# Lignin-acrylamide-diallyl dimethylammonium chloride copolymer: Mechanism of Polymerization and Purification

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# Dedications

To my parents and my daughter for all of their love, support, patience and encouragement.

# Abstract

The world economy is growing faster than ever, while environmental issues are getting worse. The use of ecologically friendly materials has increased as a result of environmental concerns. Promoting ecologically friendly products will be greatly aided by biomass, which is a renewable resource. Lignin, as a main component of lignocellulosic biomass and one of the most abundant biopolymers, is utilized extensively in a variety of industrial domains. It has been proposed that lignin can be grafted with a variety of monomers, including glycidyl-trimethylammonium chloride (GTMAC), acrylamide (AM), and acrylic acid (AA) to produce water soluble products.

In this study, a detailed investigation was carried out on the free-radical polymerization of kraft lignin, acrylamide (AM), and diallyl dimethylammonium chloride (DADMAC) to generate flocculants for wastewater treatment. To comprehend the physicochemical properties of this copolymerization system, *in-situ* nuclear magnetic resonance (NMR), rheological analysis, and particle size techniques were conducted. The copolymerization of lignin-AM and lignin-DADMAC had an activation energy of 65.7 and 69.3 kJ/mol, respectively, and followed the first-order kinetic model, which was monitored by *in-situ* $^1$H NMR results. The highest conversions of AM and DADMAC were 96% and 68%, respectively, in the copolymerization of lignin, AM, and DADMAC at the molar ratio of 5.5: 2.4: 1, pH 2 and 85°C. It evidenced that the participation of AM in the reaction was essential for polymerizing DADMAC to lignin due to less steric hindrance of AM than DADMAC facilitating its bridging performance. Lignin acted as an inhibitor in the copolymerization reaction, indicated by the monomer conversion ratio and dynamic rheology of the reaction system.

Purification is an essential step of many polymerization processes to produce highly pure polymers. In this study, different purification methods for purifying the product of lignin, acrylamide (AM), and diallyl dimethylammonium chloride (DADMAC) copolymerization reaction were examined in detail. It had been confirmed that ethanol extraction and membrane filtration were the most effective processes for producing lignin-p(AM)-p(DADMAC) by the charge density, yield, molecular weight, and solubility analyses. Membrane dialysis was considered as the most effective way to remove unreacted AM and DADMAC monomers from the reaction medium, which was proved by $^1$H NMR analysis. The ethanol-extracted and dialyzed samples showed better properties in solubility, yield, degradation temperature and use in flocculation than the acidified samples. Above all, membrane filtration with a larger molecular weight cut-off could be an environmentally friendly method for effectively purifying lignin-p(AM)-p(DADMAC) to obtain a high molecular weight copolymer. In addition to fundamental discoveries, the results of this thesis work confirmed that the polymerization and purification processes of lignin for producing sustainable flocculants is viable in a one-step aqueous system.

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# Table of contents

# List of Figures

# List of Tables

# Chapter 1: Introduction

## 1.1 Overview

Nowadays with growing environmental problems in global, ecologically friendly materials and green chemicals are essential to be investigated and applied. In order to promote environmentally friendly products, biomass will be crucial as a renewable resource. Lignocellulosic biomass contains three major components which are lignin, cellulose and hemicelluloses. Lignin is considered as a waste product in most of processes of lignocellulosic materials fractionation, and it is mostly used for energy generation. Just 1.5% of the 40 to 50 tons of lignin produced annually by the pulp and paper industry are utilized in the material production. Due to its variety of functional groups, the usage of lignin is widely distributed in various fields including oil extraction, dye production, and production of agricultural chemicals (Yang et al., 2007). Furthermore, lignin as the most plentiful source of aromatic compounds in nature has the ability to produce a significant number of chemical reagents or adhesives that can take the place of those made from oil (EI Mansouri & Salvadó, 2006).

Lignin has been suggested to be grafted with many monomers, such as acrylic acid (AA), acrylamide (AM), glycidyl-trimethylammonium chloride (GTMAC), diallyl dimethylammonium chloride (DADMAC), DADMAC with AM, dimethylamine-acetone-formaldehyde (DAF) to produce green chemicals (Lappann et al., 1997; Li, et al., 2016; Mai et al., 2000; Rong, et al., 2013; Wang et al., 2016; Fang et al., 2010). For example, lignin was polymerized by AM or AM and DADMAC and the product was used as a coagulant aid for the removal of aluminum sulfate and polyaluminum chloride, and humic acid from wastewater (Rong et al., 2013; Li et al., 2016). Soda lignin was reacted with acrylamide and the product was used as a strength additive in papermaking (Wang et al., 2016; Lin et al., 2011). In the previous work, lignin was synthesized with DADMAC and AM to produce cationic water-soluble flocculants for wastewater treatment (Price et al., 2018). Comparing with other cationic flocculants, lignin-AM-DADMAC required a lower dosage to achieve an effective removal without the need of a dual polymer system because of its high molecular weight and a comparable charge density (Price et al., 2018). Despite the promising flocculation results, the fundamentals and preparation process of generating lignin-AM-DADMAC in three-component system have not been studied in detail. Therefore, lignin-grafted- p(AM)-p(DADMAC) was chosen to be stuided in my research.

In-situ polymerization analysis has been considered as an advanced technique for understanding the kinetics of polymerization systems. For example, the in-situ $^1$H NMR analysis of the copolymerization of methyl methacrylate (MMA) and DADMAC revealed monomer consumptions for determining the reactivity of monomers in the reaction (Feng et al., 2018). As NMR can provide real-time data on the monomer concentrations, in-situ NMR monitoring was followed in this work to determine the details of the monomer conversions in the copolymerization of lignin with AM and DADMAC in order to synthesize the optimal products.

Generally, purification process is regarded as the next step after the polymerization to remove unreacted monomers, undesired chemicals and impurities. There are variable purification methods including solvents, acidification and membrane dialysis. Solvent is widely used in purification of polymers. Acetone was used for the extraction of lignin-AM and the solid precipitated at the bottom were collected (Rong et al., 2013). For xylan-METAC, i.e., similar to lignin-DADMAC, after adding the solvent, the suspension was centrifuged in order to increase the settling speed (Wang, 2015). Acidification is used as a purification method for lignin-AA and lignin-AM (Kong et al., 2015; Wang et al., 2016). Dialysis membrane tube is another method for purification, previously used in lignin-DAF, lignin-GTMAC and lignin-AM (Kong et al., 2015; Fang et al., 2010; Mai et al., 2000). According to the literature, different purification methods of lignin-p(AM)-p(DADMAC) were studied in detail as well as flocculation performance of the purified samples.

## 1.2 Objectives

The objectives in the thesis are shown as following:

- To study the kinetics and rheology of the copolymerization process of lignin with AM and DADMAC.
- To compare the polymerization processes of AM, DADMAC, AM and DADMAC with and without lignin.
- To compare different purification methods of lignin-p(AM)-p(DADMAC).
- To use lignin-p(AM)-p(DADMAC) as cationic flocculants for wastewater treatment.

## 1.3 Novelties

To the best of our knowledge, the following aspects in the study have not been investigated in previous studies:

- The suspension polymerization reactions that contain lignin have not been investigated by *in-situ* rheological study.
- The change of particle sizes during the polymerization process via FBRM have not been studied.
- The different purification methods of lignin-p(AM)-p(DADMAC) have not been studied previously.

In this MSc thesis, the kinetics and mechanism of copolymerization process of lignin with AM and DADMAC was investigated. The transformation in rheology and particle size of lignin during the polymerization reaction of lignin-p(AM)-p(DADMAC), as well as analogical homopolymers and copolymers were observed through in-situ the rheology and FBRM studies. In addition, purification is an essential step of many polymerization processes to produce highly pure polymers and the selection of purification methods plays a significant role in controlling the desired properties of the lignin-based polymeric products; therefore, the different purification methods of lignin-p(AM)-p(DADMAC) were studied through the analysis of chemical and physical aspects of lignin derivatives. In the chapter, a brief summary of this MSc thesis will be introduced, and the objectives and novelties of the study will be provided.

Chapter two introduces the basic knowledge of lignin that was used in this study. A literature review is provided for the in-situ polymerization, rheology and particle size analyses of lignin coploymerization.

In chapter three, free-radical copolymerization of three component systems of lignin, AM and DADMAC was studied in detail. Also, the physicochemical characteristics of this polymerization process were investigated in an in-situ format by nuclear magnetic resonance (NMR), rheological analysis and particle size techniques. This analysis provided fundamental evidence of lignin polymerization for generating cationic lignin-derived polymers.

In chapter four, various purification methods were considered at laboratory scales for purifying the product of lignin, AM and DADMAC copolymerization reaction. The effects of different separation methods on the charge density, solubility, and yield of the products were discussed in detail. Also, the structural and elemental analyses of the purified polymers were conducted using NMR, thermogravimetric analysis (TGA), static light scattering (SLS), and rheological analyses. The efficiency of the products as flocculants was evaluated using a dynamic drainage jar (DDJ) in kaolinite systems as well. The results of this work provide insights into the development of an efficient purification pathway for producing lignin-derived polymers with desired properties.

In chapter five, the overall conclusions are stated and the recommendations for further work are provided.

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# Chapter 2: Literature Review

## 2.1 Introduction

With the rapid development of global economy, environmental problems grow. Environmental concerns have promoted the application of environmentally friendly materials. Biomass as a renewable product will play an important role in promoting environmentally friendly products. Lignin, cellulose and hemicelluloses are three major components of lignocellulosic biomass. Lignin, as one of the most abundant biopolymers, is widely used in various industry fields. In this chapter, the basic knowledge of lignin including its chemical structure, application in industry and fractionation approaches will be introduced.

Lignin has been suggested to be grafted with many monomers, such as acrylic acid (AA), acrylamide (AM), glycidyl-trimethylammonium chloride (GTMAC). Lignin copolymers and their separation methods will be described in the following contents. In addition, fundamentals about in-situ polymerization process will be studied and discussed. Due to promising application of lignin-based polymers in flocculation, flocculation process will be discussed.

## 2.2 Lignin

Lignin is a hydrophobic material existing in the cell wall of wood helping with the strength of plants (Thakur et al., 2014). Due to the condensation of phenylpropane units, lignin has a complex three-dimensional polymeric structure. Lignin is macromolecular material composed of crosslinked aromatic monomers of including p-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S), as shown in Figure 2.1 (Clifford, 2017). Lignin isolated from herbaceous crops is made of 10 to 25% H-structure and 25 to 50% G- and S-structure (Clifford, 2017). The prominent structure in softwood lignin is coniferyl alcohol, 90 to 95% (also called guaiacyl), while both coniferyl and sinapyl alcohols are the building blocks of hardwood lignin (Clifford, 2017).

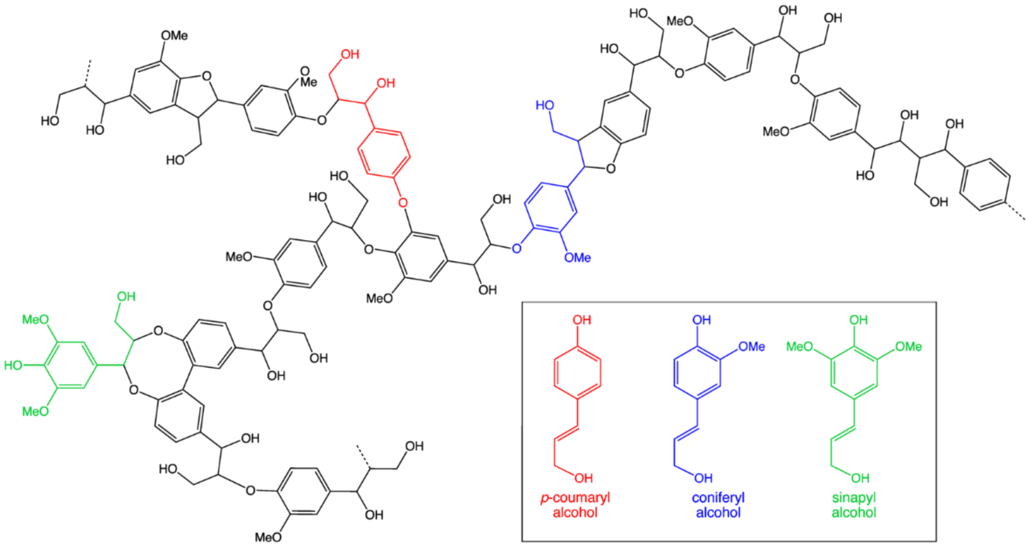

**Figure 2.1.** Lignin chemical structure (Clifford, 2017)

Lignin is considered as a waste product in most of processes of lignocellulosic materials fractionation, and it is only used for energy generation. In the pulp and paper industry, about 40 to 50 million tons of lignin is produced per year but only 1.5% is used for material production. The diversity of functional groups in lignin allows for the utilization of lignin as oil well dispersants, dyestuff, coal water slurry dispersants, agricultural chemicals and other industrial binders (Yang et al., 2007). In addition, lignin as the most abundant source of aromatic compounds in nature can generate a large amount of chemical reagents or adhesives to replace those derived from oil (EI Mansouri & Salvadó, 2006).

Lignin has a wide range of molecular weight, i.e., 1,000 Da to 100,000 Da; therefore, fractionation has become one of the best ways to obtain lignin with narrow molecular weights (Toledano et al., 2010). There are three major methods of lignin separation and fractionation. The first one is the pH induced precipitation (Sun & Tomkinson, 2001). However, the method may result in the formation of lignin colloids, causing the complication of filtration and separation process that affects the purity of produced lignin. The second fractionation method is the solvent precipitation. Lignin with low molecular weight, which is used for synthesizing high-value-added products, can be separated following this method. The drawback of this method is its high operation cost, solvent recovery and environmentally concern related to solvent use (Kilpeläinen et al., 2007). The last method is membrane technology, which can fractionate lignin with certain range of molecular weight (Colyar et al., 2008). Generally, the membrane filtration can be classified as microfiltration, ultrafiltration, nanofiltration and reverse osmosis (Kubota et al., 2008). These four types of membranes have been studied in lignin separation, especially ultrafiltration (Alén et al., 1986; Bhattacharjee, Sarkar et al., 2006; Keyoumu, et al., 2004; Paleologou, et al., 1994; Wallberg, et al., 2003).

### 2.2.1 Kraft lignin production

Lignin is generally isolated from wood through the pulping processes, such as kraft or sulfite pulping (Gellerstedt, 2015). In decades, commercial softwood kraft lignin (Indulin AT) had only been produced by the MeadWestvaco kraft mill in Charleston, SC, USA, and extracted through a two-step acidification (US Patent No. 3223697, 1965). Presently, LignoBoost and LignoForce are widely used for kraft lignin extraction from the black liquor of kraft pulping process (WO Patent No. 2006/031175, 2006; Tomani, 2010). These separation methods rely on pH induced precipitation and filtration (Vistal & Kraslawski, 2011).

## 2.3 Free-radical polymerization

Free-radical polymerization is one of the most common polymerization processes in solution or suspension reactions. The major mechanism of chain formation in a free-radical polymerization are composed of free-radical generation, initiation, propagation and termination, in which free radicals can react in different manners involving addition, coupling, disproportionation, abstraction, fragmentation and transmutation (Gurrero-Santos et al., Free redical polymerization, 2013). The first stage in a chain reaction is the initiation step, which is to add initiating free radicals to monomers (Gurrero-Santos et al., Free redical polymerization, 2013). The addition process begins with the monomer addition on the propagating radicals so that the polymer chains are formed (Gurrero-Santos et al., Free redical polymerization, 2013). During propagation, the stability of a radical is not changed by adding monomer units to the radical but is altered by the group substituent meaning (Gurrero-Santos et al., Free redical polymerization, 2013). At last, free-radical polymerization is terminated by coupling or disproportionation (Gurrero-Santos et al., Free redical polymerization, 2013).

