. <https://doi.org/10.1002/masy.201200045>
- Ponrasu, T., Chen, B.-H., Chou, T.-H., Wu, J.-J., & Cheng, Y.-S. (2021a). Fast Dissolving Electrospun Nanofibers Fabricated from Jelly Fig Polysaccharide/Pullulan for Drug Delivery Applications. *Polymers*, 13(2). <https://doi.org/10.3390/polym13020241>
- Popescu, I., Constantin, M., Bercea, M., Coşman, B.-P., Suflet, D. M., & Fundueanu, G. (2023). Poloxamer/Carboxymethyl Pullulan Aqueous Systems—Miscibility and Thermogelation Studies Using Viscometry, Rheology and Dynamic Light Scattering. *Polymers*, 15(8). <https://doi.org/10.3390/polym15081909>
- Pourmadadi, M., Farokh, A., Rahmani, E., Eshaghi, M. M., Aslani, A., Rahdar, A., & Ferreira, L. F. R. (2023). Polyacrylic acid mediated targeted drug delivery nano-systems: A review. *Journal of Drug Delivery Science and Technology*, 80, 104169.

- Prajapati, V. D., Jani, G. K., & Khanda, S. M. (2013). Pullulan: An exopolysaccharide and its various applications. *Carbohydrate Polymers*, 95(1), 540-549. <https://doi.org/https://doi.org/10.1016/j.carbpol.2013.02.082>
- Priyadarshi, R., Riahi, Z., & Rhim, J.-W. (2022). Antioxidant pectin/pullulan edible coating incorporated with Vitis vinifera grape seed extract for extending the shelf life of peanuts. *Postharvest Biology and Technology*, 183, 111740. <https://doi.org/https://doi.org/10.1016/j.postharvbio.2021.111740>
- Purohit, P., Bhatt, A., Mittal, R. K., Abdellattif, M. H., & Farghaly, T. A. (2023). Polymer Grafting and its chemical reactions. *Frontiers in Bioengineering and Biotechnology*, 10, 1044927.
- Qin, Zeyu, Jia, X., Liu, Q., Kong, B., & Wang, H. (2020). Enhancing physical properties of chitosan/pullulan electrospinning nanofibers via green crosslinking strategies. *Carbohydrate Polymers*, 247, 116734. <https://doi.org/https://doi.org/10.1016/j.carbpol.2020.116734>
- Qin, Ze-yu, Jia, X.-W., Liu, Q., Kong, B., & Wang, H. (2019). Fast dissolving oral films for drug delivery prepared from chitosan/pullulan electrospinning nanofibers. *International Journal of Biological Macromolecules*, 137, 224-231. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.06.224>
- Rahmayetty, Sukirno, Prasetya, B., & Gozan, M. (2017). Synthesis and characterization of L-lactide and polylactic acid (PLA) from L-lactic acid for biomedical applications (Vol. 1817, p. 020009). Communication présentée au AIP Conference Proceedings, AIP Publishing LLC.
- Ran, Q., Wu, S., Shen, J., Ran, Q., & Shen, J. (2007). Effects of poly (acrylic acid) on rheological and dispersion properties of aqueous TiO₂ suspensions. *Polymer-Plastics Technology and Engineering*, 46(11), 1117-1120.
- Raychaudhuri, R., Naik, S., Shreya, A. B., Kandpal, N., Pandey, A., Kalthur, G., & Mutalik, S. (2020). Pullulan based stimuli responsive and subA cellular targeted nanoplatfoms for biomedical application: Synthesis, nanoformulations and toxicological perspective. *International Journal of Biological Macromolecules*, 161, 1189-1205. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.05.262>
- Rezabeigi, E., & Demarquette, N. R. (2019). Ultraporous Membranes Electrospun from Nonsolvent-Induced Phase-Separated Ternary Systems. *Macromolecular Rapid Communications*, 40(9), 1800880.

