



Synthesis and characterization of pullulan-graft-poly (N-vinyl caprolactam) via RAFT/MADIX polymerization

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ABSTRACT

This study presents a novel synthetic strategy for preparing graft copolymers based on a pullulan backbone. The approach relies on reversible addition-fragmentation chain transfer (RAFT) radical polymerization as the key step. This significant advancement enables the synthesis of the temperature-responsive copolymer pullulan-graft-poly(N-vinyl caprolactam) (PULL-g-PNVCL), leveraging the renewable, biocompatible, and biodegradable nature of pullulan to access this previously challenging target. 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 from the pullulan macro-chain transfer agent. Comprehensive characterization, including Gel Permeation Chromatography (GPC) and Proton Nuclear Magnetic Resonance spectroscopy (¹H NMR), confirmed successful graft copolymerization. The results showed decreased thermal stability of PULL-g-PNVCL compared to pullulan. Dynamic light scattering (DLS) analyses demonstrated a thermosensitive 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. The obtained LCST values were lower than physiological temperature, which embeds PULL-g-PNVCL with the potential for expanding pullulan applications, particularly in biomedical fields such as stimuli-responsive drug delivery systems. Integrating chemical principles with polymer design, this research showcases the development of bio-based functional materials, an objective aligned with the sustainable use of industrial crops.

1. Introduction

The growing environmental concerns and depletion of petrochemical resources have intensified the search for sustainable polymer materials, and polysaccharides represent the most abundant class of biopolymers available in nature. Among these, Pullulan (PULL), a biopolymer that can be produced from various carbon sources such as glucose and lactose, has garnered significant interest due to its biocompatibility, biodegradability, and low toxicity [1–3]. These properties make its use very attractive in the food, cosmetic, and biomedical industries [3–7]. However, the practical implementation of PULL, especially in drug delivery systems, is often limited by its native properties, such as water solubility. Fortunately, the hydroxyl groups on the PULL backbone serve as reactive sites for chemical modifications aimed at tuning PULL's

properties. Indeed, grafting offers a promising approach for the modulation of PULL's properties. Grafted modulators, including functional groups or polymers, enable the development of novel materials that valuably combine the properties of different materials for specific applications that are not accessible to native PULL [1,7].

Graft copolymerization can be accomplished through two main strategies: 'grafting onto' and 'grafting from', both yielding macromolecular architectures consisting of a backbone with multiple side chains. Among these, the 'grafting from' method usually achieves higher grafting efficiency and offers superior control over the final material's molar mass and distribution through careful optimization of reaction conditions [7–9]. Previously grafted copolymers involving PULL as the backbone, accessing diverse architectures, have been reported [10–18]. These studies are summarized in Scheme 1. Notably, many of these

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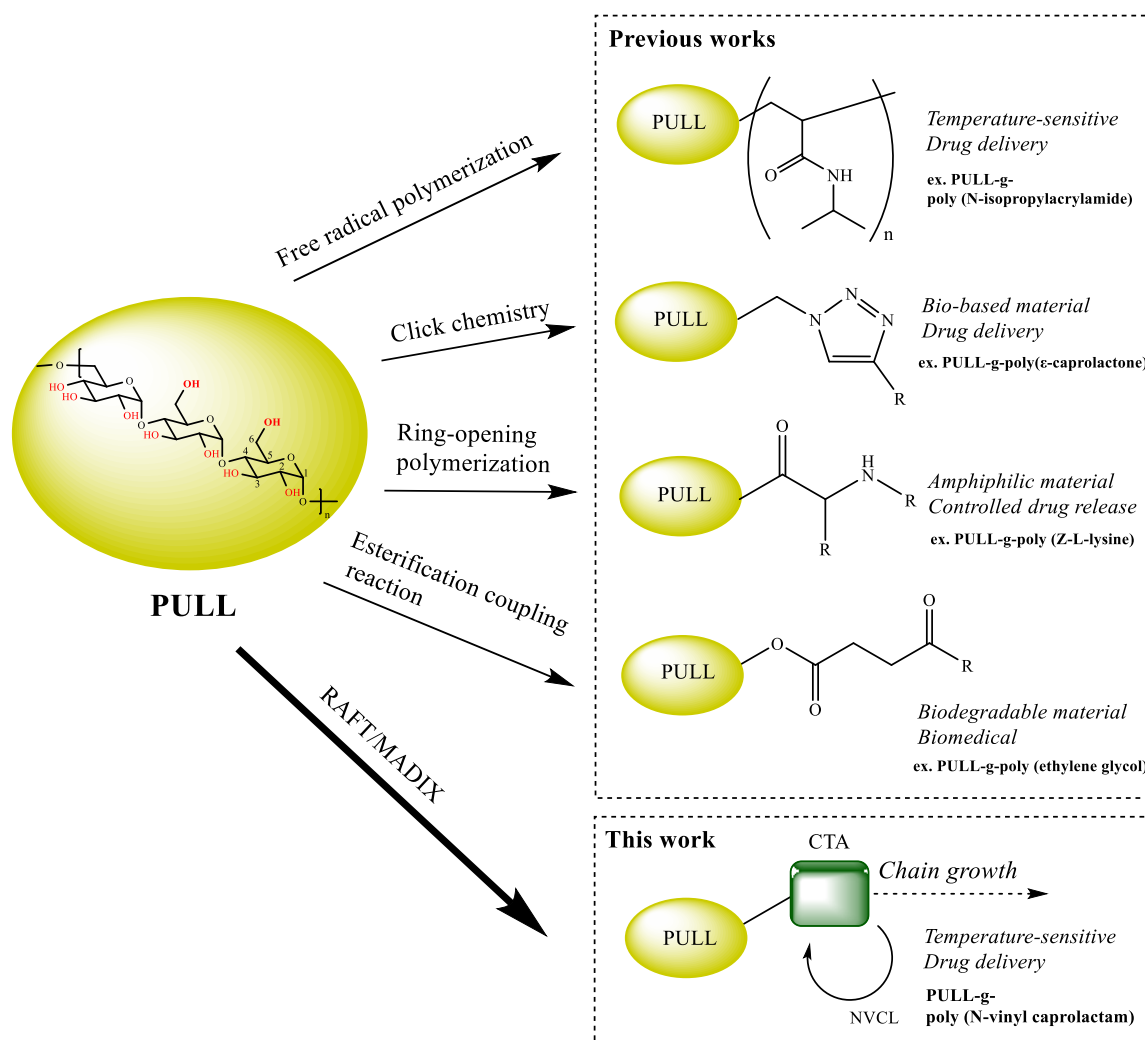
PULL-based graft copolymers exhibit unique physicochemical properties and self-aggregation behavior in aqueous media, enabling the formation of particles suitable for drug delivery applications [10,19,20].

Concomitantly, stimuli-responsive polymers have attracted considerable attention due to their capacity to incorporate and release bioactive compounds [21]. Among these, temperature-sensitive polymers are particularly notable, as they exhibit reversible alterations in their structure, conformation, and solubility. This phenomenon arises from the polymer's solubility with a given solvent below the phase transition temperature, whereas above this threshold, thermodynamically favorable polymer-polymer interactions lead to a compact form of the polymer chains, thus reducing its solubility [21–23]. The temperature at which this transition occurs is known as lower critical solution temperature (LCST) and was shown to be molar mass dependent [23–26]. Among these temperature-sensitive polymers, poly(N-vinyl caprolactam) (PNVCL) stands out as a biocompatible thermosensitive polymer with a phase transition temperature closely matching physiological conditions, making it particularly advantageous for drug delivery applications [22,27–29].

For PNVCL, the reversible addition-fragmentation chain transfer (RAFT) radical polymerization technique is particularly interesting as it enables precise control over molar mass, molar mass distribution, and polymer architecture, such as stars, branched, block, and graft copolymers [24,26,30,31]. The RAFT technique achieves this control

through its “living” characteristic, which stems from the reversible transfer of growing radical chains to a chain transfer agent (CTA), ensuring that the polymer chain remains “alive” for extended periods, allowing the synthesis of longer PNVCL chains [32–34]. However, N-vinyl caprolactam (NVCL), as a monomer, generates an unstable and highly reactive propagating radical, which typically results in shorter chains compared to more stabilized radicals [22,30]. This makes NVCL polymerization inherently challenging, requiring a CTA capable of stabilizing the process, thus ensuring adequate control over the polymerization process.

In this study, xanthate was chosen as the CTA due to its proven ability to effectively manage NVCL polymerization for grafting applications [25,35], a process specifically referred to as MADIX (macromolecular design by the interchange of xanthate). Accordingly, a PULL-based macro-RAFT chain transfer agent was designed to enable the grafting of PNVCL chains directly from the pullulan backbone. This strategy was developed to introduce temperature-responsive PNVCL grafts to the pullulan backbone. We envision that grafting the well-studied thermosensitive polymer PNVCL on a PULL backbone will modulate the physicochemical properties of the resulting pullulan-graft-poly(N-vinyl caprolactam) (PULL-g-PNVCL) system. Moreover, given the synthetic challenges in current grafting strategies, we hypothesize that RAFT/MADIX polymerization would be ideal to grow PNVCL with controlled chain lengths from a PULL backbone.



Scheme 1. – Synthesis of PULL-based copolymers via different methods. The schematic highlights various synthetic strategies for grafting PULL-based copolymers, including free-radical polymerization, click chemistry, ring-opening polymerization, and esterification coupling reactions.

Despite the range of grafting strategies previously applied to PULL (Scheme 1), most reported approaches rely on conventional free-radical polymerization, click chemistry, or coupling reactions, which typically afford limited control over graft chain length, molar mass distribution, and overall copolymer architecture. Controlled radical polymerization techniques, such as RAFT, address these limitations in synthetic polymer systems, but their application to polysaccharide backbones such as PULL remains comparatively underexplored. To the best of our knowledge, few studies have explored RAFT polymerization on a polysaccharide backbone [31,33,36,37]. In particular, no one reported the polymerization mediated by a RAFT chain transfer agent on a PULL substrate. This study addresses that gap by reporting the first synthesis of PULL-g-PNVCL copolymer via RAFT polymerization of NVCL mediated by a PULL-based macro-RAFT chain transfer agent, aiming to combine PULL's properties with PNVCL's temperature-sensitive behavior. These systems are important for a variety of uses, including smart drug delivery [38,39] and biosensors [40], where both biopolymer origin and thermal responsiveness are crucial. Their implementation enables precise, stimuli-triggered therapeutic strategies and significantly enhances treatment efficiency. Moreover, the natural and renewable origin of PULL highlights its value as a sustainable platform for designing polymeric materials. The present work provides a foundation for exploring polysaccharide-based graft copolymers with tailored functionalities and underscores the potential of RAFT/MADIX polymerization in advancing macromolecular design. Furthermore, this study aims at valorizing renewable carbohydrate resources by converting them into functional polymers with tunable, application-relevant properties for industrial use.