The mechanisms and kinetics of free-radical polymerization were investigated in many research (Salas et al., 2019; Krys & Matyjaszewski, 2017; Naguib et al., 2009; Braun & Hu, 2005; Benoit, et al., 2000; Fukuda, et al., 1996). In these studies, free radical polymerization processes were usually investigated by experimental methods and simulated based on calculation methods with known kinetic parameters, such as propagation rate constant ($k_p$), decomposition rate constants ($k_d$) and initiator efficiencies (f). In Jung's study (2019), a free radical polymerization algorithm provided a molecular dynamics model of poly (methyl methacrylate) (PMMA), which simulated the chain growth of the thermoplastic network started from the initiator radical molecule (Jung, Park, & Yun, 2019). In a terpolymerization of methyl methacrylate, acrylomitrile and indene system, the rate of the copolymerization of the three monomer pairs was observed, and the reactivity ratios for the dual systems were calculated through the Kelen-Tudos model (Naguib et al., 2009). The study of free radical terpolymerization of trans-anethole (ANE), maleic anhydride (MSA) and n-ethylmaleimide (NEMI) indicated that the equilibrium constants for complex formation between two monomers were measured by $^1$H NMR and the simulation of participation of free monomers and complexes was built on kinetic measurements (Braun & Hu, 2005). The *in-situ* polymerization processes of lignin-derived copolymers have rarely been studied in the literature.

### 2.3.1 Rheology

Rheology is one of the most common aspects of polymerization processes. The structural characteristics of different materials, such as polymers, microemulsions or surfactants, as well as their molecular interactions can be studied via monitoring the rheology behavior, e.g., viscoelasticity parameters including dynamic modulus, complex viscosity and loss tangent, of the reaction media (Wang et al., 2016). The dynamic viscosity or complex viscosity during the reaction reflects the network formation or crosslinking in the system (Fei, et al., 2014; Zhang & Easteal, 2004; Haraguchi et al., 2005). The rheological studies can reflect how lignin can be polymerized with monomers.

### 2.3.2 Particle size distribution

Mostly combined with particle size distribution (PSD), the morphology of the polymerization systems is studied by image analysis, such as SEM or TEM (Raoufian et al., 2018; Hamzehlou et al., 2017; Hu et al., 2013; Cui et al., 2007; Soto et al., 2004). For example, acrylamide grafted styrene-copolymer/2-hydroxyethyl acrylate (P(St-co-HEA)) was studied by SEM and DLS(Xia et al., 2002). SEM and PSD were also used for poly(methyl methacrylate) (PMMA) synthesized in an emulsion polymerization system, demonstrating that the emulsion polymerization started at the dispersed water droplets and the PMMA latex was formed through a homogeneous nucleation mechanism (Bao et al., 2004).

In-situ focused beam reflectance measurement system (FBRM) is widely used for studying crystallization and flocculation processes (Eggerta et al., 2019; Jiang et al., 2019; Sodhi et al., 2019; Kyoda et al., 2019; Ng et al., 2018; Kazzaza et al., 2018) but it has rarely been used for investigating polymerization. FBRM offers better images and statistical results for the chord length distribution of particles in solutions (Emmerich et al., 2019). The in-situ FBRM is the most commonly used technique for monitoring real-time particle size change in the system (Kougoulos et al., 2005). Therefore, it can be used for studying the particle size of lignin in the polymerization systems.

## 2.4 Lignin copolymerization

The modifications of lignin with acrylic acid (AA), acrylamide (AM), diallyl dimethylammonium chloride (DADMAC), DADMAC with AM, dimethylamine-acetone-formaldehyde (DAF) were studied in the past (Lappann et al., 1997; Li, et al., 2016; Mai et al., 2000; Rong, et al., 2013; Wang et al., 2016; Fang et al., 2010). For example, lignin was polymerized by AM or AM and DADMAC and the product was used as a coagulant aid for the removal of aluminum sulfate and polyaluminum chloride, and humic acid from wastewater (Rong et al., 2013; Li et al., 2016). Soda lignin was reacted with acrylamide and the product was used as a strength additive in papermaking (Wang et al., 2016; Lin et al., 2011) . In the previous work, lignin was synthesized with DADMAC and AM to produce cationic water-soluble flocculants for wastewater treatment (Price et al., 2018). Comparing with other cationic flocculants, lignin-p(AM)-p(DADMAC) copolymers required a lower dosage to achieve an effective removal without the need of a dual polymer system due to its high molecular weight and a comparable charge density (Price et al., 2018). Except the promising flocculation results, the fundamentals and preparation process of generating lignin-AM-DADMAC in three-component system have not been studied in detail. Therefore, lignin-grafted- p(AM)-p(DADMAC) was chosen to be stuided in this paper.

### 2.4.1 Lignin copolymer separation

In previous studies, many types of purification processes were utilized as the last step of polymer synthesis for removing monomers, impurities, or undesired polymers. Solvents, e.g., acetone and ethanol, are widely used in purification of polymers. According to Rong (2013), lignin-acrylamide (lignin-AM) polymer was precipitated by acetone after the mixture was cooled to room temperature; acrylamide-[2-(methacryloyloxy)ethyl] trimethylammonium chloride-methacryloxypropltri-methoxy silane polymer (P(AM-DMC-MAPMS)) was collected by using mixture of acetone and ethanol from the reaction system (Shang et al., 2009). In Wang's study (2015), xylan-[2-(methacryloyloxy)ethyl] trimethylammonium chloride (xylan-METAC) was extracted by ethanol. Acidification was used as another purification method for polymers. In cases of lignin-acrylic acid (lignin-AA) and lignin-AM (Kong et al., 2015; Wang et al., 2016), acidification was used as a method for lignin polymer recovery. Dialysis membrane is another method for purification. It was previously used for lignin-glycidyl-trimethylammonium chloride (lignin-GTMAC), lignin-dimethylamine-acetone-formaldehyde (L-DAF), and lignin-AM recovery (Kong et al., 2015; Fang et al., 2010; Mai et al., 2000). In some studies, two or more purified methods were combined to reach higher purification efficiency. For example, the combination of acidification and dialysis was utilized for lignin-AM separation (Wang et al., 2016). The copolymer of acrylamide and acryloylamino-2-hydroxypropyl trimethyl ammonium chloride (AM-AMHP) was firstly dialyzed by membrane of 4.2 KDa, and then the solution was extracted in acetone and separated by filtration. Although different separation methods were used as the last step in the polymerization reaction, there is few research to investigate the benefits of different methods and the properties of separated samples by these methods.

### 2.4.2 Lignin copolymer characterization

Charge density, organic elements, molecular weight, chemical structure and thermal stability are important properties of lignin derived materials. Charge density is one of the primary parameters that impact the flocculation performance of polymers. Generally, charge density of lignin copolymers may be higher or lower than that of unmodified lignin. As charge density cannot be used as the only parameter to prove the reaction occurrence, the chemical structure of the copolymers should be studied by nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR), for example. FTIR can characterize functional groups in lignin samples and differentiate chemical composition of organic compounds (Thermo Nicolet Corporation, 2001). Typical lignin peaks referred by wavelength indicate structure correspondences (Lora & Glasser, 2002). $^1$H NMR can determine protons on carbon, oxygen and nitrogen in lignin samples (Glasser et al., 1993; Schnell & Spiess, 2001) so that the chemical structure of lignin can be drawn based on chemical shifts. $^{31}$P NMR can identify hydroxyl groups in lignin by using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphopholane as the phosphorylation reagent, cyclohexanol as the internal standard and chromium(III) acetylacetonate as a relaxation reagent (Granata & Argyropoulos, 1995; Streubel, 2016).

Organic element of lignin copolymers can be detected by an elemental analyzer via combustion method. According to Kong et al. (2015), the degree of substitution of GTMAC on lignin can be calculated based on the nitrogen content of the copolymer and molecular weight of lignin and GTMAC.

Molecular weight is another major parameter that impacts the flocculation mechanism of polymers and is generally determined by a gel permeation chromatography (GPC) or static light scattering (SLS). GPC, also called size exclusion chromatography, can be used to measure molecular weight distribution of lignin including number-average ($\overline{\mathrm{M}}_{\mathrm{n}}$), weight-average ($\overline{\mathrm{M}}_{\mathrm{w}}$) molecular weight and polydispersity ($\overline{\mathrm{M}}_{\mathrm{w}}/\overline{\mathrm{M}}_{\mathrm{n}}$) based on the size of polymer passing through a column having various pore sizes (Runyon et al., 1969). The principle of SLS is to measure the intensity of scattered light through a molecule by different angles and to calculate molecular weight by using the relationship of the light intensity of a molecule and its molecular weight based on Rayleigh theory. Larger molecules scatter more light than smaller molecules from the same light source. The molecular weight of a molecule is proportional to the scattered light intensity of the molecule.

For studying thermal characteristics of lignin or lignin copolymers, differential scanning calorimetry (DSC) and thermo-gravimetric analysis (TGA) can be used. DSC can determine the thermal behavior associated with physical changes of a substance with a constant rate of temperature increasing or decreasing (Menczel et al., 2009). In the literature, lignin introduced into the polymers increased glass transition temperature because of its condensed rigid phenolic moieties and strong intermolecular hydrogen bonding interactions (Chung et al., 2013). TGA can study the thermal stability and degradation of lignin samples (Seoa et al., 2010). TGA for lignin is achieved by heating samples from 25 °C to 800 °C at a constant temperature-increasing rate under nitrogen atmosphere (Toledano et al., 2010). TG curves indicate the relationship between the weight loss of substances and the temperature of thermal degradation. Also, the first derivative of the TG curves (DTG) describes the weight loss rate versus the degradation temperature.

## 2.5 Flocculation

Generally, industrial wastewater contains suspended solids, colloid particles, dissolved solids, organic and inorganic matters, and other impurities (Bratby, 2006). In various wastewater treatment systems, flocculation is commonly used in water treatment to improve the clarification of water prior to filtration processes. Flocculation is a widely used solid-liquid separation process for the removal of suspended solids, colloid particles, dissolved solids and organic matter (Radoiu et al., 2004). The flocculation processes normally start by the addition of flocculants, follow by rapid mixing and then slow stirring, and end by settling flocs (Lee et al., 2014).

Flocculants, as the most important part in the flocculation processes, can be classified as organic and inorganic flocculants. Inorganic flocculants are widely applied in wastewater treatment due to their very low price (Lee et al., 2014). Nowadays, the use of organic flocculants, also called polymeric flocculants, involving synthetic, natural and grafted flocculants, have been increasingly. Synthetic flocculants are linear polymers formed by repeating units of monomers like acrylamide and acrylic acid (Suopajärvi et al., 2013). The synthetic flocculants can be divided by charge types including cationic, anionic, amphoteric and non-ionic. The flocculation efficiency mainly depends on charge types, charge density and molecular weight. These flocculants are water soluble, highly efficient with small quantities and have no effect on solution pH, but may cause environmental problems (Lee et al., 2014). Cationic synthetic flocculants usually have higher toxicity than anionic and non-ionic ones (Bolto & Gregory, 2007). Due to the demand for ecofriendly materials, natural flocculants have been used in wastewater treatment. They are based on natural polymers like starch, cellulose, lignin, natural gums, and their derivations etc. (Sharma et al., 2006). The natural flocculants are safe and biodegradable (Renault et al., 2009).

There are three types of flocculation mechanisms: charge neutralization, bridging and electrostatic patch. Molecular weight and charge density play an important role in controlling the acting mechanism. Charge neutralization is main mechanism for flocculation. It is caused by the reduction in the electric double layer repulsion between particles (i.e., reduction of zeta potential) due to the adsorption of highly charged polyelectrolytes or inorganic salt on oppositely charged particles (Zhou & Franks, 2006). The reduction of zeta potential leads to the formation of van der Waals force of attraction to initiate aggregation of colloidal and fine suspended materials. To optimize the charge neutralization performance, the dosage of flocculant should reduce the zeta potential close to zero (isoelectric point). However, if the addition of flocculant is excess, a charge reversal can occur. The flocs formed from charge neutralization are fragile, packed loosely and settle down slowly. To achieve better flocculation, charge neutralization is applied along with bridging and electrostatic patch (Lee et al., 2014). Flocculation may also take place through bridging process where high molecular weight of long chain polymer is introduced to a dispersion system. These polymers act as bridging agents by adsorbing onto more than one dispersed species at a time with a significant portion of the polymer chain remaining in the aqueous phase. Furthermore, bridging can generate aggregates (flocs) more resistance to breakage at high shear level (Lee et al., 2014). The electrostatic patch flocculation is thought to be operative for polymers of very high charge density interacting with oppositely charged particles of low charge density. The net residual charge of the polymer patch on one particle surface can attach to the bare part of an oppositely charged particle (Zhou & Franks, 2006).

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# Chapter 3: In situ copolymerization studies of lignin, acrylamide and diallyl dimethylammonium chloride: Mechanism, kinetics and rheology

## Abstract

In this work, free-radical polymerization of kraft lignin, acrylamide (AM), and diallyl dimethylammonium chloride (DADMAC) was studied in detail. In situ nuclear magnetic resonance (NMR), rheological analysis, and particle size techniques were conducted to understand the physicochemical characteristics of this copolymerization system. The copolymerization of lignin-AM and lignin-DADMAC had an activation energy of 65.7 and 69.3 kJ/mol, respectively, and followed the first-order kinetic model, which was monitored by in-situ $H^1$ NMR results. The highest conversions of AM and DADMAC were 96% and 68%, respectively, in the copolymerization of lignin, AM, and DADMAC at the molar ratio of 5.5: 2.4: 1, pH 2 and 85°C. The results illustrated that the participation of AM in the reaction was essential for polymerizing DADMAC to lignin due to less steric hindrance of AM than DADMAC facilitating its bridging performance. The monomer conversion ratio and dynamic rheology of the reaction system indicated that lignin acted as an inhibitor in the copolymerization reaction. The particle size analysis of the reaction mixtures reflected the alteration in the size of particles from coarse particles (>300 μm) to fine particles (<10 μm and 10-50 μm) and suspension to colloidal systems when the reaction progressed. The oscillation study of the reaction media confirmed the gradual increase in the viscosity of the reaction media, illustrating the crosslinking of lignin, AM, and DADMAC.

## 3.1 Introduction

Lignin is an under-utilized component of woody biomass and has a complex three-dimensional polymeric structure. It was reported that the chemical pulping industry generates over 70 million tons of lignin every year worldwide (Kazzaz & Fatehi, 2020). Despite its widespread availability, only 5% of the lignin generated by the pulping industry was recovered and sold for commercial applications (Kazzaz & Fatehi, 2020). In contrast, the remaining 95% was either burned as a fuel source or disposed of (Kazzaz & Fatehi, 2020). To diversify the use of lignin in sustainable product development, lignin has been converted into other value-added products, such as lignin nanoparticles, battery binders and electrodes, emulsion stabilizers, carbon fibers, biosorbents, chemical reagents, adhesives, flocculants, paper-strength additives, gene carriers, and hydrogels (Kang et al., 2018; Li et al., 2022; Renault et al., 2009). Among them, the use of lignin for flocculant productions seems promising as challenges associated with clean and safe drinking water is a global health problem.

The current coagulants/flocculants for wastewater treatment rely heavily on either inorganic salt (i.e., alum, $FeCl_3$) or petroleum-based synthetic polymers, including poly(acrylamide) (PAM), cationic poly(acrylamide) (CPAM), and anionic poly(acrylamide) (APAM). Although these materials are efficient, inorganic salts are toxic and produce large quantities of sludge, and synthetic polymers are nonbiodegradable, and their respective monomers can be harmful (Liu et al., 2022). The diversity of functional groups in lignin has also created opportunities for producing lignin-based flocculants for use in various industrial wastewater effluents (Wang et al., 2020). The polymerization of lignin and acrylamide can lead to a more environmentally friendly flocculant than PAM. In this regard, lignin-acrylamide polymers are usually neutral or negatively charged, only suitable for flocculating cationic material, such as cationic dyes (Rong et al., 2013; Lou et al., 2018).