- Robles Grana, A. L., Maldonado-Textle, H., Torres-Lubián, J. R., St Thomas, C., Díaz de León, R., Olivares-Romero, J. L., ... Enríquez-Medrano, F. J. (2021). Controlled (Co) Polymerization of Methacrylates Using a Novel Symmetrical Trithiocarbonate RAFT Agent Bearing Diphenylmethyl Groups. *Molecules*, 26(15), 4618.
- Román, J. T., Fuenmayor, C. A., Dominguez, C. M. Z., Clavijo-Grimaldo, D., Acosta, M., García-Castañeda, J. E., ... Rivera-Monroy, Z. J. (2019). Pullulan nanofibers containing the antimicrobial palindromic peptide LfcinB (21–25) Pal obtained via electrospinning. *RSC advances*, 9(35), 20432-20438.
- Roy, S., Halder, M., Ramprasad, P., Dasgupta, S., Singh, Y., & Pal, D. (2023). Oxidized pullulan exhibits potent antibacterial activity against *S. aureus* by disrupting its membrane integrity. *International Journal of Biological Macromolecules*, 249, 126049. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.126049>
- Saber-Samandari, S., & Gazi, M. (2015). Pullulan based porous semi-IPN hydrogel: Synthesis, characterization and its application in the removal of mercury from aqueous solution. *Journal of the Taiwan Institute of Chemical Engineers*, 51, 143-151. <https://doi.org/https://doi.org/10.1016/j.jtice.2015.01.013>
- Selvakumar, G., & Lonchin, S. (2020). Fabrication and characterization of collagen-oxidized pullulan scaffold for biomedical applications. *International Journal of Biological Macromolecules*, 164, 1592-1599. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.07.264>
- Seuring, J., & Agarwal, S. (2012). Polymers with upper critical solution temperature in aqueous solution. *Macromolecular rapid communications*, 33(22), 1898-1920.
- Shady, S. F., Gaines, P., Garhwal, R., Leahy, C., Ellis, E., Crawford, K., ... McCarthy, S. P. (2013). Synthesis and Characterization of Pullulan-Polycaprolactone Core–Shell Nanospheres Encapsulated with Ciprofloxacin. *Journal of Biomedical Nanotechnology*, 9(9), 1644-1655. <https://doi.org/10.1166/jbn.2013.1654>
- Shah, N. N., Vishwasrao, C., Singhal, R. S., & Ananthanarayan, L. (2016). n-Octenyl succinylation of pullulan: Effect on its physico-mechanical and thermal properties and application as an edible coating on fruits. *Food Hydrocolloids*, 55, 179-188. <https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.11.026>
- Shibata, M., Nozawa, R., Teramoto, N., & Yosomiya, R. (2002). Synthesis and properties of etherified pullulans. *European Polymer Journal*, 38(3), 497-501. [https://doi.org/https://doi.org/10.1016/S0014-3057\(01\)00198-7](https://doi.org/https://doi.org/10.1016/S0014-3057(01)00198-7)

- Simsek, M., Asiyanbi-Hammed, T. T., Rasaq, N., & Hammed, A. M. (2023). Progress in bioactive polysaccharide-derivatives: a review. *Food Reviews International*, 39(3), 1612-1627.
- Singh, J., & Nayak, P. (2023). pH-responsive polymers for drug delivery: trends and opportunities. *Journal of polymer science*, 61(22), 2828-2850.
- Singh, Ram S., Saini, G. K., & Kennedy, J. F. (2021). Pullulan production in stirred tank reactor by a colour-variant strain of *Aureobasidium pullulans* FB-1. *Carbohydrate Polymer Technologies and Applications*, 2, 100086. <https://doi.org/https://doi.org/10.1016/j.carpta.2021.100086>
- Singh, Ram Sarup, Kaur, N., Hassan, M., & Kennedy, J. F. (2021a). Pullulan in biomedical research and development - A review. *International Journal of Biological Macromolecules*, 166, 694-706. <https://doi.org/10.1016/j.ijbiomac.2020.10.227>
- Singh, Ram Sarup, Kaur, N., Singh, D., Purewal, S. S., & Kennedy, J. F. (2023). Pullulan in pharmaceutical and cosmeceutical formulations: a review. *International journal of biological macromolecules*, 231, 123353.
- Slark, A. T., Sherrington, D. C., Titterton, A., & Martin, I. K. (2003). Branched methacrylate copolymers from multifunctional comonomers: the effect of multifunctional monomer functionality on polymer architecture and properties. *J. Mater. Chem.*, 13(11), 2711-2720. <https://doi.org/10.1039/B306037N>
- Sonmez, B., & Celikkol, A. N. (2021). Pullulan based hydrogels for the removal of various metal ions from aqueous solutions. *Journal of Environmental Chemical Engineering*, 9(5), 106188. <https://doi.org/https://doi.org/10.1016/j.jece.2021.106188>
- Souguir, Z., Roudesli, S., About-Jaudet, E., Cerf, D. L., & Picton, L. (2007). Synthesis and physicochemical characterization of a novel ampholytic pullulan derivative with amphiphilic behavior in alkaline media. *Journal of Colloid and Interface Science*, 313(1), 108-116. <https://doi.org/https://doi.org/10.1016/j.jcis.2007.03.012>
- Suenaga, R., Komuro, Y., & Terao, K. (2025). Highly soluble and well-defined polysaccharide-based micelle in aqueous media: Decyl succinic anhydride-modified pullulan. *Carbohydrate Polymers*, 358, 123504. <https://doi.org/https://doi.org/10.1016/j.carbpol.2025.123504>
- Suflet, D. M., Constantin, M., Pelin, I. M., Popescu, I., Rimbu, C. M., Horhoge, C. E., & Fundueanu, G. (2024). Chitosan–Oxidized Pullulan Hydrogels Loaded with Essential