2. Materials and methods

2.1. Materials

The reagents utilized in this study included pullulan (PULL, food grade, 95% purity (5% mono- and oligo-saccharides), 98.2 total carbohydrates (1.8% water), $\bar{M}_n = 96000$ g/mol, $\bar{M}_w = 288000$ g/mol, and $D = 3.01$ in DMF/LiBr) sourced from Hayashibara Biochemical Laboratory (Japan), 2-bromopropionyl bromide (BPB, 97%, Sigma-Aldrich), triethylamine (TEA, 99.5%, Sigma-Aldrich), anhydrous pyridine (99.8%, Sigma-Aldrich), N-vinyl caprolactam (NVCL, 98%, Sigma-Aldrich), and 2,2'-azobis(2-methylpropionitrile) (AIBN, 98%, Sigma-Aldrich). AIBN was used as received and stored refrigerated and protected from light. Additional solvents and reagents, including dried dimethyl sulfoxide (DMSO, $\leq 0.02\%$ water, Sigma-Aldrich), anhydrous ethanol ($\leq 0.003\%$ water, Sigma-Aldrich), anhydrous N, N-dimethylformamide (DMF, 99.8%, Sigma-Aldrich), hydrochloric acid (HCl, 36.5%, Sigma-Aldrich), diethyl ether (99%, Fisher Scientific), anhydrous dichloromethane

($\geq 99.8\%$, Sigma-Aldrich), anhydrous magnesium sulfate (MgSO_4 , Fisher Scientific), and methanol ($\geq 99.8\%$, Fisher Scientific) were used as received. Deuterated water (D_2O) and dimethyl sulfoxide- d_6 (DMSO-d_6) from Cambridge Isotope Laboratories, Inc., were employed for NMR spectroscopy. Whenever the use of water was necessary, Deionized water was used.

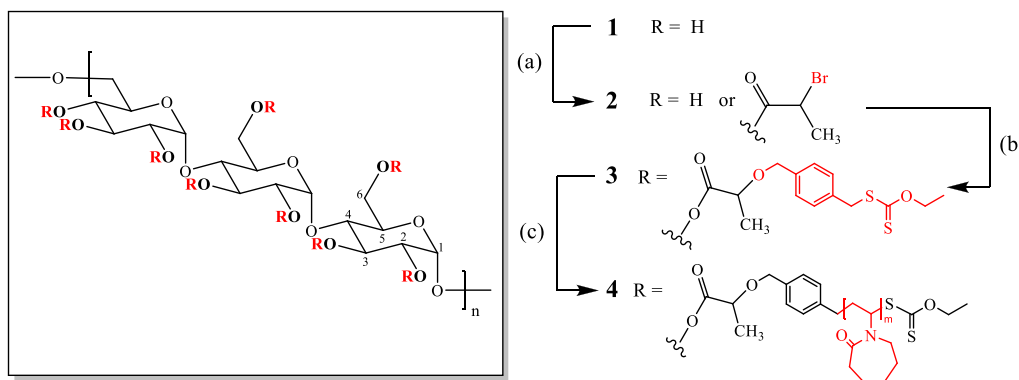
2.2. Synthesis of PULL-g-PNVCL procedures

Scheme 2 presents the synthetic sequence that was exploited to produce PULL-g-PNVCL. Each of these steps will be described in detail below. The synthesis was accomplished using a four-step process: First, the molar mass of commercially available PULL was reduced by acid hydrolysis, following procedures reported in the literature [41–44]. Second, hydroxyl groups were partially acylated with 2-bromopropionyl bromide (BPB) to afford bromide bearing PULL (PULLBr). Next, the bromopropionyl groups were partially substituted with a pre-synthesized hydroxylated CTA (xanthate-based, CTA-OH) to afford the PULL-xanthate-based macro-chain transfer agent (PULLX). Finally, the PULL-g-PNVCL copolymer was obtained from PULLX. The molecular structure and composition of the synthesized (co)polymers were characterized using multiple analytical techniques, including gel permeation chromatography (GPC), attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR), and proton nuclear magnetic resonance spectroscopy (^1H NMR). Thermal properties were evaluated through differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Additionally, PULL-g-PNVCL particles were prepared via self-assembly, and their physicochemical and thermosensitive properties were thoroughly investigated. While various conditions with different reactant ratios were explored, we chose to only detail the characterization of the most successful synthesis route in the main text. The other tested ratios encountered challenges either during synthesis or characterization, and these results are also provided in the [supplementary information](#). Similar difficulties with alternative conditions have been reported by other researchers as well [17,45]. To guide future work, we prioritized conditions that allow for better modulation and improved material properties.

Note that in order to facilitate the reading of the text, the nomenclature of the different samples is presented in [supplementary information](#) (Table S1).

2.2.1. PULL molar mass reduction

To obtain lower molar mass PULL, the polymer was hydrolyzed using a 0.025 M hydrochloric acid (HCl) solution, following protocols from previous studies [41,42,44,46]. In an oven-dried 250 mL round-bottom flask, PULL (6 g) was dissolved in deionized water (120 mL) at 60 °C for 4 h. After complete dissolution, HCl (250 μL of 0.025 M solution) was



Scheme 2. 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 PULL (Yield: 92%); 2: Bromide bearing PULL (Yield: 58–64%); 3: Xanthate-based macro-chain transfer agent PULL (Yield: 30–73%); 4: PULL-g-PNVCL (Yield: 37–38%).

added while stirring. The flask was sealed and heated at 85 °C for 1.5 h. The reaction was quenched by immersing the flask in an ice bath, and the product was recovered by slowly adding the reaction mixture dropwise into ice-cold methanol under vigorous stirring. The resulting precipitate was rinsed multiple times with cold methanol and dried under a vacuum at 40 °C over 24 h.

2.2.2. Synthesis of bromide-bearing PULL

The synthesis of **PULLBr** was adapted from the methodology of Bontempo et al. [47]. First, reduced molar mass PULL (0.250 g, 0.5138 mmol monomer units) was dissolved in DMF (5 mL) in a round-bottom flask at room temperature. In all reactions tested (and reported in the [supplementary information](#)), TEA was added in an amount twice that of BPB. TEA (0.7162 mL, 5.1387 mmol) was introduced under a nitrogen atmosphere with vigorous stirring. After 30 min, the flask was cooled in an ice bath, and BPB (0.2691 mL, 2.569 mmol, 5 molar ratio relative to a PULL monomer unit) was added dropwise to the solution. The reaction mixture was further stirred at room temperature for 72 h under a nitrogen atmosphere. The product, **PULLBr-5** (0.227 g, 64% isolated yield), was purified from the crude mixture by dialysis (MW cutoff 14 kDa) against distilled water over 3 days and subsequent precipitation in a 1:4 diethyl ether/methanol solution. The precipitates were dried under a vacuum at 40 °C over 24 h.

2.2.3. Synthesis of PULL-xanthate-based macro-chain transfer agent

The **PULLX-5** was then synthesized by reacting **PULLBr-5** with CTA-OH in the presence of pyridine at 50 °C, using DMSO as the solvent. Note that the synthetic route and the corresponding ¹H NMR spectrum of O-ethyl S-4-(hydroxymethyl) benzyl carbonodithioate (CTA-OH) are shown in [Scheme S1](#) and [Figure S1](#) ([Supplementary Information](#)) [48]. The molar feed ratio for [bromide groups in **PULLBr**]:[CTA-OH]:[pyridine] was set at 1:9:90. First, **PULLBr-5** (0.1 g, 0.0314 mmol) and CTA-OH (0.0685 g, 0.2829 mmol) were charged in an oven-dried round-bottom flask. The solids were then dissolved over 6 h in DMSO (5 mL) under a nitrogen atmosphere. Pyridine (0.2238 g, 2.8296 mmol) was then added to the solution, and the mixture was stirred at 50 °C for 24 h under a nitrogen atmosphere. The product **PULLX-5** (0.033 g, 30% isolated yield) was purified from the crude mixture by dialysis (MW cutoff 14 kDa) against distilled water over 24 h, and subsequent precipitation in cold methanol. Furthermore, to remove excess CTA-OH, the precipitates were suspended in ethanol, filtered out, and dried under vacuum at room temperature over 24 h. To ensure thorough separation from unreacted CTA-OH, the dried samples were purified by dissolving the crude mixture in a DMSO/ethanol (10:90) mixture and dialysis (MW cutoff 14 kDa) against the same solvent system over 24 h, followed by dialysis against absolute ethanol over an additional 24 h. The final materials were then dried under a vacuum at room temperature over 24 h.

2.2.4. Synthesis of PULL-g-PNVCL copolymer

To synthesize the PULL-g-PNVCL copolymer, a [CTA]:[AIBN]:[NVCL] feed molar ratio of 1:0.5:150 and 1:0.5:300 was used. The polymerization was carried out using **PULLX-5** with two NVCL molar ratios: 150 (**PULL-g-PNVCL (150)**) and 300 (**PULL-g-PNVCL (300)**), respectively. **PULLX-5** (0.100 g, containing 0.006 mmol of the xanthate group), AIBN (0.5 mg, 0.003 mmol), and DMSO (240 µL) were charged 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 (70.39 mg, 0.42866 mmol) in DMSO (10.0 mL); an aliquot of this solution (70 µL, corresponding to 0.003 mmol AIBN) was used for each polymerization, and additional DMSO was added to reach a total volume of 240 µL. Once solids were dissolved, NVCL (0.1255 g, 0.9015 mmol or 0.250 g, 1.802 mmol) was added to the solution, which was then degassed under nitrogen for 30 min at room temperature. The reaction mixture was heated at 70 °C for 24 h. The copolymers (**PULL-g-PNVCL (150)**, 38%

isolated yield) and (**PULL-g-PNVCL (300)**, 37% isolated yield) were purified from the crude mixture by dialysis (MW cutoff 14 kDa) against deionized water over 24 h and were subsequently dried under vacuum at 40 °C over 24 h. 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 reaction conditions used for each synthesis, along with the corresponding sample labels, are summarized in [Table 1](#).