Generally, a flocculant with higher cationic charges could remove more negative colloids from wastewater. To produce cationic lignin, glycidyl trimethylammonium chloride (GTMAC) was grafted on hardwood lignin to create a highly cationic lignin-based polymer suitable for sulfate removal and kaolin settling (Wahlström et al., 2017). Cationic lignin-GTMAC could also facilitate water purification by the agglomeration of viruses and other microorganisms in waster (Rivière et al., 2020). Also, lignin was copolymerized with cationic monomers to produce cationic lignin-based flocculants/coagulants. In another work, lignin-based dimethylamine–acetone–formaldehyde copolymer (L-DAF) was synthesized for the removal of anionic dyes (Acid Black 1, Reactive Red 2, and Direct Red 23) via coagulation–flocculation (Fang et al., 2010). Previous studies also demonstrated that [2-(acryloyloxy) ethyl] trimethylammonium chloride (ATAC) and [2-(methacryloyloxy)ethyl] trimethylammonium methyl sulfate (METAM) were two cationic monomers rendering lignin flocculants (Sabaghi & Fatehi, 2019). On the other hand, grafting the PAM chain onto lignin would improve lignin's solubility and molecular weight, which would be beneficial for lignin-derived flocculant fabrications(Rong et al., 2013). To make lignin with a high molecular weight and cationic charge density, the modifications of lignin with acrylamide (AM), diallyl dimethylammonium chloride (DADMAC), or [2-(methacryloyloxy)ethyl]trimethylammonium chloride (METAC) were studied in two-component systems.(Li et al., 2016; Hasan & Fatehi, 2018) In this regard, a lignin-based cationic macromolecule was produced by polymerizing diallyl dimethylammonium chloride (DADMAC) and acrylamide (AM) on lignin(Li et al., 2016). In this system, for the first time, lignin was polymerized with AM and DADMAC in an aqueous medium rather than the organic solvents to produce cationic water-soluble flocculants for wastewater treatment systems(Price et al., 2018). It was reported in our previous work that comparing with other cationic flocculants used in wastewater treatment systems (12 mg/L pAM), the synthesized lignin-AM-DADMAC flocculant required a lower dosage (1 mg/L) to achieve an acceptable removal without the need of a dual polymer system due to its relatively high molecular weight and a comparable charge density(Price et al., 2018). Despite the promising flocculation results, the fundamentals and preparation process of generating cationic lignin-AM-DADMAC copolymer in three-component systems have not been studied in detail. In this regard, the physicochemical characteristics of lignin polymerization with AM and DADMAC reactions are unavailable. Still, it is essential to investigate the mechanism of polymerization, which further help tailor lignin-derived copolymers with different compositions for various applications.

In-situ polymerization analysis has been considered as an advanced technique for understanding the kinetic of polymerization systems. For example, the polymerization of n-butyl acrylate in an aqueous suspension (Enayati&Abbaspourrad, 2018), which was studied in an in-situ form by nuclear magnetic resonance ($^1$H NMR) and scanning electron microscopy (SEM), was determined to follow a first-order kinetic model. In another work (Feng et al., 2018), the in-situ $^1$H NMR analysis of the copolymerization reaction of methyl methacrylate (MMA) and DADMAC revealed monomer consumptions for determining the reactivity of monomers in the reaction. As NMR can provide real-time data on the monomer concentrations (Feng et al., 2018; Preusser et al., 2016; Cuccato et al., 2015; Hamzehlou et al., 2014; Preusser et al., 2013), in-situ NMR monitoring was followed in this work to determine the details of the monomer conversions in the copolymerization reaction of lignin, AM, and DADMAC as one objective.

The structural characteristics of polymers, microemulsions, or surfactants and their molecular interactions can be studied by monitoring the rheological behavior, e.g., viscoelasticity parameters, of materials in solutions(Wang et al., 2016). In this respect, the rheological properties of the reaction mixture during the polymerization reaction can reflect the network formation or crosslink of monomers in the solution media(Fei et al., 2014; Zhang&Easteal, 2004; Haraguchi et al., 2005). For example, the gradual increase in the viscosity of the system during the polymerization of N-iso-propyl acrylamide, was an indicator of the formation of entanglements within polymer chains (Haraguchi et al., 2005). The rheological studies, such as viscosity profile during polymerization, provide real-time monitoring and control of polymerization processes (Ishak et al., 2006). In this work, in-situ rheological studies were utilized to understand the mechanism of polymerization of lignin, AM, and DADMAC as another objective.

This paper selected two monomers of acrylamide (AM) and diallyl dimethylammonium chloride (DADMAC) to graft with softwood kraft lignin since these monomers are routinely used for producing uncharged and charged water soluble polymers. Furthermore, the kinetics of three component systems of lignin, AM, and DADMAC was studied in detail. Also, the physicochemical characteristics of the polymerization of softwood kraft lignin, AM, and DADMAC were investigated in an in-situ format by $^1$H NMR and rheometer. Also, the information provided by a focused beam reflectance measurement (FBRM) assisted with understanding the particle size variations in the solutions during the polymerization. This analysis offered fundamental evidence of lignin polymerization for generating cationic lignin-derived polymers, which is crucial for reaction monitoring and system control, enabling the development of copolymers with desired compositions for specific end-use applications, e.g., flocculation systems.

## 3.2 Materials and Methods

### 3.2.1 Materials

Softwood kraft lignin was supplied by FPInnovations from its LignoForce pilot plant facilities in Thunder Bay, ON. Diallyl dimethylammonium chloride (DADMAC) (65 wt%. in water), acrylamide (AM), sodium persulfate, sodium hydroxide, $KNO_3$, sulfuric acid, deuteroxide and 3-(trimethylsilyl) propionic-2,2,3,3-$d_4$ acid sodium salt, all analytical grades, were purchased from Sigma Aldrich and used without further purification. Ethanol (95 vol.% in water) was obtained from Fisher Scientific.

### 3.2.2 Copolymerization

In this set of experiments, 2.0 g of lignin and 40 mL of water were added to a three-neck flask. The specified amounts of AM and DADMAC were added to the flask to generate a 5.5: 2.4: 1 molar ratio of lignin: AM: DADMAC (i.e., equivalent to 6.67:1.06:1 weight ratio based on the molar mass (196 g/mol) of C9 unit of lignin) (Bourbonnais, 1997), which was the optimum molar ratio based on our previous work (Price et al., 2018), and the pH of the suspension was adjusted to 2 while stirring at 500 rpm. The reaction mixtures were purged with nitrogen for 20 min, and then 30 mg of $Na_2S_2O_8$ as the initiator was added to the reaction mixtures. The mixture was purged with nitrogen for an additional 10 min, while stirring at 500 rpm. The reactions were carried out at 85ºC for 2 h to obtain a lignin-AM-DADMAC copolymer. After the reaction, the mixture was cooled to room temperature and mixed with 400 mL of ethanol, which induced the precipitation of the copolymer. The precipitated copolymer was washed with 50 mL of ethanol and then dried in a freeze-dryer for 24 h. For comparison, the copolymerization of AM with DADMAC (i.e., pAM-DADMAC), lignin with AM (i.e., lignin-AM), lignin with DADMAC (i.e., lignin-DADMAC), and homopolymerization of AM monomer (i.e., pAM)) and DADMAC monomer (i.e., pDADMAC) were performed under the same reaction conditions stated above.

### 3.2.3 Molecular weight

The molecular weight of lignin, pAM-DADMAC, and lignin-AM-DADMAC was analyzed by a static light scattering (SLS) system (Brookhaven Instruments BI-200SM) that was attached to a goniometer system, Holtsville, USA. In this analysis, the samples were prepared in 0.5 M of NaOH and 10 mM of $KNO_3$ at 5 different concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 g/L, while stirring at 300 rpm overnight for dissolution. Before measurement, the sample solutions were filtrated twice with 0.45 µm syringe filters to remove impurities. The refractive index increment of the lignin derivative samples (dn/dc) was determined by a differential refractometer (Brookhaven Instruments BI-DNDC, Holtsville, USA), which was 0.13 for pAM-DADMAC and 0.14 for lignin-AM-DADMAC. In the molecular weight measurement using static light scattering, time-averaged intensity measurements were made at variable angles from 15° to 155°. The light scattering data that depended on concentrations and angles was fitted into the equation using the Zimm plot software of the instrument(Wang et al., 2016; Andersson et al., 2003).

### 3.2.4 In-situ copolymerization reaction in NMR instrument

In this experiment, lignin and monomers were dissolved in $D_2O$ under the conditions stated in section 2.2 in the nuclear magnetic resonance, NMR, tubes. After adding the initiator, 1 mL of the reaction mixture was added to the 5 mm NMR tube. The sample cavity of the NMR instrument was equilibrated at desired reaction temperatures by the temperature control unit of the NMR instrument, and the tubes containing the reaction mixtures were placed into the cavity. The copolymerization reaction was conducted in the NMR tubes at different reaction temperatures from 60°C to 85°C for 2 h. The sample was scanned every 10 minutes by an INOVA-500 MHz machine (Varian, USA) for $^1$H NMR analysis. The concentration of monomers during the copolymerization reaction was determined via $^1$H NMR analysis.

The copolymerization degree of lignin is difficult to measure because of its heterogeneous structure, as it contains various substructures arranged randomly. Therefore, the copolymerization process was studied based on the conversion ratio of AM and DADMAC monomers calculated considering their initial and final concentrations in the polymerization reaction system. To determine the concentration of AM and DADMAC, the areas under the peaks associated with different groups of AM (peak g, h, f) and DADMAC (peak a and b) were determined as shown in Figure S1a and calibrated with that of the internal standard, 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid sodium salt (TMS) (Abdollahi et al., 2013). Figure S1b shows the $^1$H NMR spectra for the copolymerization of lignin, AM, and DADMAC as a function of time at 85°C, as an example. The initial and final concentrations of AM and DADMAC monomers in the $^1$H NMR spectrum (at 0 min and 120 min, respectively) were calculated by considering the integrated area of g, h, f, and a, b, respectively in Figure S1b. The conversion rate of each monomer was calculated based on the amounts of consumed monomers determined by the difference between initial and final concentrations of AM and DADMAC monomers during the polymerization reaction.

### 3.2.5 Rheology

#### *3.2.5.1 Dynamic viscosity of polymerization system*

The viscosity changes of a solution containing monomers could indirectly indicate the difference in the molecular weight of polymers generated during polymerization. The rheological behavior of the copolymerization and homopolymerization reactions were monitored by conducting the polymerization reactions inside the chamber of a rheometer, DHR-2 (TA Instruments Co., USA) using a cylinder cup (with a diameter of 30.37 mm) and a vane rotor (with a bob diameter of 28 mm and bob length of 42 mm) in an in-situ manner. In this case, the reaction mixture containing 2 g of lignin and monomers (molar ratio of lignin: AM: DADMAC: 5.5: 2.4: 1) with the initiator was mixed manually in a 40 mL glass flask at pH 2, purged with nitrogen for 30 min, then transferred to the cup of the rheometer and blended at

100 rpm for 2 h. The rheometer monitored the viscosity change of the system. In addition, the rheology of copolymerization of lignin, AM, and DADMAC was studied from 60 °C to 85 °C for 2 h.

#### *3.2.5.2 Dynamic viscosity and zeta potential of lignin solution*

The rheometer determined the dynamic viscosity of lignin solutions in the pH range of 2-11. The concentration of lignin solution was 0.025 g/mL. The procedure to prepare lignin samples was the same as the polymerization process but without any monomers of AM and DADMAC. The zeta potential of the lignin solutions was measured by a zeta potential analyzer (Nanobrook Omni, Brookhaven Instruments).

#### *3.2.5.3 Oscillation study during and after polymerization*

The rheological behavior of produced lignin-AM-DADMAC copolymer was studied using the oscillation technique of the rheometer at 85 °C using the same cylinder cup and a DIN rotor (with a bob diameter of 28.03 mm and bob length of 41.96 mm). The reaction media's linear viscoelastic region (LVR) was studied for selecting the proper strain and stress results, which were 159% and 46.5 Pa for strain and stress, respectively (Figure S2 in supplementary material). The time sweep test ran under the strain of 10 %, and the stress during the process did not exceed 15Pa to ensure that the linkage formation would not be destroyed during the test. To observe the accurate viscoelastic behavior of the polymer, the mutation number ($N_{mu} = t_{exp} \times \frac{\partial \ln(x)}{\partial t}$, x = G' or G") was introduced to ensure the time of measurement has to be lower than the characteristic evolution time of the system(Pibre et al., 2008). Thus, the angular frequency, ω = 10 rad/s = 1.59 Hz, was set in the experiment to have a mutation number lower than 0.1 as recommended. The frequency sweep experiment was studied right after the reaction at 85°C and 10 rad/s.

### 3.2.6 In situ particle size distribution measurement

A focused beam reflectance measurement system (FBRM), Mettler-Toledeo AutoChem, USA, also studied the copolymerization and homopolymerization processes in real time. This instrument detects the chord length of the particles in the polymerization reaction systems in a real-time(Barros et al., 2021). Due to the sensitivity of the FBRM probe to the concentration of analysis media, the reaction systems were diluted by adding an extra 40 mL of water, and the other conditions were kept the same as the original reaction (i.e., the molar ratio of lignin: AM: DADMAC = 5.5: 2.4: 1, pH 2, reaction time: 2h). After adding the initiator, the reaction mixture was purged with nitrogen for 10 min and then the FBRM probe was put into the mixture while blending at 200 rpm. The reaction temperature was adjusted to 70 ºC. The beaker was sealed by parafilm during the measurement to isolate the reaction system, and the FBRM instrument monitored the entire 2 h of the copolymerization process.

The particle size of the unmodified kraft lignin at different pH values was determined by a Malvern Mastersizer 2000 instrument (Worcestershire, UK). In this analysis, the kraft lignin suspensions (1 g/L) were adjusted to various pH values (i.e., pH 2, 4, 7, 9, 11). About 10 mL of these suspensions were taken and added to 600 mL of deionized water, which was adjusted to the corresponding pH values. The diluted samples were directly used for measuring the particle size distribution at the stirring rate of 250 rpm in the detection range of 0.02-2000 μm.

## 3.3 Results and discussion

### 3.3.1 Mechanism of copolymerization

The reaction mechanism of lignin, AM and DADMAC was a free-radical polymerization, and $Na_2S_2O_8$ was used as the initiator. The major chain formation in a free-radical polymerization is composed of free-radical generation, initiation, propagation, and termination, in which free radicals can react with different manners involving addition, coupling, disproportionation, abstraction, fragmentation and transmutation (Gurrero-Santos et al., 2009). In this reaction, sodium persulfate is thermally decomposed into sulfate radical anions, $SO_4^{-\cdot}$. Then, the free radicals are transferred to lignin, AM or DADMAC monomers in the reaction media (Prince et al., 2018). The free radicals transferred to lignin generate phenoxy radicals by abstracting unstable hydrogen in phenyl-hydroxyl groups (Ye et al., 2014). In coniferyl alcohol groups of lignin, the radicals derived from hydroxyl groups on the phenyl ring instead of aliphatic groups due to the higher stability of phenoxy radicals(Pan et al., 2006). In the addition stage, the phenoxy radicals attached to AM monomers form lignin-pAM chains, which are then covalently bound with DADMAC monomers to generate the final product, lignin-AM-DADMAC. The free-radical sites in both AM and DADMAC monomers were methylene groups on their backbone. In the propagation stage, the stability of a radical is not changed by adding monomer units to the radical but is altered by the group substituent, implying that every chemical has its unique propagation rate constant (Gurrero-Santos et al., 2009). The propagation rate constants ($k_p$) of AM monomers at 50°C is $2\times10^4$ L/(mol·s), which is much greater than $k_p$ of DADMAC monomers, 90 L/(mol·s) (Wandrey et al., 1999).

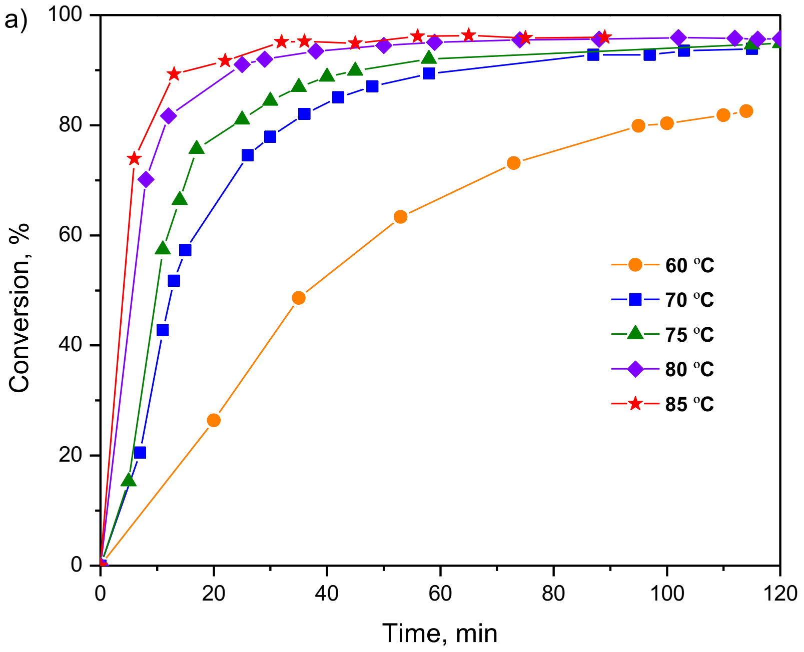
For this reason, lignin radicals react with AM more preferably than with DADMAC. Furthermore, lignin-AM-DADMAC was not the only copolymer formed in the reaction media, and the propagation of AM-DADMAC was also achieved. Finally, the free-radical polymerization was terminated by coupling or disproportionation (Gurrero-Santos et al., 2009), in which lignin-AM-DADMAC radicals and AM-DADMAC radicals combined into lignin-AM-DADMAC copolymers or broke into copolymers or homopolymers.