Clove Oil: Synthesis, Characterization, Antioxidant and Antimicrobial Properties. *Gels*, 10(4). <https://doi.org/10.3390/gels10040227>

Sui, X., Yuan, J., Zhou, M., Zhang, J., Yang, H., Yuan, W., ... Pan, C. (2008). Synthesis of Cellulose- *graft* -Poly(*N* , *N* -dimethylamino-2-ethyl methacrylate) Copolymers via Homogeneous ATRP and Their Aggregates in Aqueous Media. *Biomacromolecules*, 9(10), 2615-2620. <https://doi.org/10.1021/bm800538d>

Sütekin, S. D., & Güven, O. (2018). Radiation-induced controlled polymerization of acrylic acid by RAFT and RAFT-MADIX methods in protic solvents. *Radiation Physics and Chemistry*, 142, 82-87. <https://doi.org/https://doi.org/10.1016/j.radphyschem.2017.01.046>

Tan, G., Wang, L., Pan, W., & Chen, K. (2022). Polysaccharide electrospun nanofibers for wound healing applications. *International Journal of Nanomedicine*, 17, 3913.

Tang, J., Hua, D., Cheng, J., Jiang, J., & Zhu, X. (2008). Synthesis and properties of temperature-responsive chitosan by controlled free radical polymerization with chitosan-RAFT agent. *International Journal of Biological Macromolecules*, 43(4), 383-389. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2008.07.019>

Taton, D., Baussard, J.-F., Dupayage, L., Gnanou, Y., Destarac, M., Mignaud, C., & Pitois, C. (2006). Direct synthetic strategies to hydrophilic starlike polymers by MADIX. (S.l.) : ACS Publications.

Taton, D., Wilczewska, A., & Destarac, M. (2001). Direct synthesis of double hydrophilic statistical di-and triblock copolymers comprised of acrylamide and acrylic acid units via the MADIX process. *Macromolecular Rapid Communications*, 22(18), 1497-1503.

Teixeira do Nascimento, A., Stoddart, P. R., Goris, T., Kael, M., Manasseh, R., Alt, K., ... Moulton, S. E. (2025). Stimuli-responsive materials for biomedical applications. *Advanced Materials*, 37(36), e07559.

Teramoto, N., & Shibata, M. (2006a). Synthesis and properties of pullulan acetate. Thermal properties, biodegradability, and a semi-clear gel formation in organic solvents. *Carbohydrate polymers*, 63(4), 476-481.

Teraoka, I. (2002). *Polymer solutions*. (S.l.) : (s.n.).