2.3. Polymeric particle preparation

Polymeric particles were prepared using a self-assembly procedure [49,50]. The copolymer (15 mg) was dissolved in DMSO (1 mL). This solution was then added dropwise into deionized water (3 mL). To remove DMSO, the mixture was dialyzed (MW cutoff 14 kDa) against deionized water over 24 h. The resulting particle dispersion was transferred to a 15 mL volumetric flask and diluted to the calibration mark with deionized water, yielding a final particle concentration of 1 mg/mL.

2.4. Characterization

2.4.1. Gel permeation chromatography (GPC)

The weight-average molar mass ($\overline{M}_w$), number-average molar mass ($\overline{M}_n$), and dispersity ($\text{Đ} = \overline{M}_w/\overline{M}_n$) of PULL, reduced molar mass PULL, PULLBr, PULLX, and PULL-g-PNVCL were determined by gel permeation chromatography (GPC). The GPC system included a Tosoh ECOSEC Elite HPLC 8420 equipped with an automatic injector and a refractive index detector. Separation was carried out using two Tosoh Bioscience TSKgel Alpha-M columns (7.8 mm × 30 cm, 13 µm particle size), calibrated with PULL standards (molar mass range of 667–107,000 g/mol). Due to the limited water solubility of the PULLBr samples at high DS of BPB, as well as PULLX and PULL-g-PNVCL, dimethylformamide (DMF) was selected as a partial solvent for molar mass analysis. Additionally, the molar masses of PULL and its reduced molar mass were analyzed in DMF to enable consistent comparison. Therefore, a solution of DMF containing 0.1% (v/v) lithium bromide (LiBr) was used as the mobile phase at 60 °C, with a flow rate of 0.8 mL/min and an injection volume of 100 µL. Prior to injection, the samples were dissolved in the mobile phase and filtered through a PTFE membrane filter (0.2 µm).

2.4.2. Nuclear magnetic resonance (NMR)

The chemical structures of the materials were characterized using nuclear magnetic resonance (NMR) spectroscopy. All analyses were performed on a Bruker Avance III spectrometer for proton nuclear magnetic resonance (¹H NMR) and ¹H-¹H correlation spectroscopy (COSY) at a 300 MHz frequency, and on a Bruker Avance III HD for diffusion-ordered spectroscopy (¹H DOSY) at a 600 MHz frequency. Measurements were performed at room temperature using either deuterated dimethyl sulfoxide (DMSO-d₆, δ = 2.50 ppm) or deuterated water (D₂O, δ = 4.79 ppm) as solvents; all chemical shifts are reported in parts per million (ppm). ¹H NMR spectra of the modified PULL samples

Table 1

Reagent quantities used for the synthesis of PULL-g-PNVCL 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 DMSO
PULL-g-PNVCL (150)	0.100 g, 0.006 mmol	0.5 mg, 0.003 mmol	0.125 g, 0.901 mmol	240 µL
PULL-g-PNVCL (300)	0.100 g, 0.006 mmol	0.5 mg, 0.003 mmol	0.250 g, 1.802 mmol	240 µL

at each modification step were used to estimate the degree of substitution of hydroxyl groups on the PULL backbone. Spectral data were processed using MestreNova software, with baseline and phase corrections applied to all spectra before analysis.

DOSY experiments were conducted to determine whether we had a blend of PULLX-5 and PNVL or if PNVL was indeed grafted from PULLX-5. They were conducted using a bipolar longitudinal eddy current delay pulse sequence with spoil gradients, following the methodology of Wu et al. [51]. The gradient duration (δ) was set to 4 ms, the diffusion time (Δ) was 175 ms, and the gradient strength (g) was varied linearly from 0.96 G/cm to 47.19 G/cm over 64 increments. These parameters were selected to ensure at least 50% signal attenuation for the slowest diffusing species. The recycle delay was set at 5 s. Data acquisition and analysis were performed using MestreNova software. DOSY spectra were generated using the peak fit method, producing 2D spectra with NMR chemical shifts along one axis and calculated diffusion coefficients along the other. It should be mentioned that no standard was used to calibrate the diffusion coefficient in the DOSY analysis; therefore, the scale depicted in the spectrum serves only for relative comparison.

2.4.3. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra of the samples were recorded using a Perkin-Elmer FTIR spectrometer (Spectrum Two™), equipped with a diamond crystal. The measurements were conducted over the wavenumber range of 400–4000 cm^{-1} , with a resolution of 4 cm^{-1} .

2.4.4. Thermal analysis

Differential scanning calorimetry (DSC) was conducted using a TA Instruments DSC 2500 model, equipped with a cooling accessory. Approximately 4 mg of the samples were placed in a T-Zero premium lid-punctured pan, while an empty pan served as a blank sample. The samples were heated at a rate of 10 $^{\circ}\text{C}/\text{min}$. The first heating scan was performed from 40 $^{\circ}\text{C}$ to 240 $^{\circ}\text{C}$ to eliminate any thermal history and moisture from the samples, followed by a second scan ranging from –40 $^{\circ}\text{C}$ to 250 $^{\circ}\text{C}$, which was followed by cooling back to 40 $^{\circ}\text{C}$. All measurements were conducted under a nitrogen flow of 50 mL/min, maintaining a consistent heating/cooling rate of 10 $^{\circ}\text{C}/\text{min}$.

The thermal stability of both homo- and copolymers was assessed using thermogravimetric analysis (TGA) with a Pyris Diamond TG/DTA instrument (Perkin Elmer® Inc.). TGA was performed over a temperature range of 30 $^{\circ}\text{C}$ to 600 $^{\circ}\text{C}$ under nitrogen flow at 100 mL/min, with a heating rate of 10 $^{\circ}\text{C}/\text{min}$.

2.4.5. Particle size analysis

The hydrodynamic average diameter (Z_{av}) and size distribution (polydispersity index, PDI) of the particles in deionized water were analyzed using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS instrument. The Z_{av} of particle dispersions at a concentration of 1 mg/mL was measured in polystyrene cuvettes at 24 $^{\circ}\text{C}$. The viscosity of water at the specified temperature was identified, and the samples were allowed to equilibrate for 2 min prior to measurement. Each analysis was conducted in triplicate.

2.4.6. Temperature sensitivity analysis

To evaluate the temperature sensitivity of the copolymer PULL-g-PNVCL, the Z_{av} , and PDI of the particles in water at a concentration of 1 mg/mL were analyzed by DLS across a temperature range of 20 $^{\circ}\text{C}$ to 44 $^{\circ}\text{C}$, with increments of 2 $^{\circ}\text{C}$. Each temperature point was analyzed in triplicate, and the transition temperature was defined as the point at which a sharp increase in particle size was observed. Additionally, cooling and heating cycles at temperatures below and above or close to the LCST were conducted to investigate the reversible thermal phase transition of the copolymer.

To further confirm the temperature sensitivity of the copolymer,

PULL-g-PNVCL and PULLX-5 samples were analyzed using DSC [52–56]. 4 mg of the copolymer or PULLX-5 with 40 μL of water was placed in a T-Zero premium hermetic unpunctured pan, with an empty pan serving as the blank sample. Measurements were conducted over a temperature range of 20 $^{\circ}\text{C}$ to 50 $^{\circ}\text{C}$ at a heating and cooling rate of 1 $^{\circ}\text{C}/\text{min}$. All runs were performed under a nitrogen flow of 50 mL/min and repeated three times.

3. Results and discussion

3.1. Reduced molar mass PULL characterization

Fig. 1 presents the GPC chromatograms of commercial PULL and the reduced molar mass PULL. In this case, it is shown that PULL exhibits a high average molar mass and broad distribution. High molar mass polymers are expected to exhibit a higher level of chain entanglement, which may reduce the effective accessibility of hydroxyl groups and lead to less efficient and more heterogeneous chemical modification along the backbone [57]. To optimize the reactivity and dispersity of PULL, we aimed to achieve shorter and better-defined chains compared to the commercial PULL. This was accomplished through a 1.5-hour acid hydrolysis, which cleaves the maltotriose units in PULL to afford a lower molar mass PULL as the starting point of the synthetic sequence depicted in Scheme 2. The GPC data in Table 2 also show a decrease in molar mass and dispersity of the commercial PULL following acid hydrolysis. Furthermore, ^1H NMR analysis (Figure S2) showed that the maltotriose subunit was unaltered throughout the polymer, following the molar mass reduction reaction. Thus, the characteristic peaks associated with PULL were consistently observed in the spectra. The ^1H NMR spectrum (300 MHz, ppm, D_2O) revealed the following chemical shifts, δ : 5.25–5.5, 4.95 (3 H, anomeric protons of α -(1 $\rightarrow$ 4) and α -(1 $\rightarrow$ 6), respectively).

3.2. Bromide-bearing PULL characterization

Aiming to synthesize PULL-g-PNVCL, the precursor reduced molar mass PULL was first acylated with BPB, as illustrated in Scheme 2. The structure of PULLBr-5 was confirmed using ^1H NMR spectra, as shown in Fig. 2 and Figure S4. 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 Fig. 2, Figure S3, and Figure S4). 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 PULL. This is consistent with observations from other studies on cellulose or PULL with BPB [15,59].

Moreover, the influence of varying the BPB equivalents on the degree of substitution (DS) was examined. 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 (5.25–5.5 ppm) of PULL, serving as the reference integrals. Details of the DS calculations can be found in the supplementary information (Figure S5). The DS on hydroxyl groups of PULL by bromopropionyl units in PULLBr-5, chosen for further synthesis, was determined to be 15 per 100 hydroxyl groups or 136 per 100 monomer units. As was expected, and as Table S2 presents, higher DS is achieved by increasing the concentration of the BPB in the reaction media. The reliability of the reported bromide substitution was further confirmed through independent batch repetitions, which yielded consistent DS values.