The presence of lignin in the radical polymerization was reported to either promote or inhibit polymerization in previous studies.(Kong et al., 2015; Mai et al., 2000; Kaur et al., 2010; Chen et al., 1998; Phillips et al., 1973). As a chain transfer reagent in free-radical polymerization (Phillips et al., 1973), lignin promotes the molecular weight development of the polymer. However, in this study, the lignin-containing copolymer had a lower molecular weight ($6.53\times10^5$ g/mol) and a lower charge density (1.43 meq/g) than the lignin-free AM-DADMAC copolymer (molecular weight of $2\times10^6$ g/mol and charge density of 1.88 meq/g), indicating that lignin inhibited the polymerization of the two monomers. The reason for this phenomenon could be that, in the initiation stage of the lignin-containing reaction, many phenoxy radicals may be generated, but these radicals are more stable than the other monomers. Thus, lignin does not promote the polymerization reaction as effectively as the other monomers(Phillips et al., 1973). The quinoid structure of lignin may influence the molecular weight of the resulting copolymer(Mai et al., 2000). The formation of covalent bonds between lignin and AM may provide steric hindrance for the growing chains of AM or DADMAC, especially that of DADMAC, due to its relatively low reactivity.

### 3.3.2. Kinetics of copolymerization

The conversions of AM and DADMAC in the copolymerization process of AM and DADMAC with lignin at different temperatures are shown in Figure 3.1a and 3.1b. Overall, the highest conversion ratio of DADMAC was 68%, which was lower than that of AM, 96%. Generally, the conversion of AM is faster than that of DADMAC at any temperature. The increase in temperature improved the conversion rates of both monomers, and the rate change was more dramatic for AM than for DADMAC. The reactivity ratio (i.e., the ratio of the rate constant for a reactive propagation species adding its own type of monomer to the rate constant for its addition of the other monomer) of DADMAC was reported to be between 0.03 and 0.12

and that of AM was between 6 and 7 in the polymerization reaction of AM and DADMAC in water (Rintoul&Wandrey, 2009; Avic&Bayir, 2002; Bi&Zhang, 2012). Therefore, the lignin-AM-DADMAC copolymer contained more AM with a greater reactivity ratio than DADMAC with a lower reactivity ratio(Dube et al., 2013). AM monomers are depleted quickly in the early stage of copolymerization, followed by the attachment of DADMAC monomers(Dube et al., 2013). The reactivity ratio of DADMAC is close to 0, indicating that it prefers cross-propagation to homopropagation(Dube et al., 2013). Non-polar AM has less steric hindrance than DADMAC. Thus, it can readily participate in the polymerization reactions to form high molecular-weight polyelectrolytes(Abdollahi et al., 2013). DADMAC with small reactivity has bulky and charged organic side groups promoting self-inhibition and electrical repulsion (Wang et al., 2011), and hampering the progress of the polymerization reaction. For comparison, the copolymerization of AM and DADMAC without lignin was studied, as shown in Figure 3.1c and 3.1d. The conversion rate calculations depicted opposite trends for the conversion of AM and DADMAC with temperature, and the monomers were consumed faster as temperature decreased. AM monomers' conversion ratio reached a relatively stable value in 5 min at 60°C and in 40 min at 75°C. DADMAC monomers had a similar trend except at 70°C. For the copolymerization system without lignin, the polymerization of acrylamide was reported to be highly exothermic (Orbán et al., 1999; Mahdavian&Zandi, 2003), leading to decreasing monomer conversion rates in the copolymerization system as the reaction temperature increased. On the contrary, the polymerization system with lignin were found to be endothermic(Price et al., 2018; Guo et al., 2020; Wang et al., 2018). The formation of radicals is accelerated at a higher temperature, which promotes the chain transfer and termination.(Kong et al., 2015). Also, at a higher temperature, the viscosity of the reaction solution is known to reduce, facilitating the access of monomers onto lignin backbone. The reaction at 60°C generated the highest conversion of AM and DADMAC monomers, which were respectively 100% and 88% (i.e., higher than that in the lignin-AM-DADMAC copolymers), demonstrating that lignin acted as an inhibitor of the polymerization in these reactions. The presence of stable radicals in the macromolecules might be the reason for this behavior(Mai et al., 2000). However, at each temperature, the addition of lignin reduced the conversion of DADMAC (e.g., from 60 % to 42 % at 75°C) more than that of AM (e.g., from 99 % to 95 % at 75°C) (Figure 3.1), suggesting lignin has a more significant impact on the conversion of DADMAC monomers than that of AM monomers.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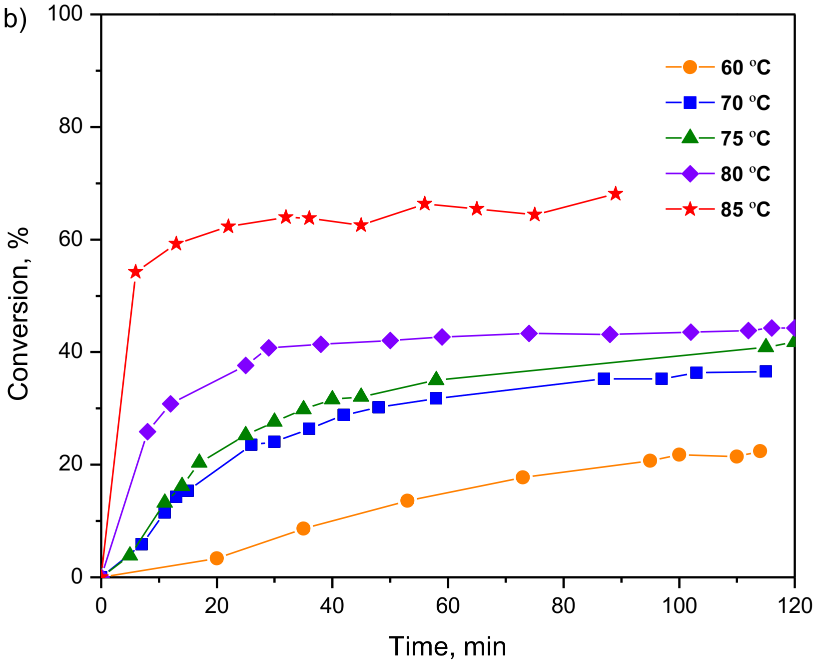
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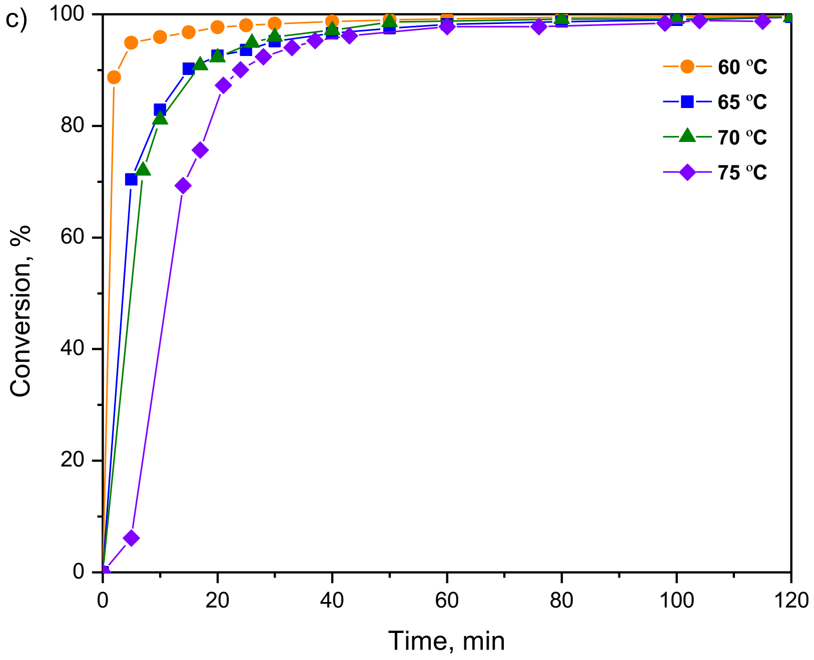


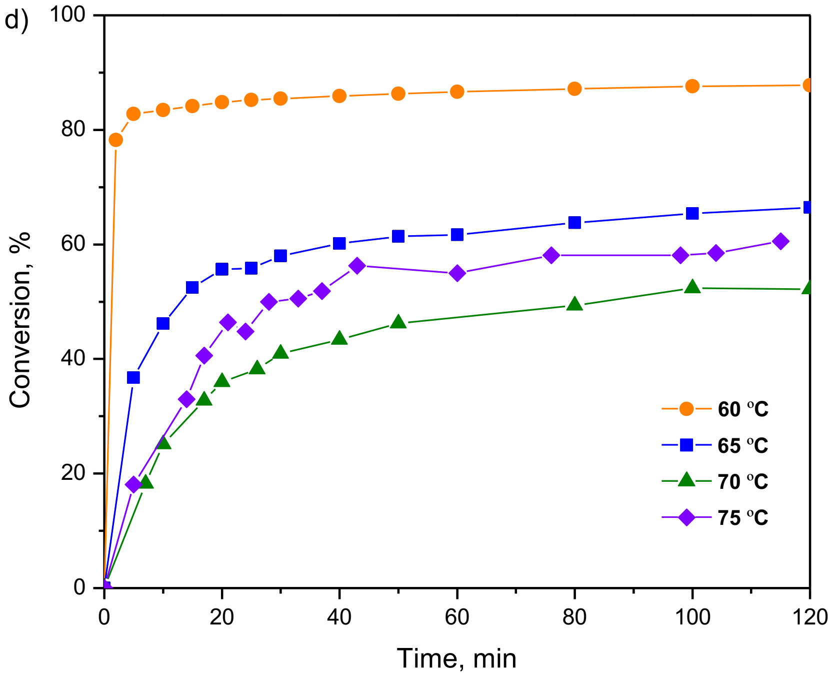
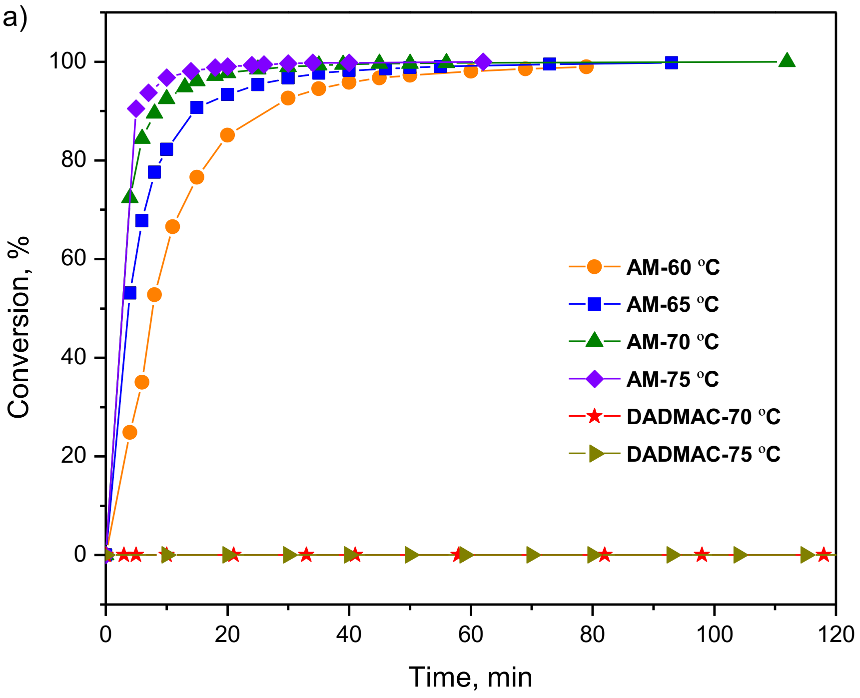


**Figure 3.1.** Monomer conversion of a) AM and b) DADMAC in the copolymerization process of lignin, AM and DADMAC (LAD) at different temperatures. Monomer conversion of c) AM and d) DADMAC in the copolymerization process of AM and DADMAC without lignin (AD) at different temperatures.

The homopolymerization processes of AM and DADMAC and the copolymerization of lignin with AM or DADMAC in two-component systems were studied under the same reaction conditions of the copolymerization, and the results are shown in Figure 3.2. AM monomers in the homopolymerization reacted more rapidly than that in the copolymerization. They reached a 100% conversion at the equilibrium stage. AM monomers had the lowest conversion rate when reacting with lignin, which indirectly indicates that the involvement of DADMAC in the copolymerization improved the reaction ratio of AM and lignin (Figure 3.1 and 3.2). DADMAC monomers did not form pDADMAC, prohibiting the progress of lignin-DADMAC (Figure 3.2b). Therefore, the participation of acrylamide is required for the reaction of lignin and DADMAC.



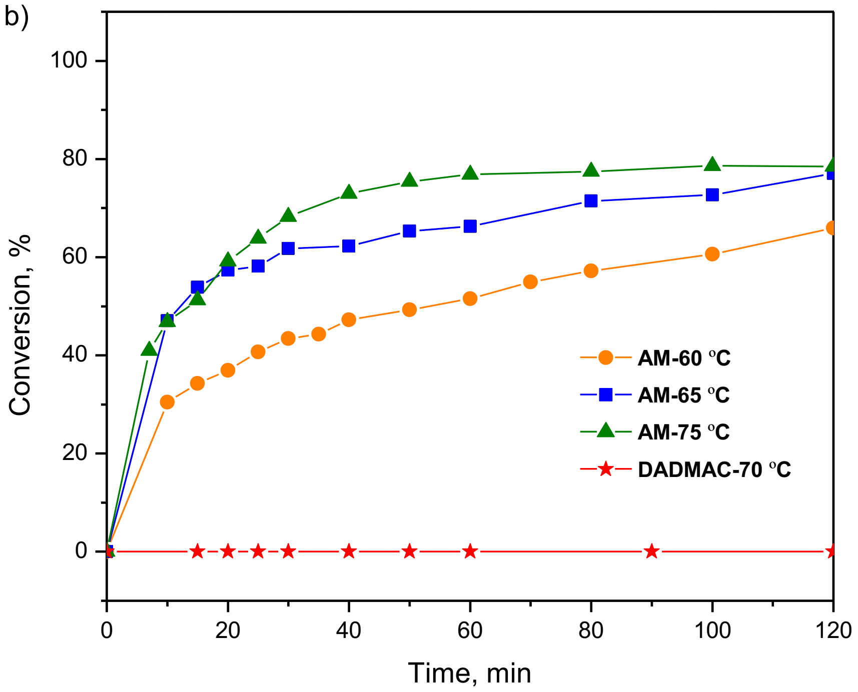


**Figure 3.2.** Monomer conversion of AM and DADMAC in a) homopolymerization process to generate pAM or p-DADMAC; b) copolymerization to form Lignin-AM or Lignin-DADMAC at different temperatures.