- Thakur, A., Sharma, S., Naman, S., & Baldi, A. (2023). Pullulan based polymeric novel drug delivery systems: A review on current state of art and prospects. *Journal of Drug Delivery Science and Technology*, 90, 105117. <https://doi.org/https://doi.org/10.1016/j.jddst.2023.105117>
- Tian, X., Ding, J., Zhang, B., Qiu, F., Zhuang, X., & Chen, Y. (2018). Recent Advances in RAFT Polymerization: Novel Initiation Mechanisms and Optoelectronic Applications. *Polymers*, 10(3). <https://doi.org/10.3390/polym10030318>
- Tiwari, S., Patil, R., Dubey, S. K., & Bahadur, P. (2019a). Derivatization approaches and applications of pullulan. *Advances in Colloid and Interface Science*, 269, 296-308. <https://doi.org/10.1016/j.cis.2019.04.014>
- Torres, F. G., Troncoso, O. P., Pisani, A., Gatto, F., & Bardi, G. (2019). Natural polysaccharide nanomaterials: an overview of their immunological properties. *International journal of molecular sciences*, 20(20), 5092.
- Truong, N. P., Jones, G. R., Bradford, K. G., Konkolewicz, D., & Anastasaki, A. (2021). A comparison of RAFT and ATRP methods for controlled radical polymerization. *Nature Reviews Chemistry*, 5(12), 859-869.
- US-FDA, US food and drug administration, center for food safety and applied nutrition, office of food safety, agency response letter. (2002). GRAS Notice No. GRN 000099.
- Vieira, T. A., Matos, L., Carvalho, L. T., Alves, G. M., Lacerda, T. M., & Medeiros, S. F. (2024). Self-assembled nanoparticles based on pullulan- G -poly(Z-L -lysine) for controlled drug delivery: Synthesis, characterization and preliminary studies on the encapsulation of indomethacin. *Journal of Applied Polymer Science*, 141(28), e55632. <https://doi.org/10.1002/app.55632>
- Wan, D., Zhou, Q., Pu, H., & Yang, G. (2008). Controlled radical polymerization of N -vinylcaprolactam mediated by xanthate or dithiocarbamate. *Journal of Polymer Science Part A: Polymer Chemistry*, 46(11), 3756-3765. <https://doi.org/10.1002/pola.22722>
- Wang, C., Wang, W., Qi, H., Dai, Y., Jiang, S., Ding, B., ... Wu, T. (2025). Electrospinning and electrospun nanofibers: From academic research to industrial production. *Progress in Materials Science*, 101494.

- Wang, K. L., Jiang, N., He, B. B., & Kang, D. X. (2017). Regiocontrol synthesis cellulose-graft-polycaprolactone copolymer (2,3-di-O-PCL-cellulose) by a new route. *Express Polymer Letters*, 11(12), 991-1002. <https://doi.org/10.3144/expresspolymlett.2017.94>
- Wang, M., Zhao, Y., Shen, A., Liu, Y., Yao, Y., & Wu, H. (2024). Preparation of polyacrylic acid thickener with electrolyte resistance for carpet ink-jet printing. *Progress in Organic Coatings*, 194, 108538. <https://doi.org/https://doi.org/10.1016/j.porgcoat.2024.108538>
- Ward, M. A., & Georgiou, T. K. (2011). Thermoresponsive Polymers for Biomedical Applications. *Polymers*, 3(3), 1215-1242. <https://doi.org/10.3390/polym3031215>
- Wei, K., Wang, L., Li, L., & Zheng, S. (2015). Synthesis and characterization of bead-like poly(N-isopropylacrylamide) copolymers with double decker silsesquioxane in the main chains. *Polym. Chem.*, 6(2), 256-269. <https://doi.org/10.1039/C4PY00786G>
- Wei, M., Gao, Y., Li, X., & Serpe, M. J. (2017). Stimuli-responsive polymers and their applications. *Polymer Chemistry*, 8(1), 127-143. <https://doi.org/10.1039/C6PY01585A>
- Winniger, J., Iurea, D. M., Atanase, L. I., Salhi, S., Delaite, C., & Riess, G. (2019). Micellization of novel biocompatible thermo-sensitive graft copolymers based on poly(ϵ -caprolactone), poly(N-vinylcaprolactam) and poly(N-vinylpyrrolidone). *European Polymer Journal*, 119, 74-82. <https://doi.org/https://doi.org/10.1016/j.eurpolymj.2019.07.015>
- Wolf, B. W., Garleb, K. A., Choe, Y. S., Humphrey, P. M., & Maki, K. C. (2003). Pullulan Is a Slowly Digested Carbohydrate in Humans. *The Journal of Nutrition*, 133(4), 1051-1055. <https://doi.org/https://doi.org/10.1093/jn/133.4.1051>
- Wu, D. H., Chen, A. D., & Johnson, C. S. (1995). An Improved Diffusion-Ordered Spectroscopy Experiment Incorporating Bipolar-Gradient Pulses. *Journal of Magnetic Resonance, Series A*, 115(2), 260-264. <https://doi.org/https://doi.org/10.1006/jmra.1995.1176>
- Wu, S., Jin, Z., Kim, J. M., Tong, Q., & Chen, H. (2009). Graft copolymerization of methyl acrylate onto pullulan using ceric ammonium nitrate as initiator. *Carbohydrate Polymers*, 76(1), 129-132. <https://doi.org/https://doi.org/10.1016/j.carbpol.2008.10.002>