Additionally, the COSY spectra (Figure S6 and Figure S7 show the COSY spectra of PULL and PULLBr-5) of PULLBr show the same coupling patterns for the glucose ring protons as observed prior to modification, confirming the preservation of the maltotriose unit.

Fig. 3 presents the ATR-FTIR spectra of the reduced molar mass PULL and PULLBr-5. The characteristic peaks are described in Table S7. In this

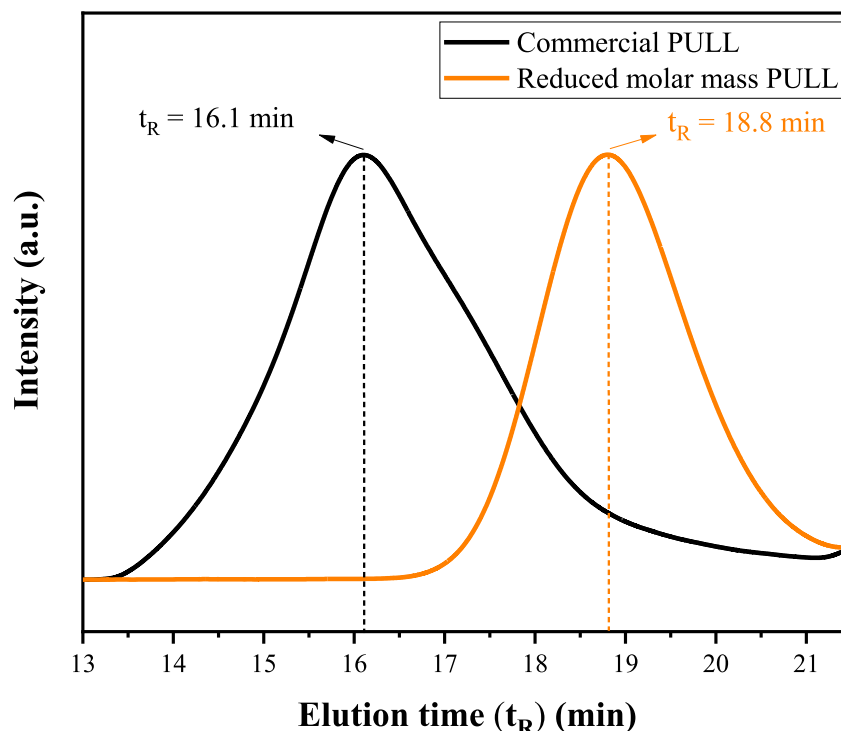


Fig. 1. GPC chromatograms of commercial PULL and reduced molar mass PULL in DMF/LiBr.

Table 2

Molar masses ($\overline{M}_n$ and $\overline{M}_w$), dispersity, and percentage of PNVL in the copolymer for PULL, reduced molar mass PULL, 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*}
PULL	96	288	3.01	-----
Reduced molar mass PULL	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 PNVL in copolymer (%) = $(\overline{M}_n(\text{PULL-g-PNVCL}) - \overline{M}_n(\text{PULLX})) / \overline{M}_n(\text{PULLX}) \times 100$ [58], * The percentage of PNVL in the copolymers was estimated from the difference in molar masses between the copolymers and PULLX as determined by GPC. Because conventional GPC calibrated with linear standards provides relative rather than absolute molecular weights for branched graft architectures, these values represent semi-quantitative estimations of copolymer composition. Although these values are not absolute, the observed increases were significant enough to confirm the presence of PNVL in the copolymer structure.

case, a new band appearing at 1740 cm⁻¹ (C=O stretch) provides further indications of the bromopropionyl grafting onto the reduced molar mass PULL backbone.

3.3. PULL-xanthate-based macro-chain transfer agent characterization

Fig. 4 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 (see Figure S1), 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 Figures S8 and S9 in the Supplementary Information for detailed calculations). The integration value for the combined resonances at 4.87–5.25 ppm (anomeric protons, 2 H) was used as the reference to estimate the DS based on the peak integration from 7.10 to 7.36 ppm. Partial overlap of the anomeric reference signal with neighboring resonances causes the calculated DS values to be underestimates. The difference in anomeric-proton chemical shifts between PULLX-5 and PULLBr-5 spectra arises from the use of different NMR solvents. The DS on bromopropionyl units by CTA-OH in PULLX-5 was determined to be 4.5 per 100 monomer units. The results showed partial substitution of the bromopropionyl groups on the PULLBr-5 backbone by the CTA-OH, leading to a lower number of CTA-OH units grafted to the PULLX-5 than the bromopropionyl groups grafted to the PULLBr-5, despite using CTA-OH in significant excess (9 equivalents). Similar findings were reported by Pal et al. [31], 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 molecules compared to the bromopropionyl group is not surprising. This strategy of converting a hydroxyl- or halide-functionalized polymer backbone into a xanthate-based macro-CTA for subsequent RAFT/MA-DIX grafting has been similarly employed to construct graft copolymers from polyepichlorohydrin backbones [60–62].

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

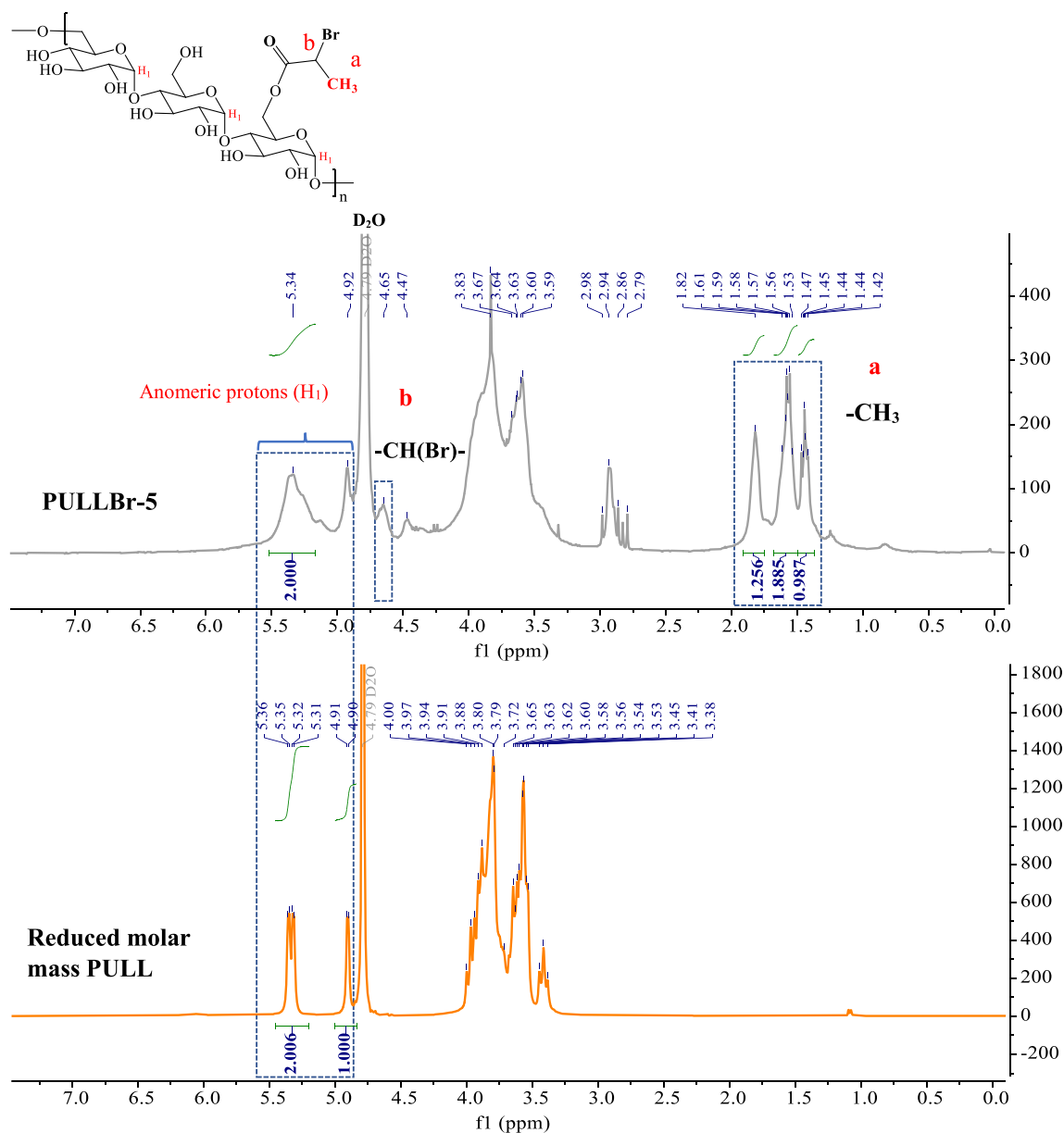


Fig. 2. ^1H NMR spectra of reduced molar mass PULL and PULLBr-5 in D_2O .

reaction. However, the CTA molecules were presented in sufficient numbers to promote RAFT/MADIX polymerization in the last synthetic step.

3.4. PULL-g-PNVCL characterization

Fig. 5 shows the ^1H NMR spectra of PULL-g-PNVCL (150) and PULL-g-PNVCL (300). We were delighted to observe the characteristic resonances of PNVCL between 1.50–2.00 ppm and 2.20–2.40 ppm (the caprolactam ring and the backbone chain of the polymer), as well as the typical resonances of the maltotriose unit of PULL. Therefore, these findings confirmed the successful polymerization of NVCL from the PULL backbone. Additionally, it was observed that increasing the concentration of NVCL monomer in the reaction media generates longer PNVCL chains that translate into higher integral values for the PNVCL signals. Accordingly, the results showed 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) which was determined by ^1H

NMR analysis with anomeric resonances as the integral reference peaks.

A DOSY NMR experiment was performed to further evaluate the effective formation of the PULL-g-PNVCL copolymer. Fig. 6(a) and (b) present 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 greatly reducing 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 polymer would be filtered out in the dialysis step. Indeed, the dialysis step (14 kDa MW cutoff) performed after polymerization effectively removed any ungrafted and smaller polymer chains, as shown in Fig. 6 (b). Therefore, the dominant species align with a single diffusion coefficient that is correlated to the signals corresponding to both PNVCL and PULL, 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.