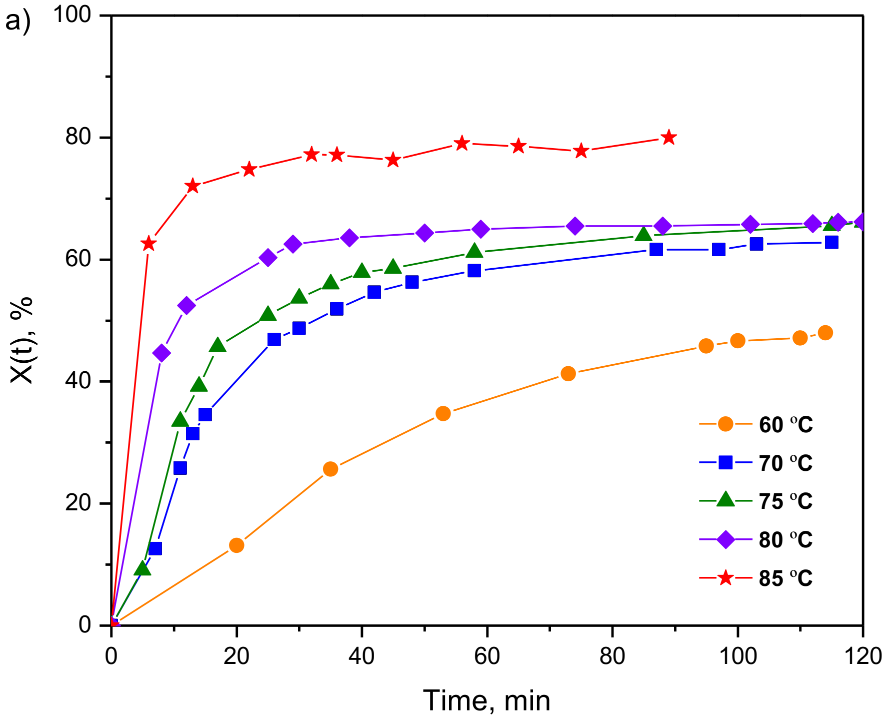
The overall conversion of AM and DADMAC monomers was calculated following Equation 1 (Abdollahi et al., 2013):

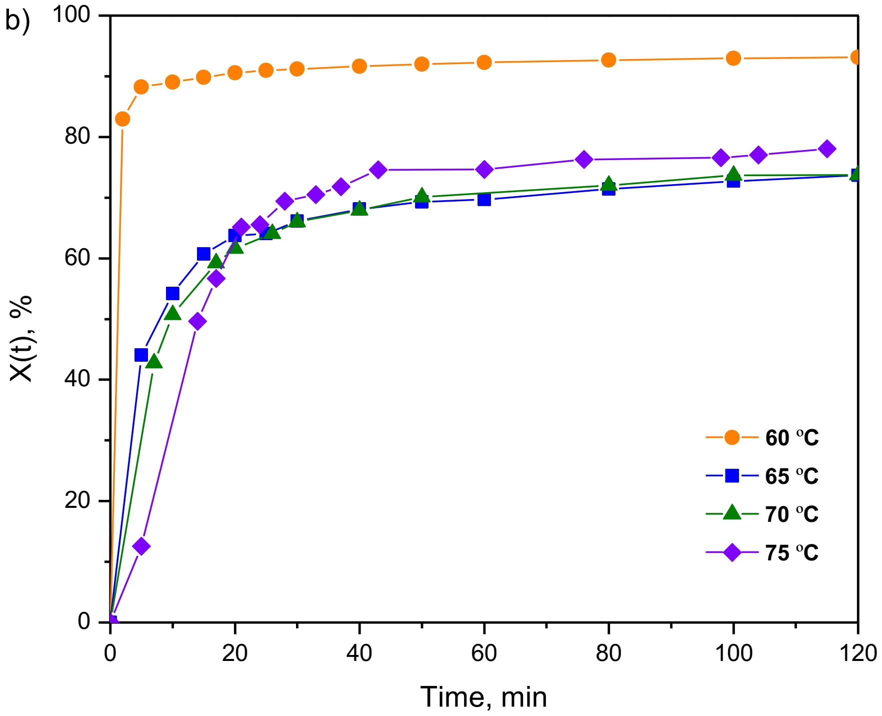
$$X(t) = f_{AM}^{0}X_{AM}(t) + f_{DADMAC}^{0}X_{DADMAC}(t) \qquad (1)$$

where X(t) is the overall conversion, %; $f_{AM}^{0}$ and $f_{DADMAC}^{0}$ are the initial mole fractions of AM and DADMAC monomers, respectively; $X_{AM}$ (t) and $X_{DADMAC}$ (t) are the individual conversion rate of AM and DADMAC at a specific time, respectively. The overall conversion of monomers is shown in Figure 3.3a and 3.3b as a function of time at different temperatures, which were calculated considering the results in Fig 3. For the three-component system of lignin, AM, and DADMAC, the overall monomer conversion of 77 % was reached in 30 min at 85 °C (Figure 3.3a). The higher reaction temperature generated a higher conversion. The trend of the overall conversions in Figure 3.3a is very similar to the plot of DADMAC conversion in Figure 3.1b. In the two-component system of AM and DADMAC (Figure 3.3b), the lowest reaction temperature revealed the highest overall conversion, which is similar to the trend in the plot of

DADMAC conversion in Figure 3.1d. These results confirm that DADMAC played a major role in the overall conversion of monomers in copolymerization in the presence and absence of lignin.

The consumption rates of monomers are shown in Figure 3.3c and 3.3d as a function of time at different temperatures. The consumption rate, Rp, defined as the rate of monomer cusuming in the reaction system, X(t)/t, reached the maximum value in 10 min except at 60 °C. In both reaction systems of lignin-AM-DADMAC and AM-DADMAC, the reaction can be defined into two stages: auto-acceleration and self-deceleration. In the first stage (auto-acceleration), the copolymerization ratio rapidly increased to the maximum value. Then, the Rp started to reduce, but the conversion was still increasing. In this stage, the polymers in the reaction medium probably had low reactive mobility, and the intermolecular bonds were converted to intramolecular covalent bonds.(Chartier et al., 2012)

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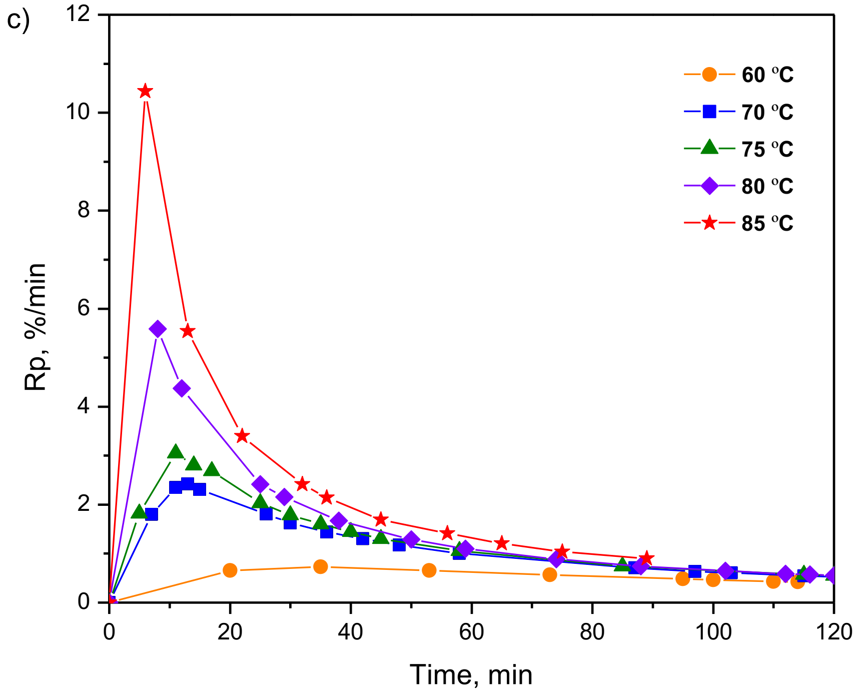
c)
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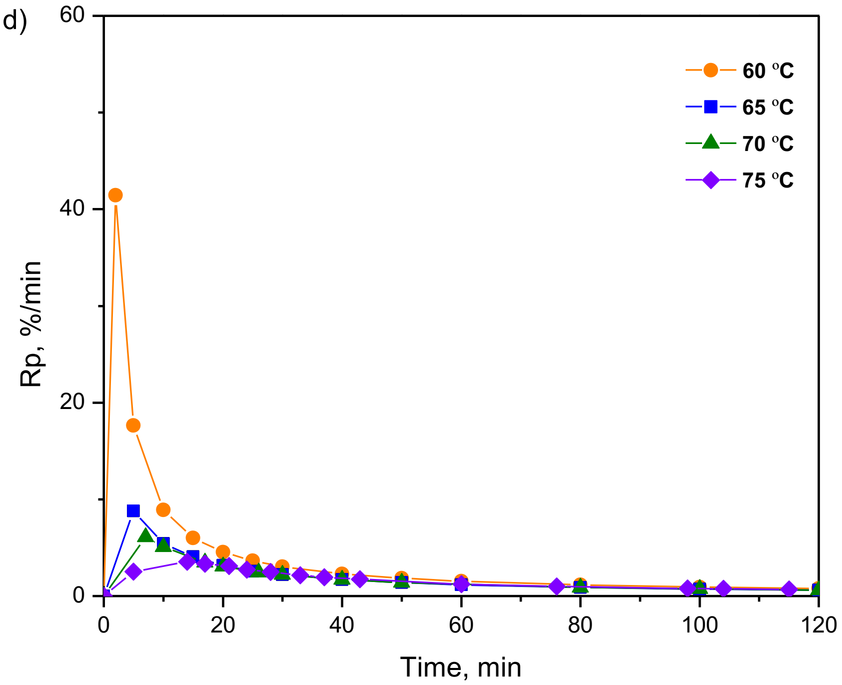


**Figure 3.3.** Overall monomer conversion (X(t)) for the systems of AM and DADMAC a) with lignin and b) without lignin. Consumption rate (Rp) for the systems of AM and DADMAC c) with lignin and d) without lignin.

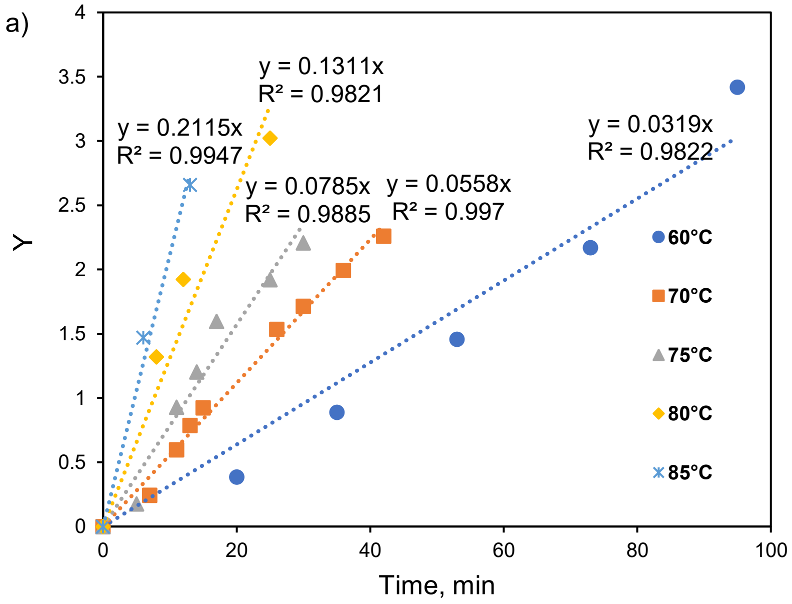
The activation energy of the copolymerization reaction for lignin-AM-DADMAC, lignin-AM, and AM-DADMAC productions, as well as of the homopolymerization for pAM was calculated based on the monomer conversions at different reaction temperatures (Figure 3.1 and 3.2) and the results are shown in Figure 3.4. The copolymerization of monomers in Figure 3.4a-d was fitted into the first order kinetics described in Equations 2 and 3. Then, the activation energy was determined using Equation 4. (Pibre et al., 2008)

$$-\frac{d[Mo]}{dt} = K([Mo] - [Mo]_f) \qquad (2)$$

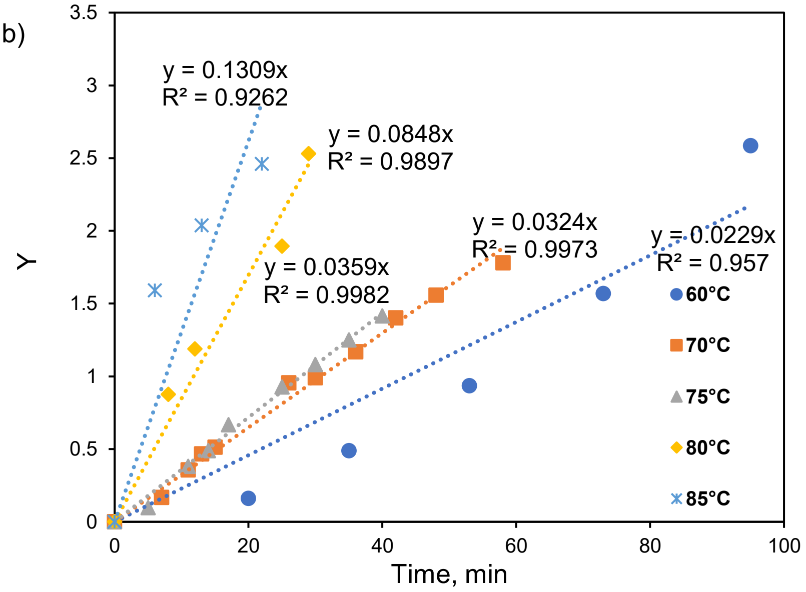
$$Y = Ln\frac{[Mo]_0 - [Mo]_f}{[Mo] - [Mo]_f} = Kt \qquad (3)$$

$$K = Ae^{-\frac{E_a}{RT}} \qquad (4)$$

where [Mo] is the monomer concentration at time t; $[Mo]_0$ is the initial monomer concentration; $[Mo]_f$ is the final monomer concentration when the equilibrium is achieved; K is the reaction rate constant; A is the collision frequency factor; and $E_a$ is the activation energy. The activation energy for AM and DADMAC in the lignin-containing copolymerization reaction systems can be identified by the plot of ln(K) versus 1/T (Figure 3.4e), which are 65.7 kJ/mol and 69.3 kJ/mol (Table 3.1), respectively. The slightly lower activation energy of AM led to a higher conversion of AM than that of DADMAC because DADMAC required more activation energy to react(Wandrey et al., 1999). As DADMAC could not produce pDADMAC (Figure 3.2) and its activation energy was higher than AM, it can be stated that the presence of AM was necessary for the copolymerization reaction. Under the same reaction conditions, the activation energy of AM in pAM system (93.4 kJ/mol) is higher than that (65.7 kJ/mol) in the copolymerization reaction of lignin-AM-DADMAC. The activation energy of AM in the lignin-AM system is 90.4 kJ/mol, which is slightly lower than that (93.4 kJ/mol) of pAM. These results imply that AM preferably reacts with lignin due to its lower activation energy with lignin than with itself.

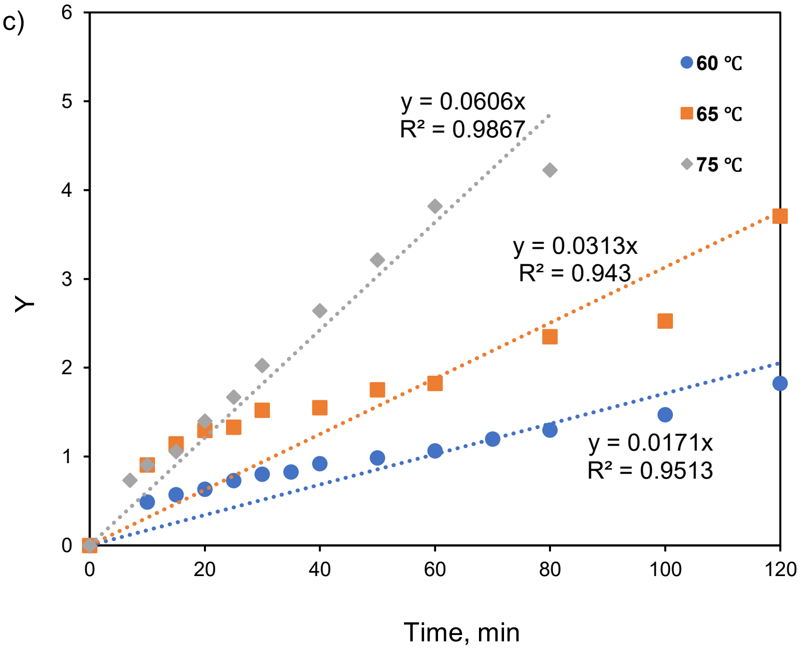


b)



3.5
3
2.5
2
1.5
1
0.5
0
Y
0
20
40
60
80
100
Time, min
y = 0.1309x
R² = 0.9262
y = 0.0848x
R² = 0.9897
y = 0.0324x
R² = 0.9973
y = 0.0229x
R² = 0.957
y = 0.0359x
R² = 0.9982
● 60°C
■ 70°C
▲ 75°C
◆ 80°C
✳ 85°C

c)



6
5
4
3
2
1
0
Y
0
20
40
60
80
100
120
Time, min
● 60 ℃
■ 65 ℃
◆ 75 ℃
y = 0.0606x
R² = 0.9867
y = 0.0313x
R² = 0.943
y = 0.0171x
R² = 0.9513