- Wu, S., Jin, Z., Tong, Q., & Chen, H. (2009). Sweet potato: A novel substrate for pullulan production by *Aureobasidium pullulans*. *Carbohydrate Polymers*, 76(4), 645-649. <https://doi.org/https://doi.org/10.1016/j.carbpol.2008.11.034>
- Xu, S., Li, J., Li, J., Wu, Y., Xiao, Y., & Hu, C. (2019). D-Excess-LaA production directly from biomass by trivalent yttrium species. *Iscience*, 12, 132-140.
- Xue, J., Wu, T., Dai, Y., & Xia, Y. (2019). Electrospinning and electrospun nanofibers: methods, materials, and applications. *Chemical reviews*, 119(8), 5298-5415.
- Yin, X., Hoffman, A. S., & Stayton, P. S. (2006). Poly (N-isopropylacrylamide-co-propylacrylic acid) copolymers that respond sharply to temperature and pH. *Biomacromolecules*, 7(5), 1381-1385.
- Yuan, Y., Raheja, K., Milbrandt, N. B., Beilharz, S., Tene, S., Oshabaheebwa, S., ... Karayilan, M. (2023). Thermoresponsive polymers with LCST transition: synthesis, characterization, and their impact on biomedical frontiers. *RSC Applied Polymers*, 1(2), 158-189. <https://doi.org/10.1039/D3LP00114H>
- Zhang, H., Gao, F., Liu, L., Li, X., Zhou, Z., Yang, X., & Zhang, Q. (2009). Pullulan acetate nanoparticles prepared by solvent diffusion method for epirubicin chemotherapy. *Colloids and Surfaces B: Biointerfaces*, 71(1), 19-26.
- Zhang, L., Liu, J., Zheng, X., Zhang, A., Zhang, X., & Tang, K. (2019). Pullulan dialdehyde crosslinked gelatin hydrogels with high strength for biomedical applications. *Carbohydrate Polymers*, 216, 45-53. <https://doi.org/https://doi.org/10.1016/j.carbpol.2019.04.004>
- Zhao, L., Tong, Q., Wang, H., Liu, Y., Xu, J., & Rehman, A. (2020). Emulsifying properties and structure characteristics of octenyl succinic anhydride-modified pullulans with different degree of substitution. *Carbohydrate Polymers*, 250, 116844. <https://doi.org/https://doi.org/10.1016/j.carbpol.2020.116844>
- Zhao, R.-X., Li, L., Wang, B., Yang, W.-W., Chen, Y., He, X.-H., ... Jiang, S.-C. (2012). Aliphatic tertiary amine mediated synthesis of highly branched polylactide copolymers. *Polymer*, 53(3), 719-727. <https://doi.org/https://doi.org/10.1016/j.polymer.2011.12.045>
- Zhao, X., Coutelier, O., Nguyen, H. H., Delmas, C., Destarac, M., & Marty, J.-D. (2015). Effect of copolymer composition of RAFT/MADIX-derived N-vinylcaprolactam/N-vinylpyrrolidone statistical copolymers on their thermoresponsive behavior and

hydrogel properties. *Polymer Chemistry*, 6(29), 5233-5243.
<https://doi.org/10.1039/C5PY00606F>

Zhou, D., Zhu, X., Zhu, J., & Yin, H. (2005). Influence of the chemical structure of dithiocarbamates with different N-groups on the reversible addition–fragmentation chain transfer polymerization of styrene. *Journal of Polymer Science Part A: Polymer Chemistry*, 43(20), 4849-4856.

Zorin, I. M., Fetin, P. A., Mikusheva, N. G., Lezov, A. A., Perevyazko, I., Gubarev, A. S., ... Tsvetkov, N. V. (2023). Pullulan-Graft-Polyoxazoline: Approaches from Chemistry and Physics. *Molecules*, 29(1), 26. <https://doi.org/10.3390/molecules29010026>