The ATR-FTIR spectra for PULLX-5, PULL-g-PNVCL (150), PULL-g-

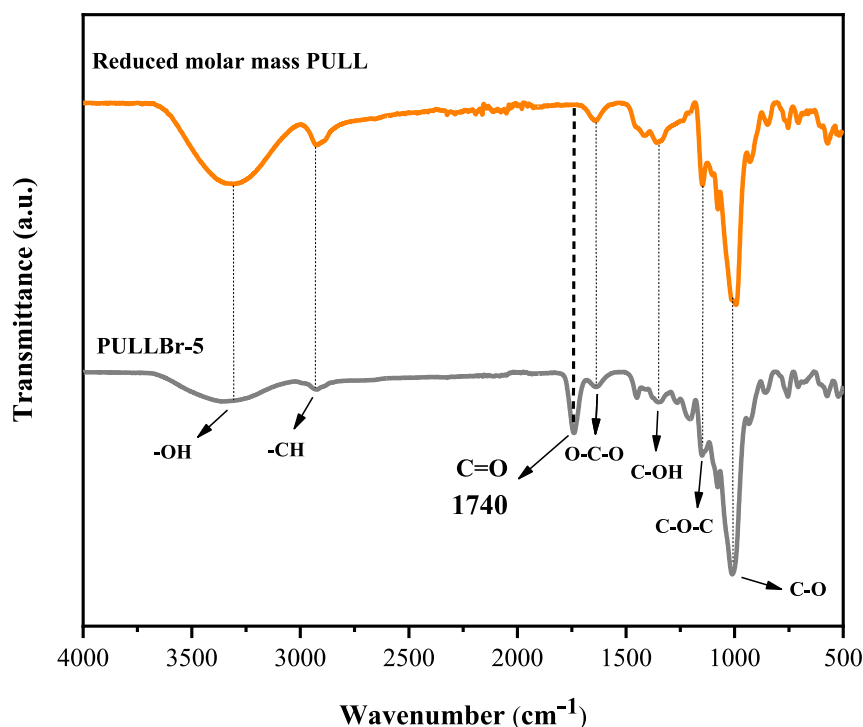


Fig. 3. ATR-FTIR spectra of reduced molar mass PULL and PULLBr-5.

PNVCL (300), and the NVCL monomer are shown in Fig. 7. Extending the spectral range from 1500 to 1700 cm^{-1} reveals a wavenumber shift and an increased intensity for carbonyl peaks ($\text{C}=\text{O}$) characteristic of the caprolactam function, probably due to stacking contributions of grafted PNVCL chains with the O-C-O peak from PULL. These findings align with the observations from the NMR analysis.

GPC analyses of all grafted materials were conducted to further investigate the NVCL polymerization from the PULL backbone. Fig. 8 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 grafted PNVCL growth from the PULLX-5 (Fig. 8 and Figure S11).

The molar masses, dispersity, and PNVCL percentage in copolymers values are summarized in Table 2 and Table S6. Results depicted that the molar masses of all graft copolymers exceed those of the PULL-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 narrow molar mass distributions ($D \leq 1.83$), when compared to the typical dispersity values observed for branched copolymers synthesized via non-controlled polymerization methods (often more than 5) [63–66]. Furthermore, taking into account the inherent dispersity of the PULL backbone, the dispersity values obtained for the PULL-g-PNVCL copolymers are consistent with expectations for such grafted systems. This suggests effective CTA-mediated RAFT polymerization, 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

(Table S6). This trend aligns with prior observations from the ^1H NMR analysis of PULL-g-PNVCL (150) and PULL-g-PNVCL (300). Because the macro-CTA functionalization degree was held constant, the number of active grafting sites per pullulan backbone remained fixed. Consequently, the observed molecular weight growth directly reflects the uniform extension of PNVCL side chains. More interestingly, an increase in molar mass from reduced molar mass PULL to PULLBr-5 and PULLX-5 samples indicates successful grafting of bromopropionyl and CTA groups' side chains, as previously observed in ^1H NMR and ATR-FTIR analyses. This trend is consistent with other studies on polysaccharide modifications (e.g., PULL and dextran), which report stepwise increases in the molar mass following small organic modifications (e.g., acetyl chloride and bromoacetyl bromide) [33,45]. Although isolated yields of modified PULL were in the 37–73% range (Table S7), 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.

3.5. Thermal analyses

Fig. 9(a) presents the thermograms obtained by DSC for PULL, reduced molar mass PULL, PULLX-5, and PULL-g-PNVCL (300), which reveal distinct thermal behaviors for each sample. It was not possible to determine the glass transition temperature (T_g) of PULL [15,45]. Determining transition temperatures for polysaccharides sourced from industry and nature is often challenging due to their high molar mass distribution, a phenomenon also observed in cellulose [15,67]. However, the T_g could be determined for reduced molar mass PULL, PULLX-5, and PULL-g-PNVCL (300). They are listed in Table 3. The

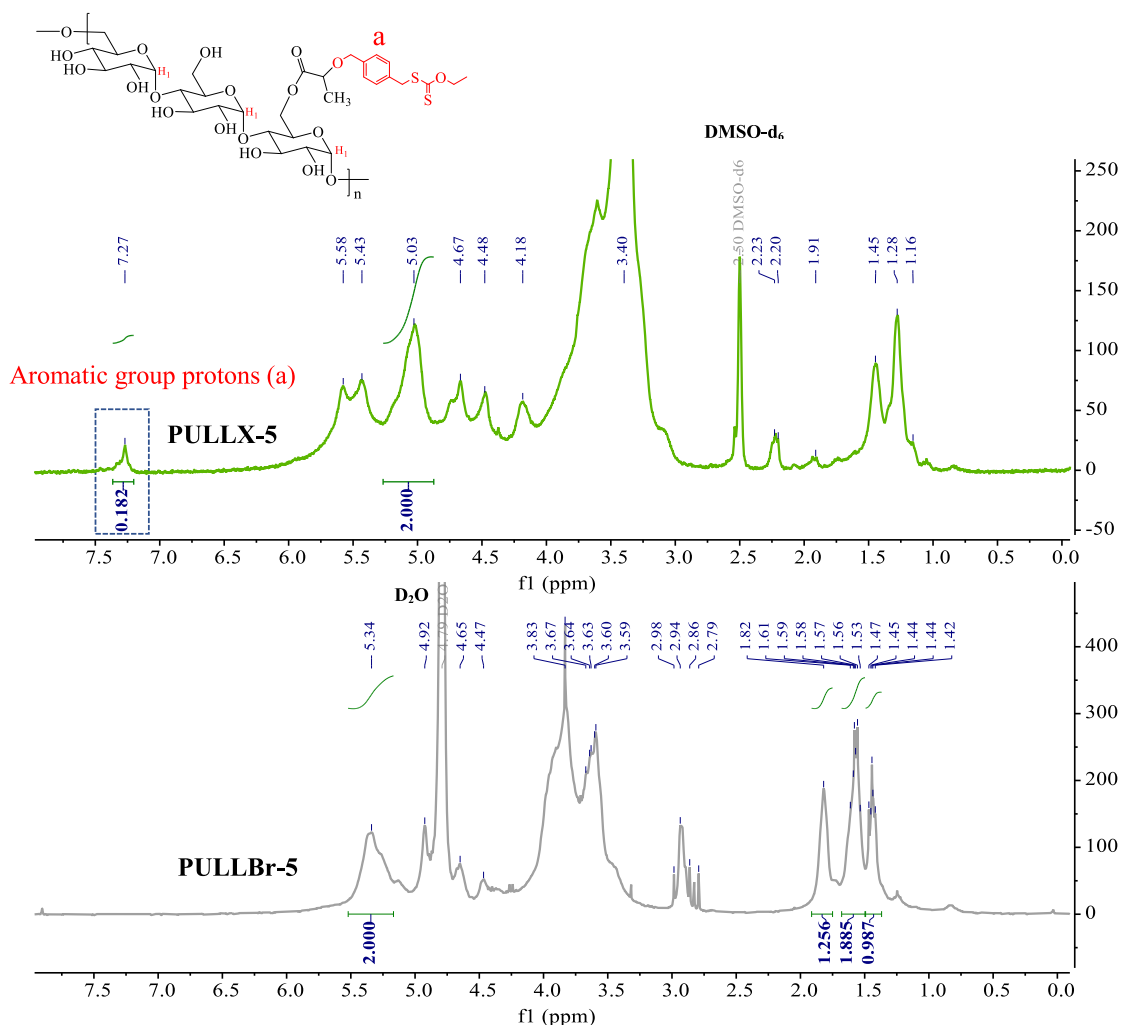


Fig. 4. ^1H NMR spectra of PULLBr-5 in D_2O and PULLX-5 in DMSO-d_6 .

noticeable T_g for all PULL-modified substrates also provided evidence of the successful synthetic sequence. The PULLX-5 had a T_g increase to 233 $^\circ\text{C}$, and after graft copolymerization with PNVC, a T_g reduction to 189 $^\circ\text{C}$ was observed. This decrease can be explained by the branched structure of PNVC, which may diminish intermolecular interactions and enhance chain mobility. A similar trend was reported by T. Carvalho et al. [18] for PULL-g-poly(Z-L-lysine).