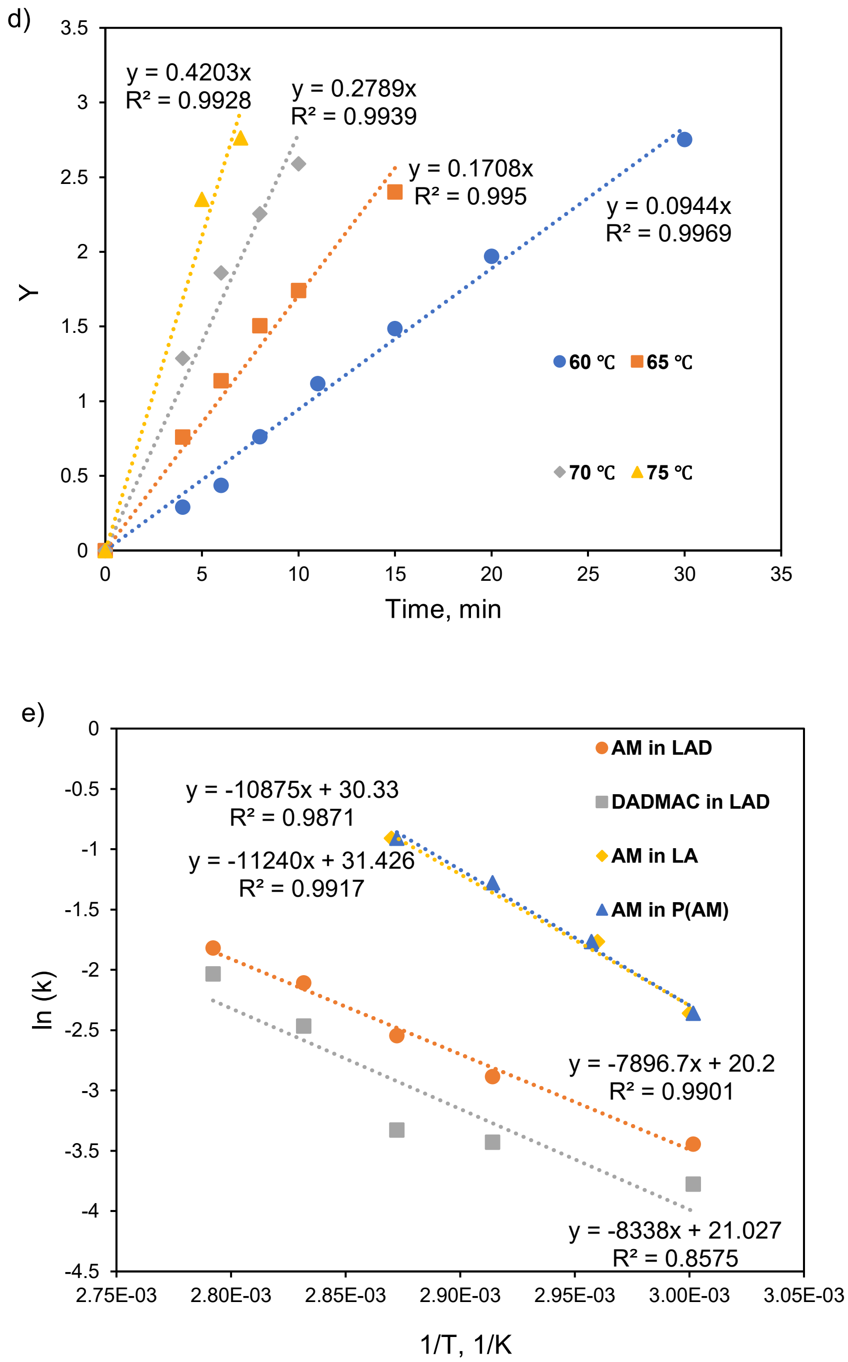


**Figure 3.4.** Arrhenius plot for a) AM and b) DADMAC in the copolymerization of lignin-AM-DADMAC, c) AM in lignin-AM and d) AM in pAM (Y is expressed in Equation 3). e) The plot of ln(K) versus 1/T for determination of activation energy.

**Table 3.1.** Summary of rate constants and activation energy of AM and DADMAC in LAD, AM in LA, AM in pAM

| | Temperature, °C | Rate constant (K) | Activation energy (Ea), kJ/mol |
|---|---|---|---|

| | | | |
|---|---|---|---|
| AM in LAD | 60 | 0.032 | 65.7 |
| | 70 | 0.056 | |
| | 75 | 0.079 | |
| | 80 | 0.131 | |
| | 85 | 0.212 | |
| DADMAC in LAD | 60 | 0.023 | 69.3 |
| | 70 | 0.033 | |
| | 75 | 0.036 | |
| | 80 | 0.085 | |
| | 85 | 0.131 | |
| AM in LA | 60 | 0.017 | 90.4 |
| | 65 | 0.032 | |
| | 75 | 0.061 | |
| AM in pAM | 60 | 0.094 | 93.4 |
| | 65 | 0.171 | |
| | 70 | 0.279 | |
| | 75 | 0.42 | |

### 3.3.3 Shear-induced viscosity analysis

Figure 3.5a describes the changes in the viscosity of the reaction media for the production of lignin copolymers and homopolymers during the polymerization reactions. Generally, the viscosity of reaction media increased as time elapsed for the production of copolymers. The increase in the viscosity clarifies the stiffening of the system due to an early-stage reaction(Kim et al., 2016). The viscosity for the polymerization of lignin, AM and DADMAC increased gradually and reached its maximum in 30 min, representing the formation of the linear polymer(Ishak et al., 2006). It was discussed that the viscosity enhancement was caused by the formation of entanglements between linear polymer chains(Ishak et al., 2006). The formation of pAM-DADMAC and pAM indicated a much steeper increase in the viscosity owing to the chemical crosslinking and subsequent gel formation(Kizilay&Okay, 2003). The viscosity of the lignin-free copolymerization system was ten times as much as that of the lignin-containing system. This behavior reflects the higher molecular weight of the generated polymers in the reaction mixture of lignin-free system (AD in Figure 3.5a) than that of the lignin-containing system (LAD in Figure 3.5a), which agrees with the higher molecular weight of the pAM-DADMAC copolymer ($2\times10^6$ g/mol) than the lignin-AM-DADMAC copolymer ($6.53\times10^5$ g/mol). For the homopolymerization system containing AM, the viscosity increased at the beginning of the reaction and then decreased after 10 min of the reaction. The molecular weight of the pAM solution in the first 10 min was $1.88\times10^6$ g/mol, which was greater than that after 2h of reaction ($1.67\times10^6$ g/mol). This behavior confirms that the degradation of pAM molecules causes a decrease in viscosity. Nagashiro and Tsunoda reported that the viscosity decrease of pAM molecules in aqueous solutions was attributed to the change in the dissolved state or shear degradation(Nagashiro&Tsunoda, 1977). It is also noticeable that the viscosity change was dramatic for the two-component system of AM and DADMAC or a single system of AM. The lignin-containing system had a slower pace in the viscosity increase (Figure 3.5a). These results may show that the homopolymerization of AM or copolymerization of AM and DADMAC was quick. Still, the presence of lignin in the system reduced the polymerization rate. This behavior may be related to the stability of phenol radicals of lignin and their resistance in participating or initiating the polymerization reactions. The reaction system containing only DADMAC did not have a noticeable viscosity change. The reaction progress (Figure 3.2) implied that the homopolymerization of DADMAC did not proceed under the conditions studied in the rheometer.

Furthermore, Figure 3.5b describes the copolymerization and homopolymerization systems of lignin, AM, and DADMAC without the initiator. Except for the AM system, the rest had no change in viscosity. AM started to form pAM after 45 min when the reaction temperature was higher than its melting point of 84.5°C (Sigma-Aldrich, accessed 21 December 2022). Acrylamide easily polymerizes at its melting point in solution or under ultraviolet light (Sigma-Aldrich, accessed 21 December 2022). Candau et al.(1985) also found that acrylamide could polymerize even in the absence of an initiator due to impurities (e.g., oxygen, peroxides, and emulsifier) present in the reaction medium. The results in Fig 6b report that the initiator is essential for the copolymerization processes of AM and DADMAC with and without lignin.

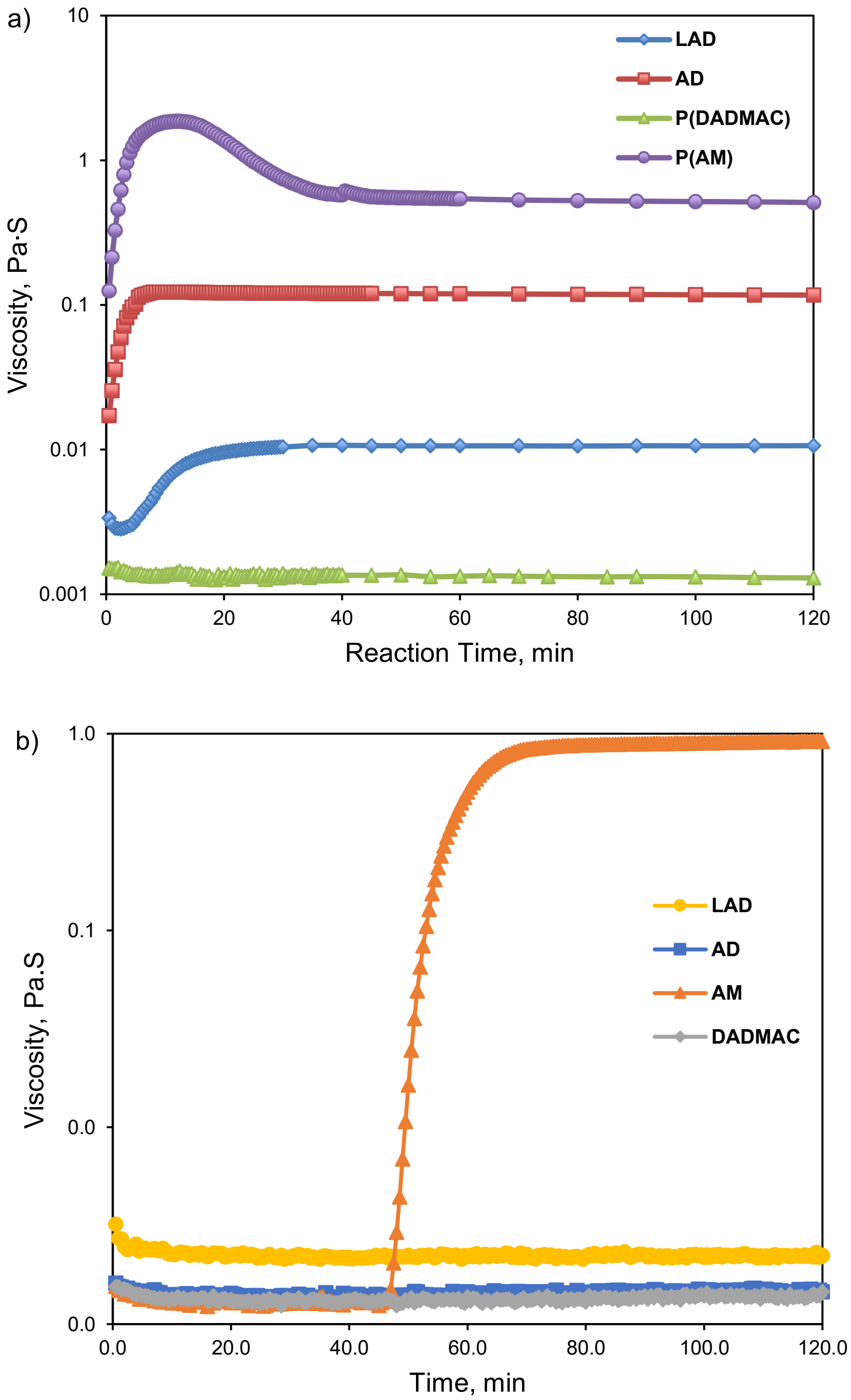


**Figure 3.5.** Viscosity changes of lignin-AM-DADMAC (LAD), AM-DADMAC (AD), pDADMAC, and pAM reaction systems a) with initiator b) without initiator as a function of time (Measurement conditions: the molar ratio of lignin: AM: DADMAC = 5.5: 2.4: 1, pH 2, 85˚C, 100 rpm, 2h).

The reaction of the system containing lignin experienced a small viscosity drop within 5 min of reaction in Figure 3.5a, which is perhaps associated with the thixotropy phenomenon defined as the breakage of lignin agglomerates caused by the applied shears at the beginning of the reaction (Figure S3) (Mewis&Wagner, 2009).

### 3.3.4 Viscosity analysis at different temperatures

The viscosity of the reaction media containing lignin, AM, and DADMAC was studied at different temperatures and time intervals, and the results are shown in Figure 3.6. The thixotropic behavior of all samples is evident in the first 5 min of the experiment. It is also seen that the system had a slower rate of viscosity gain but with overall higher viscosity at 75°C than other temperatures. Theoretically, as these reactions are generally endothermic, a higher reaction temperature provides a higher conversion of monomers to form larger polymers. Thus, the higher viscosity of the reaction mixtures(Wang et al., 2018). Meanwhile, the temperature increase itself could decrease the viscosity, and the partial hydrolysis of the copolymers at a higher temperature may occur (Kong et al., 2015), which may cause a slower rate of viscosity gain. It is also noted that the viscosity of the system first increased rapidly and then became stabilized. The reason could be that the reaction proceeded very quickly at the beginning, the formation of larger molecules hindered the progress of the reaction in the later stage, slowing down the reaction rate and hence viscosity increase.

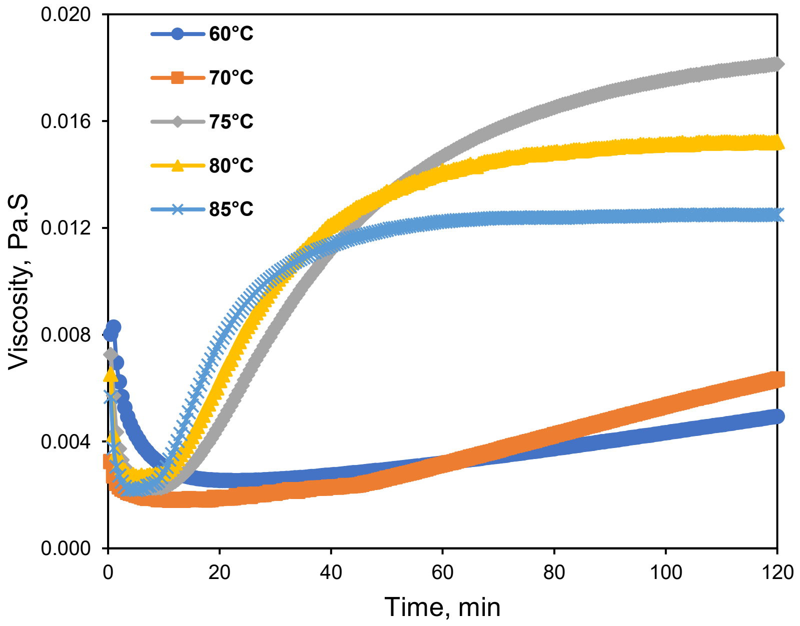


**Figure 3.6.** Viscosity changes of the reaction system containing lignin, AM, and DADMAC at different reaction temperatures (Measurement conditions: the molar ratio of lignin: AM: DADMAC = 5.5: 2.4: 1, pH 2, 100 rpm, 2h).

### 3.3.5 Rheological analysis

The rheometer investigated the oscillation response of the three-component reaction mixture of lignin, AM, and DADMAC. The change in the storage (G’) and loss (G”) modulus (called elastic and viscous modulus), as well as the complex viscosity (η) of the reaction mixture, was analyzed at 85°C in Figure 3.7. The elastic and viscous modulus and complex viscosity had a similar trend, illustrating the crosslinking of monomers in the reaction medium(Zhang&Easteal, 2004). Furthermore, the gradual increases in the G’ and G” verified the growing chain of polymers in the reaction, and this growth continued after the monomer conversion rate reached its maximum (Figure 3.1a) in the polymerization process(Cayuela et al., 2006). At the beginning of the reaction, the viscosity increased rapidly, which might be attributed to the main chain growth and network formation taking place(Zhang&Easteal, 2004). The increasing rate of viscosity slowed down, which was most likely due to the lowered monomer conversion rate in the later stage of copolymerization (Figure 3.1a). During the stabilization period, the rate of aggregation and breakdown within the microstructural network is in equilibrium(Kim et al., 2016).