The TGA results for PULL, reduced molar mass PULL, PULLX-5, and PULL-g-PNVC (300) are presented in Fig. 9(b). All samples displayed multistep weight loss during thermal decomposition. The initial weight loss, occurring between 30 $^\circ\text{C}$ and 150 $^\circ\text{C}$, is attributed to the loss of moisture [68,69]. The main weight loss observed between 200 $^\circ\text{C}$ and 300 $^\circ\text{C}$ corresponds to the degradation of the biopolymers [5,68,69]. As shown in Table 3, PULL exhibits an initial degradation temperature (T_{onset}) of 288 $^\circ\text{C}$, higher than that of reduced molar mass PULL and the modified PULL samples, indicating superior thermal stability. The T_{onset} for reduced molar mass PULL, PULLX-5, and PULL-g-PNVC (300) decreased to 281, 261, and 251 $^\circ\text{C}$, respectively. This decrease may be linked to molar mass reduction, decreased thermal stability of the CTAs, and diminished cooperative hydrogen bonding along the PULL backbone due to PNVC grafting, respectively. Additionally, it can be suggested that the inter- and intra-molecular hydrogen bonding interactions were replaced by less stable interactions, leading to the reduced thermal stability of the PULL-g-PNVC (300) compared to PULL. Furthermore, similar trends in the reduction of the temperature of maximum degradation rate (T_{max}) for reduced molar mass PULL, PULLX-5, and

PULL-g-PNVC (300) compared to PULL are shown in Table 3. These findings align with Fernández-Quiroz et al. Fernández-Quiroz et al., [69], who reported reduced thermal stability after grafting PNVC onto chitosan, and Omar-Aziz et al. Omar-Aziz et al. [70], who observed similar effects when modifying PULL with octenyl succinic anhydride. As illustrated in Fig. 9(b), the residue for final PULL, reduced molar mass PULL, 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-PNVC (300) can be mainly attributed to the higher carbon content ratio of the copolymer compared to that of the PULLX-5, reduced molar mass PULL, and PULL alone. This provides additional evidence of the successful incorporation of PNVC into the PULL chain.

3.6. Particle size characterization and temperature-sensitive phase transition behavior of PULL-g-PNVC

To evaluate the effect of copolymerization on the average particle size distribution, PULL-g-PNVC (150) and PULL-g-PNVC (300) copolymer particles were prepared via a self-assembly process and were analyzed by DLS at 24 $^\circ\text{C}$. Fig. 10 (a) and (b) show the Z_{av} of the particles. The Z_{av} of PULL-g-PNVC (150) and PULL-g-PNVC (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 [71], larger particle sizes were observed for PULL-g-PNVC (300) than for

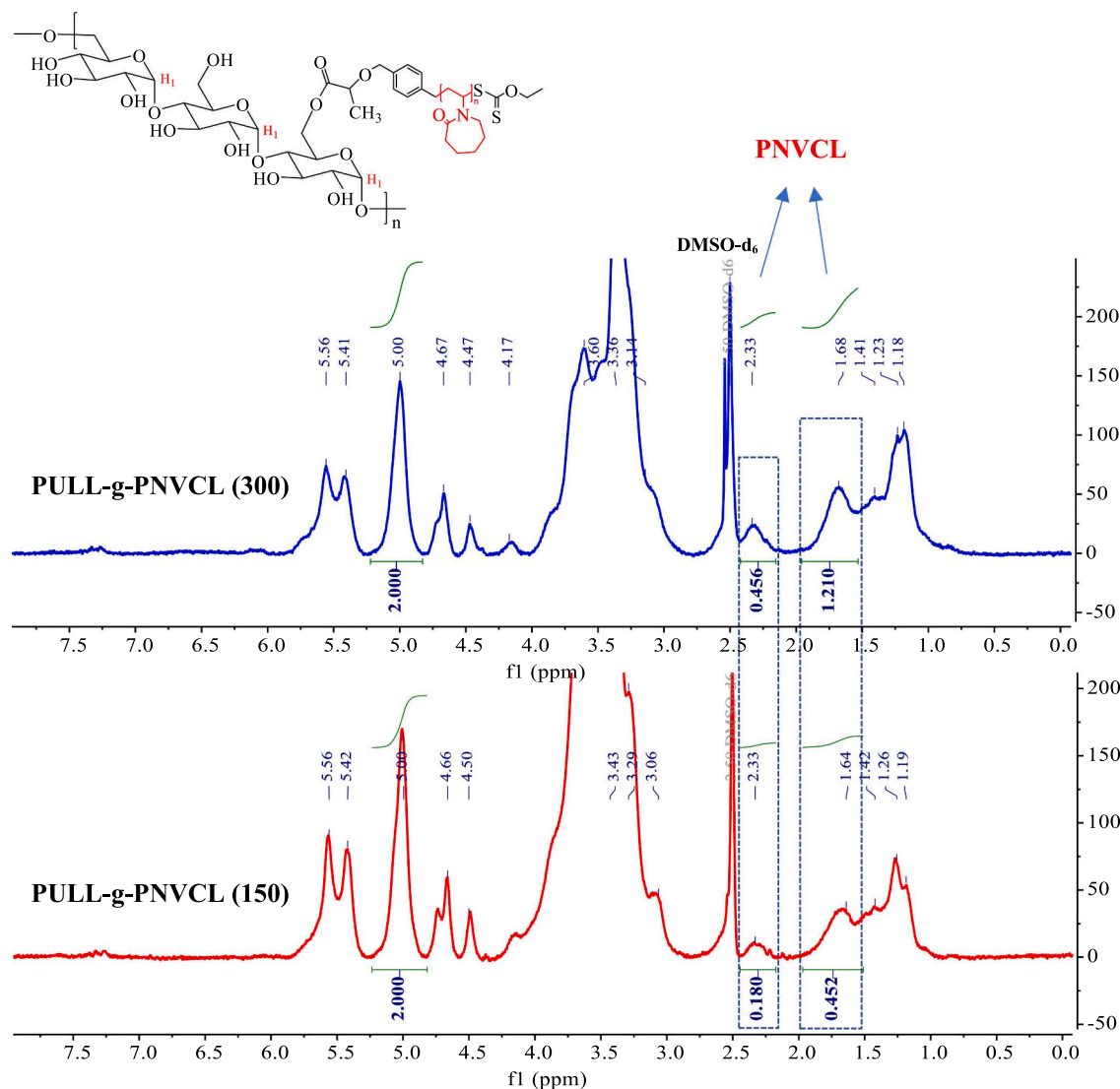


Fig. 5. ^1H NMR spectra of PULL-g-PNVCL in DMSO-d_6 .

PULL-g-PNVCL (150), yet another indication of successful graft copolymerization. This increase in size in DLS measurements, performed at a temperature below the LCST of PNVCCL, 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 thermosensitive 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 Fig. 10 (c). At 24 °C, the hydrodynamic diameters of the particles were around $(10.1 \pm 0.9) \times 10^2$ 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 approached the LCST, particle aggregation occurred, leading to the formation of large floculates, characterized by an abrupt increase in hydrodynamic diameter and a dramatic rise in the turbidity of the dispersion. 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 ranges (27 °C – 42 °C) reported for various PNVCCL copolymers [31,72,73]. These results indicate the expected LCST values for the obtained copolymers, which are summarized in Table 4. 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 $(25.5 \pm 1.7) \times 10^2$ 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 floculates. Such behavior has also been reported for PNVCCL or PNIPAM homo- and copolymers [74–76].

The observed variations in the LCST of the synthesized PULL-g-PNVCL copolymers are attributed to the balance between hydrophilic and hydrophobic interactions arising from their molecular architecture. The phase transition of poly(N-vinyl caprolactam) (PNVCCL) is governed by the competition between hydrogen bonding of the lactam carbonyl groups with water molecules and hydrophobic interactions among the caprolactam rings. Grafting PNVCCL from the pullulan backbone modifies this balance; consequently, the LCST depends on the overall copolymer composition and molecular architecture. 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 PNVCCL 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

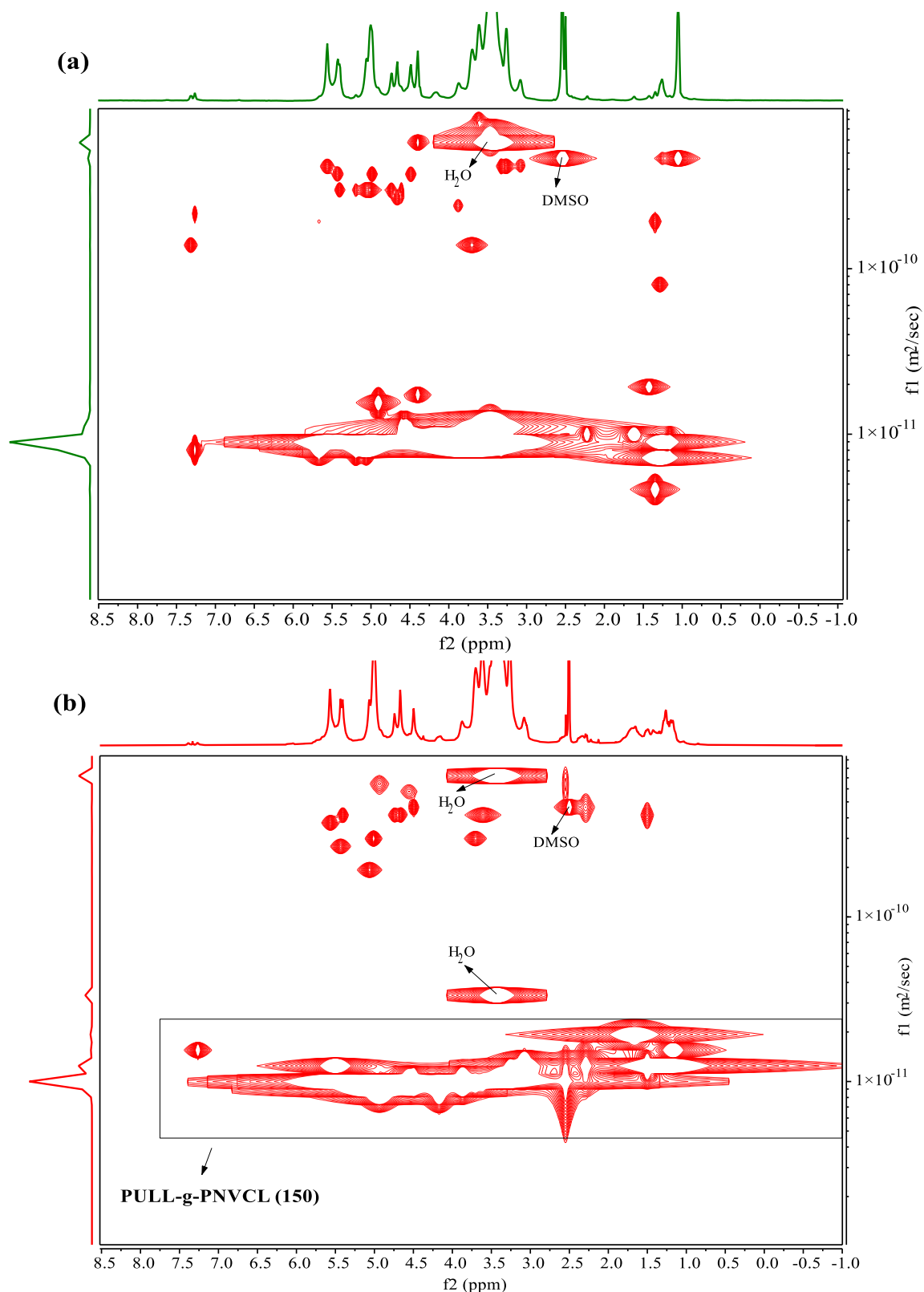


Fig. 6. DOSY spectra of (a) PULLX-5, and (b) PULL-g-PNVCL (150) in DMSO- d_6 .

PULL-g-PNVCL (300) at their respective LCST are shown as dotted lines in Fig. 10 (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.

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. Fig. 11 (a) and (b) display the hydrodynamic diameter of the particle dispersions, transitioning between temperatures below and above or close to the LCST. The

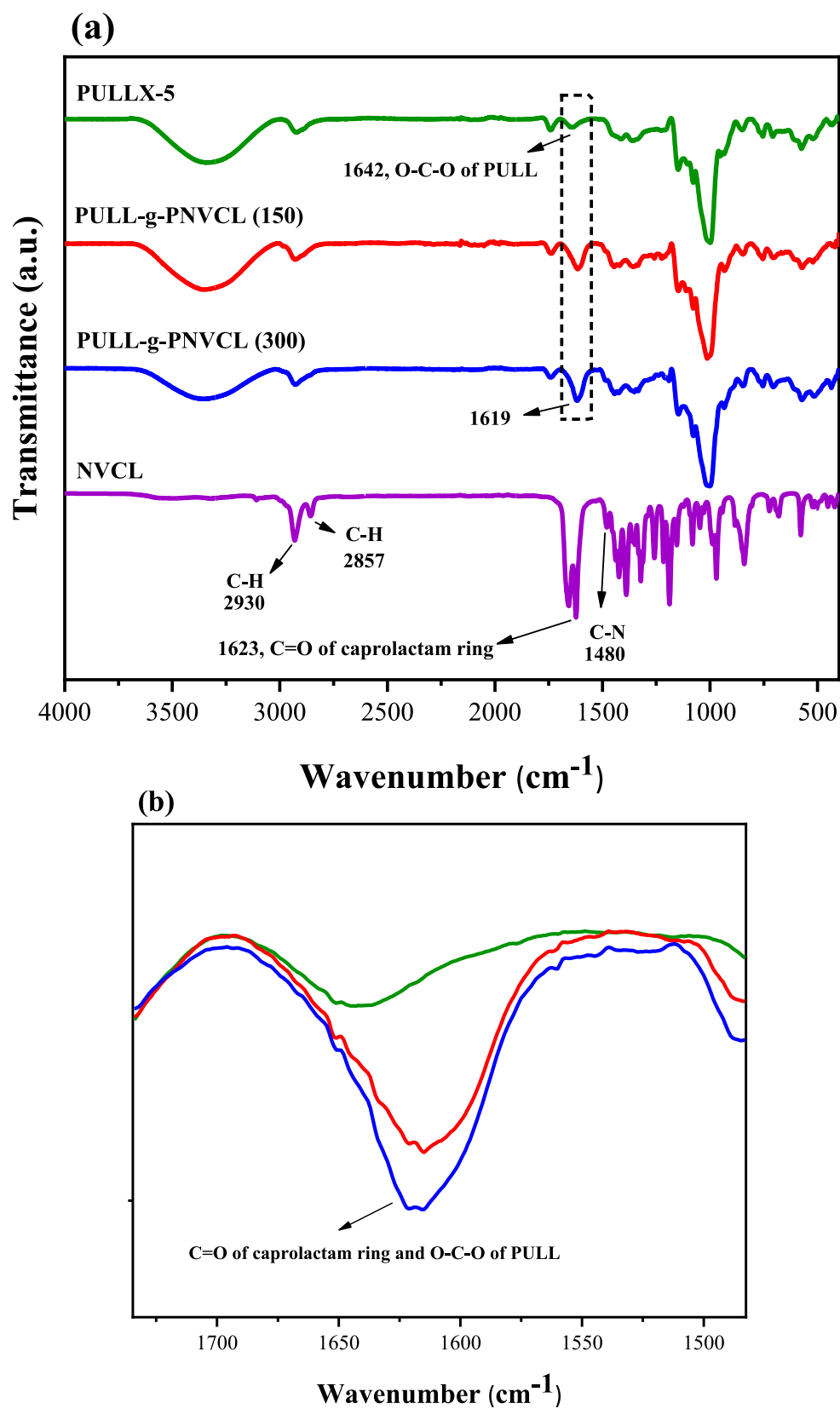


Fig. 7. ATR-FTIR spectra of PULLX-5, PULL-g-PNVCL (150), PULL-g-PNVCL (300), and NVCL monomer, (a) full spectra, and (b) zoom between 1500 cm^{-1} and 1700 cm^{-1} .

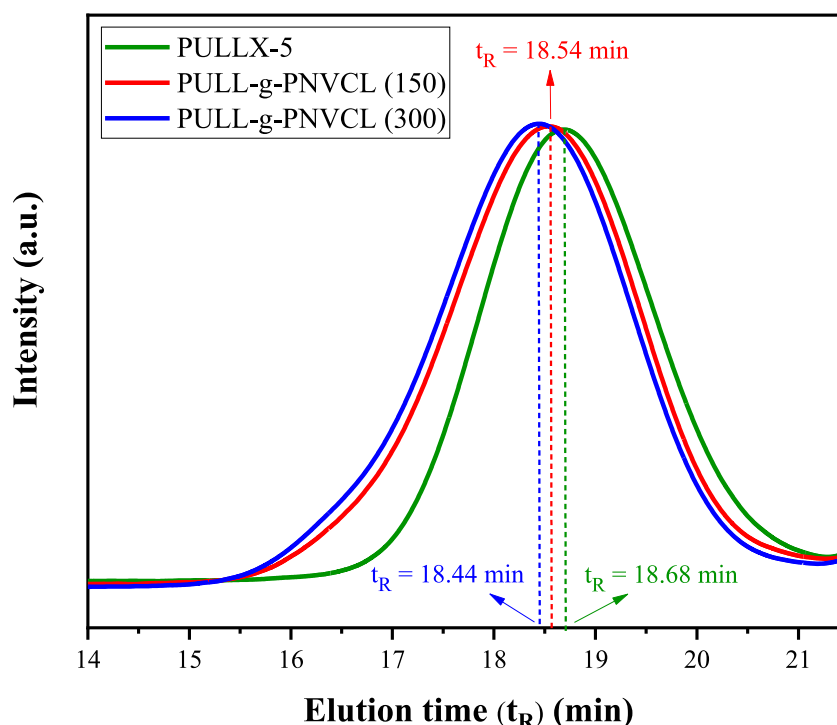


Fig. 8. GPC chromatograms of PULLX-5, PULL-g-PNVCL (150), and PULL-g-PNVCL (300) in DMF/LiBr.

hydrodynamic diameter increased at a higher temperature, returning to nearly the initial value upon cooling, as reported in similar studies [10, 24–26,72].

To further validate the LCST values obtained, DSC analyses were performed on PULLX-5, PULL-g-PNVCL (150), and PULL-g-PNVCL (300) samples. Fig. 10 (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 PNVC 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 PNVC in PULL-g-PNVCL (150) compared to PULL-g-PNVCL (300). The cooling thermograms (Figure S12) 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 PNVC functionalization of the copolymers.

4. Conclusions

Following the successful synthesis of PULL-g-PNVCL copolymers with two different PNVC chain lengths, our findings about the ability to modulate the properties of PULL enable the development of functional polysaccharide-based materials and future applications such as biomedical and drug delivery systems. Notably, the dispersity of the PULL-g-PNVCL copolymer is similar to the PULL starting material, indicating the high efficiency of the xanthate-based CTA in mediating the RAFT polymerization of NVCL. The grafting of PNVC impacted the thermal properties of PULL, leading to a slight reduction but acceptable

thermal stability after copolymerization. Furthermore, dynamic light scattering (DLS) measurements showed that the average diameter of the copolymer particles increased with longer PNVC chain length. Additionally, the lower critical solution temperature (LCST) decreased with shorter PNVC chains, as shown by both DLS and differential scanning calorimetry (DSC). This confirms that the thermosensitive behavior of the synthesized PULL-g-PNVCL copolymer is directly linked to the PNVC content, allowing the LCST to be tailored to specific temperature and application requirements. However, detailed kinetic studies, such as monomer conversion, evolution of molecular weight with conversion, and chain-end fidelity analyses, represent important directions for future work to further confirm the precise living character.

In conclusion, this study demonstrates that RAFT-mediated graft polymerization of NVCL using a PULL-based macro-chain transfer agent represents a significant advancement for future investigations into graft copolymerization of other desired monomers from PULL 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 up possibilities for encapsulation and controlled release of drugs or active substances for the biomedical industry. Overall, this research broadens the applications of pullulan and opens avenues for the development of sustainable, high-value bio-based materials from polysaccharides with potential for industrial and product-oriented applications.

Supplementary data

The following is the [Supplementary data](#) to this article:

Additional information on copolymer synthesis, calculated degree of substitution values, and yields, as well as detailed copolymer characterization by ¹H NMR, FTIR, and DSC.