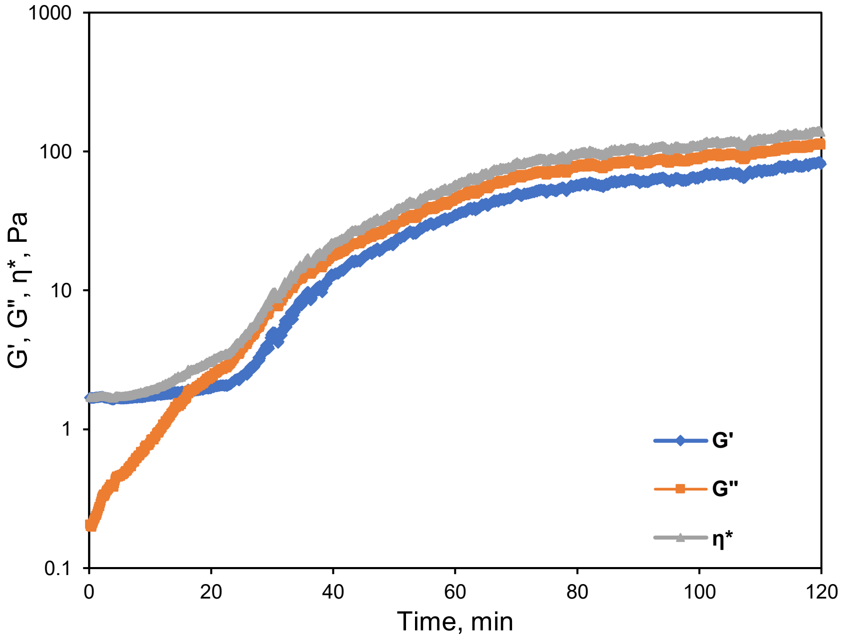
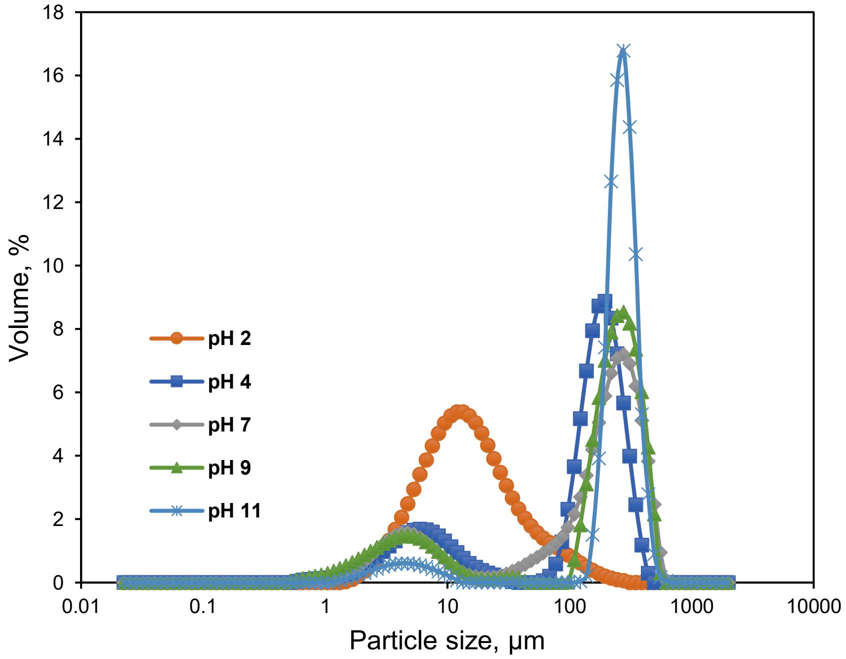


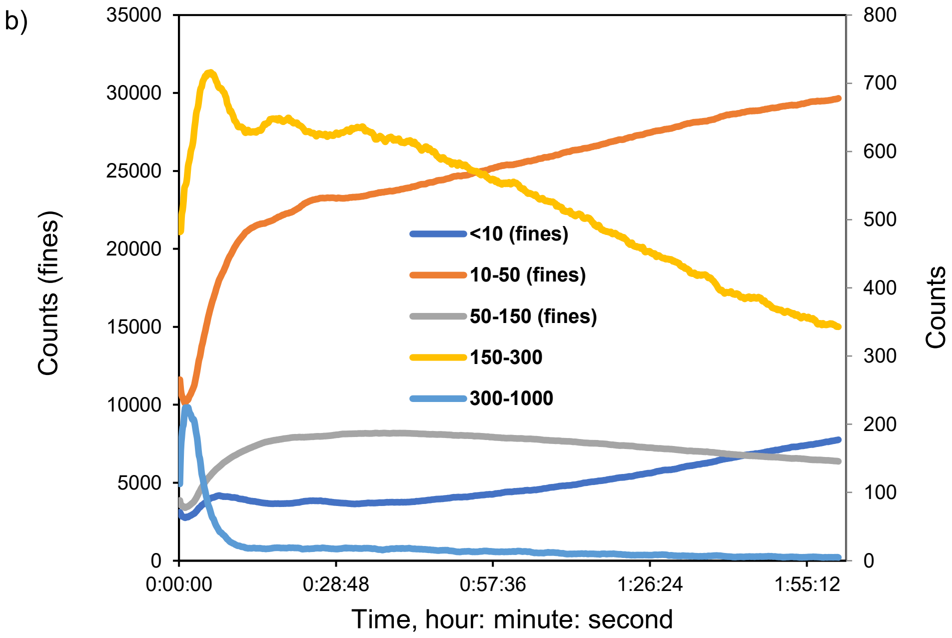
**Figure 3.7**. In situ polymerization of lignin with AM and DADMAC monomers in a rheometer chamber. Storage and loss modulus and complex viscosity as a function of reaction time during the polymerization process (Measurement conditions: the molar ratio of lignin: AM: DADMAC = 5.5: 2.4: 1, pH 2, 85℃, 100 rpm, 2h).

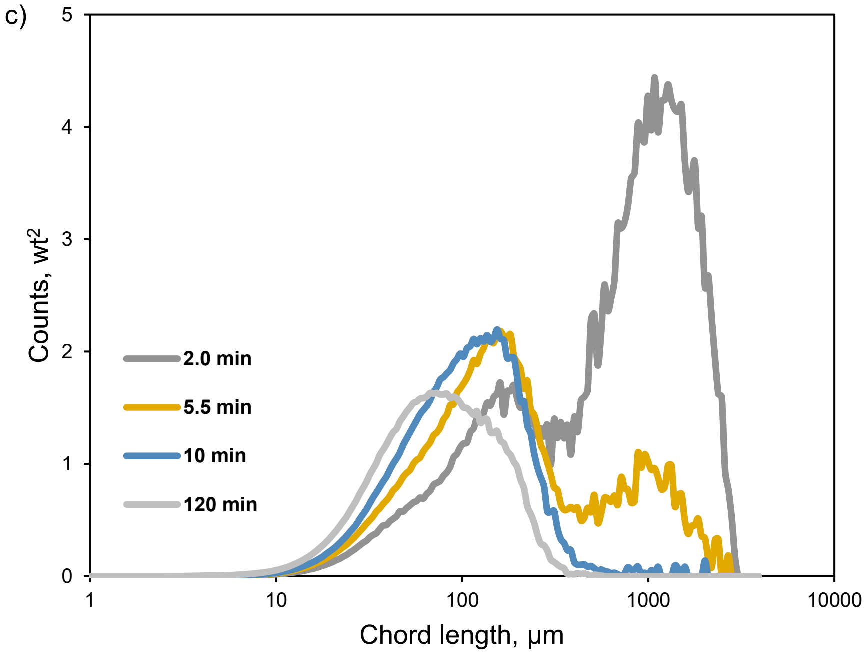
### 3.3.6 Particle size analysis

The particle size distribution of lignin at different pH values is investigated, and the results are shown in Figure 3.8a. Lignin at different pHs(or pH values) (except pH 2) showed bimodal distributions from 1 to 10 μm and from 100 to 1000 μm. The first peak of 1-10 μm was probably the size of kraft lignin particles and the second peak of 100-1000 μm was caused by aggregation of the particles in suspension systems(Norgren et al., 2002). Lignin at pH 2 only had a unimodal distribution from 1 to 100 μm owing to the less solubility of lignin and its further aggregation to the larger size (Price et al., 2018).

a)




b)
Counts (fines)
35000
30000
25000
20000
15000
10000
5000
0
800
700
600
500
400
300
200
100
0
Counts
<10 (fines)
10-50 (fines)
50-150 (fines)
150-300
300-1000
0:00:00
0:28:48
0:57:36
1:26:24
1:55:12
Time, hour: minute: second


c)
Counts, wt²
5
4
3
2
1
0
2.0 min
5.5 min
10 min
120 min
1
10
100
1000
10000
Chord length, μm

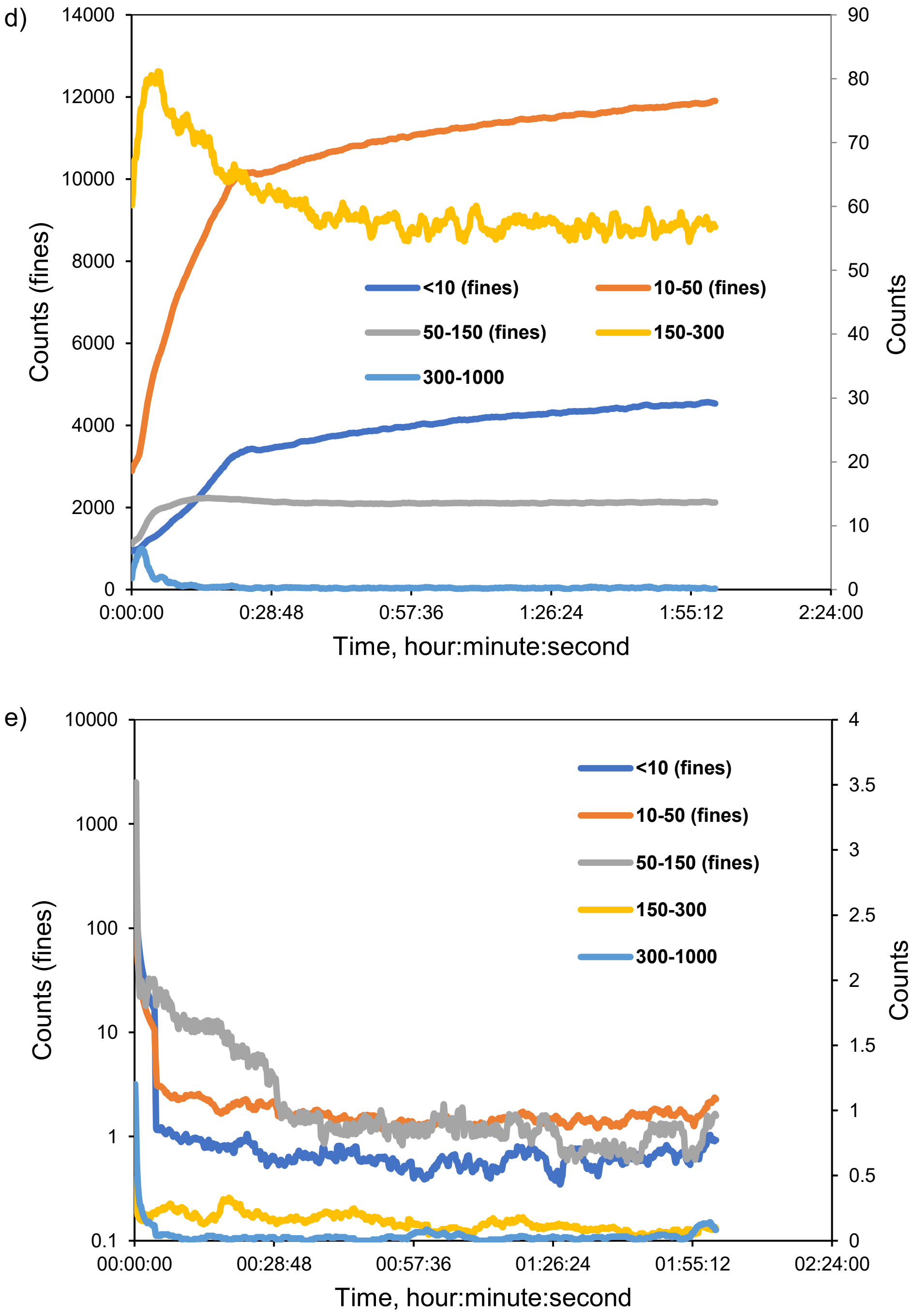


**Figure 3.8.** a) Particle size distribution of lignin at different pH; b) Particle size variations of the three-component reaction mixture of lignin, AM, and DADMAC (LAD) during the polymerization process; c) chord length distribution of the LAD reaction media. Particle size variations of the two-component reaction mixtures of d) lignin with AM e) AM with DADMAC (Measurement conditions: the molar ratio of lignin: AM: DADMAC = 5.5: 2.4: 1, pH 2, 70℃, 200 rpm, 2h)).

Figure 3.8b and 3.8c show the size of particles during the polymerization process of lignin with AM and DADMAC. It is found that the size of particles in lignin-AM-DADMAC solution became stable after 5 min confirming the results of the rheometer illustrated in Figure 3.5a. In the first 10 min time of the reaction, the number of coarse particles (those greater than 300 μm) became negligible, suggesting particle dissociation and breaking, which is in agreement with the thixotropy phenomenon causing the viscosity drop at the beginning of the reaction (Figure 3.5a). The decrease in the number of coarse particles (greater than 300 μm) and the increase in the number of fine particles (<10 μm and 10-50 μm) indicated the solubility improvement of the sample owing to the formation of the copolymers. During the reaction, the distribution of particle size displayed wide multimodal in the first 5 min. Then, the size shifted to a single peak in the finer particle size range (Figure 3.8c), indicating the reaction mixture changed from a suspension to a colloidal system along with the formation of lignin copolymer.

As illustrated in Figure 3.8d, the particle size of lignin-AM also showed a decrease in the number of coarse particles and an increase in the number of fine particles before 5 min. After 30 min, the number of particles with different sizes stabilized, which is consistent with the viscosity trend for lignin-AM in Figure 3.5a, implying that the breakdown and aggregation of microstructure is almost in equilibrium. For the AM-DADMAC in Figure 3.8e, a very limited number of coarse particles was observed in the absence of lignin, and there was almost no change in particle size after 10 min. The change of particle in the first 10 min may be caused by shear mixing. These results suggest that 1) the copolymerization of AM and DADMAC would not induce a significant particle size change since they are both water-soluble monomers and generate water-soluble polymers, and 2) the copolymerization of lignin and AM follows the same trend as that of lignin, AM, and DADMAC, and 3) the change in particle size of the systems (Figure 3.8a) originates from the solubilization of lignin when lignin reacts with AM (Figure 3.8e).

## 3.4 Conclusion

Through free-radical polymerization, Kraft lignin was synthesized with AM and DADMAC in an acidic environment. In the lignin-AM-DADMAC (LAD) polymerization system, the conversion of AM (96%) was much greater than that of DADMAC (68%) because of the higher reactivity of AM. The temperature elevation improved the conversion rates of both AM and DADMAC monomers, and the rate change was more dramatic for AM than for DADMAC. This is also confirmed by the lower activation energy of AM (65.7 kJ/mol) than

DADMAC (69.3 kJ/mol) in the LAD system. The comparison of the monomer conversion between the AM-DADMAC (AD) polymerization system and LAD system demonstrated that lignin acted as an inhibitor of the copolymerization, and lignin has a greater impact on the conversion of DADMAC monomers than that of AM monomers. The zero-monomer conversion of DADMAC in the lignin-DADMAC system and DADMAC homopolymerization system showed that DADMAC neither formed homopolymers nor directly reacted with lignin. The shear-induced viscosity analysis also proved these results. The viscosity of the lignin-containing reactions all reduced in the first 5 min, which might be attributed to the thixotropic behavior caused by dissociation and breaking of lignin agglomerates. The reaction at 75°C had an overall higher viscosity than other temperatures. The slower rate of viscosity gains at 85°C than 75°C throughout the reaction process could be attributed to the lower viscosity of the reaction system or copolymer degradation at a higher temperature. The elastic and viscous modulus verified the growing chain of polymers in the reaction, and this growth continued after the monomer conversion rate reached its maximum. The particle size analysis of the reaction mixtures reflected a decrease in the number of coarse particles (greater than 300 μm) and the increase in the number of fine particles (<10 μm and 10-50 μm), indicating the solubility improvement of the sample owing to the formation of the copolymers. When the reaction progressed beyond 5 min, the particle size and viscosity analyses reflected the alteration from a suspension to a colloidal system due to the shift in the particle size from coarse to fine particle ranges. This study offered fundamental evidence of lignin polymerization for generating cationic lignin-derived polymers, which is important for reaction monitoring and system control, enabling the development of copolymers with desired compositions for specific applications.