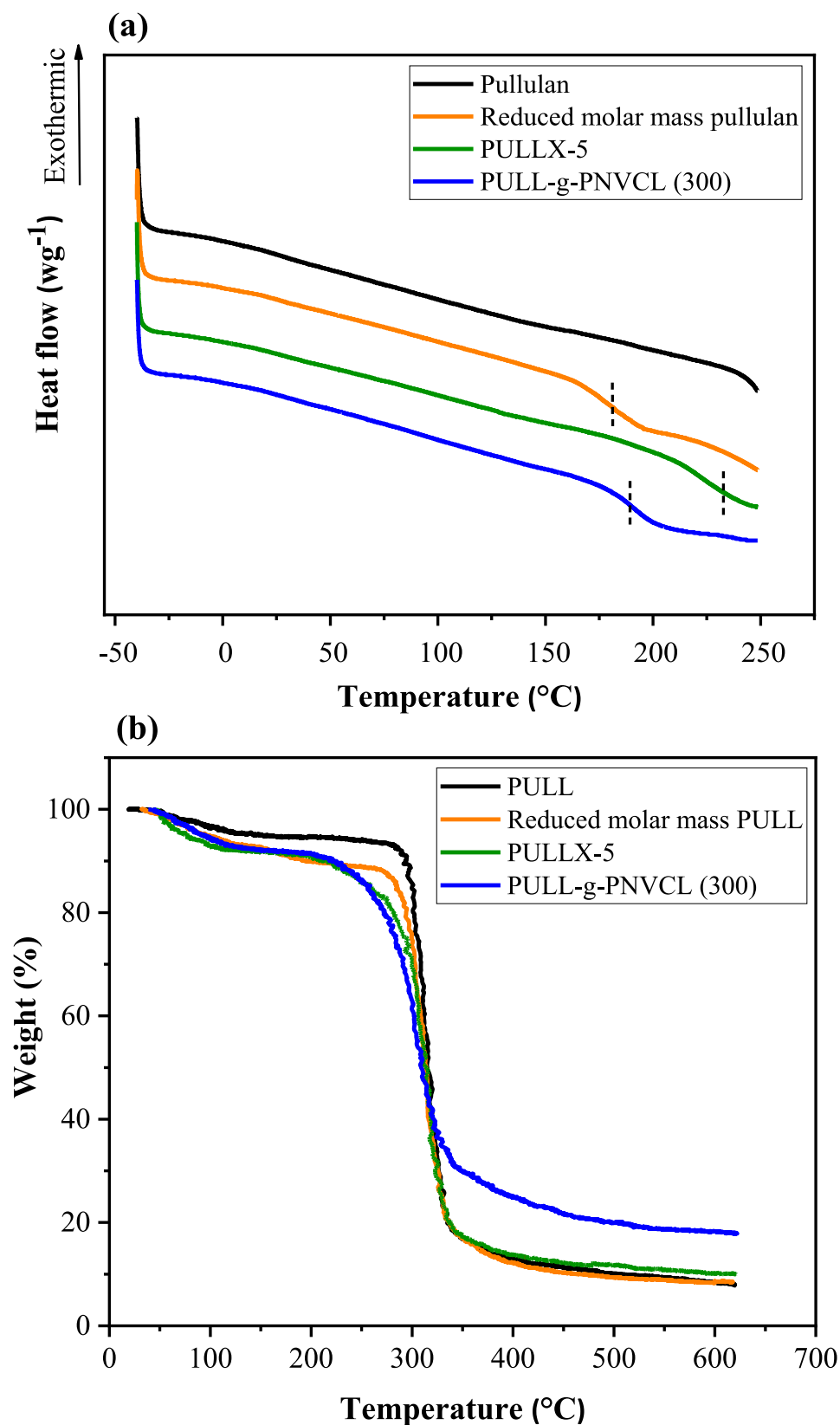


Fig. 9. (a) DSC and (b) TGA thermograms of PULL, reduced molar mass PULL, PULLX-5, and PULL-g-PNVCL (300).

Table 3

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 PULL, reduced molar mass PULL, PULLX-5, and PULL-g-PNVCL (300).

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

CRediT authorship contribution statement

Amilton Martins dos Santos: Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Milan Bergeron-Brlek:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Nicole Raymonde Demarquette:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Formal analysis. **Atefe Sadeghi Lari:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gizelda Maria Alves:** Writing – review & editing, Validation, Methodology. **Gabriela**

Aparecida Braga Silva: Writing – review & editing, Validation, Methodology, Investigation.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) in order to perform grammar checks and improve readability, with caution. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 4

LCST values of the synthesized PULL-g-PNVCL samples.

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

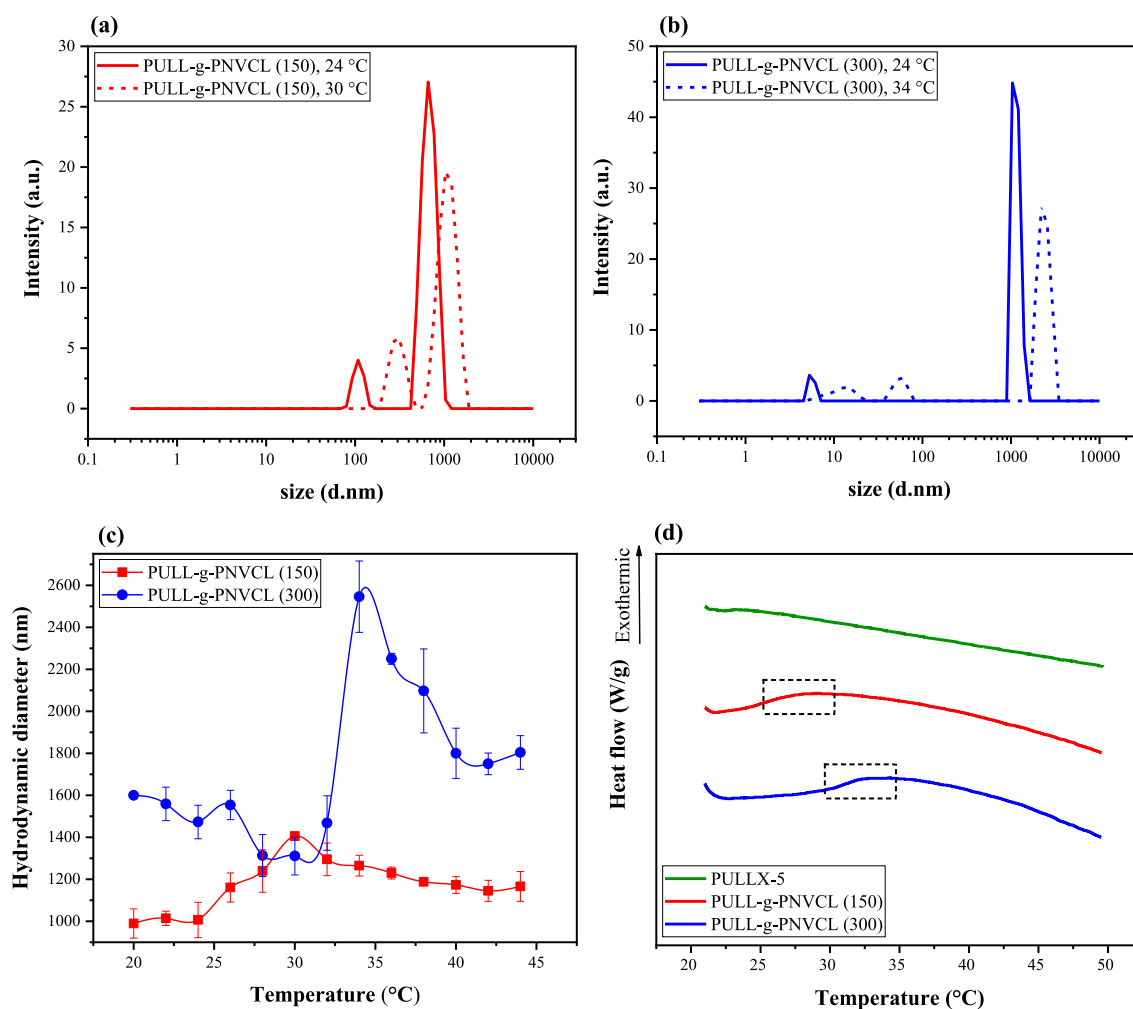


Fig. 10. DLS analyses: (a) size distribution of PULL-g-PNVCL (150) particles at 1 mg/mL at selected temperatures of 24 °C and 30 °C; (b) size distribution of PULL-g-PNVCL (300) particles at 1 mg/mL at selected temperatures of 24 °C and 34 °C; (c) hydrodynamic diameters as a function of temperature for 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.

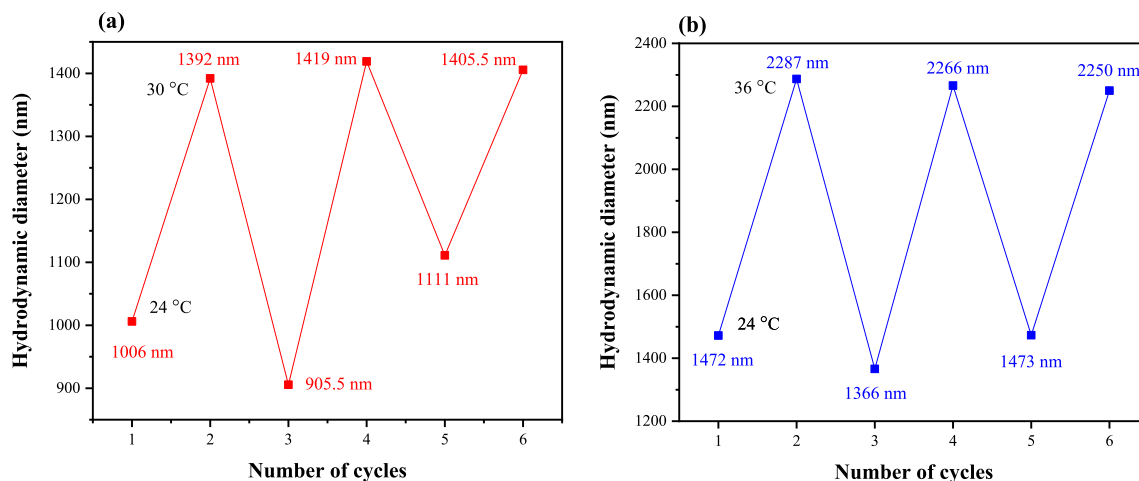


Fig. 11. (a) Hydrodynamic diameter of PULL-g-PNVCL (150) particles at 1 mg/mL as a function of temperature cycling between 24 °C and 30 °C, and (b) hydrodynamic diameter of PULL-g-PNVCL (300) particles at 1 mg/mL as a function of temperature cycling between 24 °C and 36 °C.

the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.mtcomm.2026.116041](https://doi.org/10.1016/j.mtcomm.2026.116041).

Data availability

Data will be made available on request.

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