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# Chapter 4 Purification processes for generating cationic lignin-acrylamide polymers

## Abstract

Purification is an essential step of many polymerization processes to produce highly pure polymers. This study considered various purification methods for purifying the product of lignin, acrylamide (AM), and diallyl dimethylammonium chloride (DADMAC) copolymerization reaction. The charge density, yield, molecular weight, and solubility analyses confirmed that ethanol extraction and membrane filtration were the most effective processes for producing lignin-p(AM)-p(DADMAC). The $^1$H NMR analysis revealed that the membrane dialysis effectively removed unreacted AM and DADMAC monomers from the reaction medium. The produced samples of ethanol-extraction and dialysis processes had higher solubility and yield compared with the product of the acidification process. Thermogravimetric studies confirmed that the ethanol-extracted and dialyzed samples had a higher degradation temperature (220 °C) than the acidified samples (160 °C). The rheological studies confirmed that the viscosities of the polymer solutions were influenced by the solubility more than by the molecular weight of the generated polymers. The flocculation studies confirmed that the ethanol-extracted and dialyzed polymers were more effective flocculants than the acidified samples for a kaolinite suspension. Based on the above results, membrane filtration with a larger molecular weight cut-off could be an environmentally friendly method for effectively purifying lignin-p(AM)-p(DADMAC) to obtain a high molecular weight copolymer.

## 4.1 Introduction

Lignin, as an abundant and renewable biomass, can be used in the production of value-added products, such as carbon fibers, biosorbents, platform chemicals, adhesives, flocculants, paper-strength additives, and hydrogels (Piccinino et al., 2021; Mili et al., 2022; Fang et al., 2010; Kong et al., 2015; Musilová et al., 2018; Rong et al., 2013). The diversity of functional groups in lignin has created opportunities for the polymerization of lignin, which has received attention to induce lignin polymers for potentially replacing petroleum-based products. Various anionic (e.g., acrylic acid), cationic (e.g., 2-[methacryloyloxy] ethyl) trimethyl ammonium chloride (METAC)), and non-ionic (e.g., acrylamide (AM)) monomers have been successfully grafted onto lignin via a free radical polymerization to produce value-added products (Rong et al., 2013; Yu et al., 2016; Wang et al., 2018).

Polyacrylamide (PAM) is a type of water-soluble polymer with high flocculating efficiency in water treatment (Costine et al., 2018). Grafting the PAM chain onto lignin would improve its solubility and molecular weight (as PAM is water soluble and lignin is not), which would be beneficial for creating effective linin-derived flocculants (Rong et al., 2013). However, most suspensions have negative charges, and flocculants with cationic groups are more favorable to aggregate and settle colloids from suspensions. Therefore, polymerization with cationic monomers can be explored to impart positive charges to lignin-p(AM) and improve its flocculation performance. With a high cationic charge density, poly diallyl dimethylammonium chloride (PDADMAC) is one of the effective synthetic polyelectrolytes for the flocculation process and is extensively used in industry (Razali et al., 2011). In this paper, two monomers of acrylamide (AM) and diallyl dimethylammonium chloride (DADMAC) were selected to polymerize with kraft lignin to generate a cationic flocculant (Price et al., 2018).

Several purification processes were utilized as a final step of polymer synthesis for separating the products with desired characteristics from undesired chemicals, such as unreacted monomers, undesired homopolymers, and some inorganic salts used in the reaction. The purity of copolymers can improve their efficiency and widen their applications. Solvents, such as acetone and ethanol, are widely used to purify polymers (Stejskal et al., 2014). Rong and coworkers (2013) purified lignin-acrylamide (lignin-AM) polymer with acetone. In another work, lignin-[2-(methacryloyloxy)ethyl] trimethylammonium chloride (lignin-METAC) was purified using 80/20 vol/vol ethanol/water and centrifugation, and the final precipitated lignin-METAC product had no unreacted monomers (i.e., METAC) and homopolymer (i.e., polyMETAC) (Wang et al., 2018). In another work, a mixture of acetone and ethanol was used for purifying the product of acrylamide-[2-(methacryloyloxy)ethyl] trimethylammonium chloride-methacryloxypropltri-methoxy silane polymer) (Shang et al., 2009). Acidification is a commercial process for lignin recovery in the LignoBoost$^{TM}$ and LignoForce$^{TM}$ processes (Tomani, 2010; Kouisni et al., 2012; Hubbe et al., 2019). Compared with other methods (e.g., membrane separation), acidification is easy and inexpensive to operate (Hubbe et al., 2019). In the past, lignin-acrylic acid (lignin-AA) and lignin-AM were separated from reaction media via acidification (Kong et al., 2015; Wang et al., 2016). Membrane filtration has been an effective method for purifying polymers from reaction mixtures to remove chemicals (e.g., unreacted monomers and inorganic salts) smaller than the molecular weights cut-off of the membrane. Previously, lignin-glycidyl-trimethylammonium chloride (lignin-GTMAC) and lignin-AM were purified using a membrane with molecular weights cut-off of 1000 g/mol and 12 000 - 14 000 g/mol, respectively (Fang et al., 2010; Kong et al., 2015; Mai et al., 2000). As well known, filtration with different pore sizes can produce products with altered molecular weights and properties (Sulaeva et al., 2018).

This study produced lignin-p(AM)-p(DADMAC) from polymerizing lignin, AM, and DADMAC monomers via free radical polymerization (Wang et al., 2023). Then, different ethanol extraction (as a non-toxic solvent), acidification, and membrane dialysis processes were utilized to purify the products. Although different purification processes were used to purify lignin-based polymers in the past (Gilarranz et al., 1998; Cabrera et al., 2016; Mbotchak et al., 2015; Fang et al., 2015), the effectiveness of altered processes in producing lignin-derived polymers was not studied comprehensively. However, this information will be essential for creating lignin-derived products with different properties for varied applications. This is the first report to compare the efficiency of the altered purification processes for generating polymeric lignin derivatives with different properties. The effects of different separation methods on charge density, solubility, and product yield were discussed in detail. Also, the structural and elemental analyses of the separated polymers were comprehensively investigated using nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA), static light scattering (SLS), and rheology analyses. Also, the efficiency of the products as flocculants was assessed using a dynamic drainage jar (DDJ) in kaolinite systems. The results of this work provide insights into the development of an efficient purification pathway for producing lignin-derived polymers with desired properties.

## 4.2 Materials and methods

### 4.2.1 Materials

Softwood kraft lignin was supplied by FPInnovations from the LignoForceTM pilot plant in Thunder Bay, ON. Diallyl dimethylammonium chloride (65 wt.% in water) (DADMAC), acrylamide, sodium persulfate, sodium hydroxide, kaolinite, all analytical grades, were obtained from Sigma Aldrich and used without any further purification. Membrane dialysis with molecular weight cuts off, MWCO, of 1 kg/mol, 10 kg/mol, and 25 kg/mol (1KDa, 10KDa, 25KDa) were purchased from Spectrum-Labs.

### 4.2.2 Polymerization Process

In this set of experiments, 2.0 g of lignin and 40 mL of water were added to a three-neck glass flask. A specified amount of acrylamide and DADMAC at lignin: AM: DADMAC molar ratio of 5.5: 2.4: 1 was prepared in the flask, which was the optimum conditions based on our previous work (Price et al., 2018), and the pH of the system was adjusted to 2 while stirring at 500 rpm. The reaction mixture was purged with nitrogen for 20 min, and then 30 mg of Na2S2O8 was added to the reaction mixture. The mixture was purged with nitrogen for an additional 10 min while stirring at 500 rpm. The reaction was maintained at 90ºC for 2 h to obtain lignin-p(AM)-p(DADMAC). After the reaction, the mixture was cooled to room temperature and purified following different methods.

### 4.2.3 Product Purification

Seven different methods were utilized for lignin-p(AM)-p(DADMAC) purification after reaction, including ethanol extraction (process 1), centrifugation (process 2), acidification (process 3), acidification with aeration (process 4), and membrane dialysis with MWCO of 1 kg/mol (process 5), 10 kg/mol (process 6) and 25 kg/mol (process 7).

For ethanol extraction (process 1), the reaction mixture was cooled to room temperature and mixed with ethanol. In this process, ethanol was added to the reaction medium at a volume ratio of 4:1, which made lignin polymer precipitate. The precipitate was washed with 50 mL of ethanol and dried in the oven at 60°C until constant weight. Using a lower drying temperature positively affected the polymer's properties (e.g., solubility, molecular weight), avoiding the induced polymer's physicochemical instability.(Homez-Jara et al., 2018) In process 2, the reaction mixture was centrifuged at 4000 for 10 min, and the precipitate was collected and dried at 60°C until constant weight. In process 3, the pH of the reaction mixture was adjusted to 1.5 at 90°C by adding 25% H2SO4. The mixture was kept stirring at 400 rpm for 30 min and then centrifuged at 4000 rpm for 10 min. The precipitate was collected and dried in an oven at 60°C until constant weight. In process 4, the acidification of the reaction mixture was conducted at

90°C, but the mixture was aerated by an air pump (Topfin Air-1000) at the flow rate of 0.017 L/s while stirring at 400 rpm. Afterward, the sample was centrifuged at 4000 rpm for 10 min, and the precipitate was collected and dried at 60°C in an oven until a constant weight. In the filtration method (processes, 5,6, and 7), the reaction mixtures were first adjusted to pH 7 with the help of NaOH (1 mol/L), and then kept in the cellulose dialysis tubes obtained from SpectrumLabs and dialyzed against 4 L of deionized water for 48 hours. The deionized water was changed every 12 hours during this analysis. Then, the sample was collected and dried in the oven at 60°C until constant weight.

### 4.2.4 Yield, solubility and charge density

The yield of lignin-p(AM)-p(DADMAC) production was calculated following Equation (1).

$$\text{Yield, \%} = \frac{\text{Mass of copolymer}}{\text{Mass of monomers} + \text{Mass of lignin}} \times 100 \qquad (1)$$

Where the mass of copolymer is the amount of product after purification and drying. The mass of monomers and the mass of lignin are the corresponding masses used in the reaction.

The products' charge density and solubility are essential properties for their use as flocculants. (Vajihinejad et al., 2019). For charge density and solubility measurement, 0.2 g of lignin-p(AM)-p(DADMAC) product collected following the different purification methods was added to 19.8 mL of water. Then, the suspension was shaken at 30°C and 150 rpm for 2 h and centrifuged at 1000 rpm for 5 min. After the centrifugation, the supernatant was collected for charge density and solubility analysis. The sample concentration in the supernatants was determined by drying the supernatant at 105°C to determine its solubility, which is reported as a weight percentage (wt %). (Prince et al., 2018)

The charge density measurement was performed on the supernatant obtained above by a particle charge detector, Mutek PCD 04 titrator (Arzbergerstrae, Herrsching, Germany), with PVSK (0.005 mol/L) as the titrant (Prince et al., 2018). The reported results in this paper are the averages of three runs.

### 4.2.5 $^1$H NMR analysis

The chemical structures of lignin-p(AM)-p(DADMAC) purified following different methods were recorded by INOVA-500 MHz instrument (Varian, USA) and analyzed using $^1$H NMR with a 45° pulse and a relaxation delay time of 1.0 s. First, 0.02 g of the copolymers were dissolved in 1 mL of D2O at pH 11. The copolymers were completely water-soluble at pH 11. (Prince et al., 2018) The unreacted AM and DADMAC contents still in the copolymer after purification steps were calculated according to our previous publication. (Wang et al., 2023)

### 4.2.6 Organic element analysis

The organic elements of lignin-p(AM)-p(DADMAC) polymer were detected by a Vario Micro elemental analyzer (Elementar, German). Before the elemental analysis, the samples were dried in an oven at 105°C overnight to remove any moisture. Approximately carbon, hydrogen, nitrogen, and oxygen contents of 2 mg of lignin-p(AM)-p(DADMAC) separated by different processes were measured following a method described elsewhere. (Prince et al., 2018). In lignin-p(AM)-p(DADMAC), DADMAC was the only contributor of charged group (i.e., a charge density of +1 meq/g equals to 1 mmol/g of DADMAC). Thus, the lignin copolymers' charge density (CD) was utilized to calculate the content of DADMAC in the copolymer using Equation (2). The nitrogen content of kraft lignin was negligible; thus, the nitrogen content in lignin-p(AM)-p(DADMAC) originated from nitrogen in acrylamide and DADMAC. The nitrogen content of the copolymers was used to calculate the AM content in the copolymer using Equation (3). DADMAC and AM grafting ratios were calculated according to Equations (4) and (5), respectively.

$$\text{Content of DADMAC in the copolymer}\left(\frac{\text{mol}}{\text{g}}\right) = \mathrm{C_{DADMAC}} = \frac{\mathrm{CD}}{1000} - \frac{\mathrm{U_{DADMAC}\,\%}}{\mathrm{M_{DADMAC}}} \quad (2)$$

$$\text{Content of AM in the copolymer}\left(\frac{\text{mol}}{\text{g}}\right) = \mathrm{C_{AM}} = \frac{\mathrm{N\%}}{14} - \mathrm{C_{DADMAC}} - \frac{\mathrm{U_{AM}\%}}{\mathrm{M_{AM}}} \quad (3)$$

$$\text{Grafting ratio of DADMAC} = \frac{\text{Weight of grafted DADMAC}}{\text{Weight of lignin in the polymer}} = \frac{\mathrm{C_{DADMAC}\times M_{DADMAC}}}{1-\mathrm{C_{DADMAC}\times M_{DADMAC}}-\mathrm{C_{AM}\times M_{AM}}} \times 100 \quad (4)$$

$$\text{Grafting ratio of AM} = \frac{\text{Weight of grafted AM}}{\text{Weight of lignin in the polymer}} = \frac{\mathrm{C_{AM}\times M_{AM}}}{1-\mathrm{C_{DADMAC}\times M_{DADMAC}}-\mathrm{C_{AM}\times M_{AM}}} \times 100 \quad (5)$$

Where CD is the charge density of the polymer, mmol/g; N% is nitrogen content of the copolymer; CDADMAC and CAM are the contents of DADMAC and AM in the polymer in the product, respectively, in mol/g; UDADMAC and UAM are percentages of unreacted DADMAC and AM, respectively, in %; MDADMAC and MAM are molar mass of DADMAC and AM, respectively, in g/mol.

### 4.2.7 Molecular weight analysis

The molecular weight and radius of gyration (Rg) of lignin-p(AM)-p(DADMAC) copolymer was determined by a static light scattering (SLS) instrument (Brookhaven Instruments BI-200SM Research Goniometer System, Holtsville, USA). In this analysis, the samples were prepared in 0.5 M of NaOH and 10 mM of KNO3 at 5 different concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 g/L while stirring at 300 rpm overnight for dissolution. Before measurement, the sample solutions were filtrated twice using 0.45 µm syringe filters to remove impurities. The samples' refractive index increment (dn/dc) was determined by a differential refractometer (Brookhaven Instruments BI-DNDC, Holtsville, USA) at 25°C. In the SLS measurement, time-averaged intensity measurements were made at variable angles from 15° to 155° and a wavelength of 637 nm at room temperature. The molecular weight and radius of gyration (Rg) in the study were then calculated by BIC Zimm Plot software.(Lucey et al., 2000)

### 4.2.8 Thermogravimetric analysis

The thermogravimetric analysis of lignin-p(AM)-p(DADMAC) and p(AM)-p(DADMAC) was performed using a TGA analyzer (TGA i1000 Series System, Wisconsin, USA) to characterize the thermal stability of the samples. The samples were heated from room temperature to 700°C at the heating flow rate of 20°C/min under nitrogen at the flow rate of 100 mL/min.

### 4.2.9 Rheological analysis

The rheological properties of lignin--p(AM)-p(DADMAC) were analyzed by a DHR-2 rheometer (TA Instruments Co.). In this analysis, 3 wt.% concentration of the lignin samples was prepared in deionized water at pH 6.5 and 25°C. Flow ramp experiments and oscillation measurements were carried out with a standard concentric cylinder cup (with a diameter of 30.37 mm) and a DIN rotor (with a bob diameter of 28.03 mm and bob length of 41.96 mm).

### 4.2.10 Flocculation of kaolinite suspension

The flocculation performance of lignin-p(AM)-p(DADMAC) in kaolinite suspensions was investigated by a photometric dispersion analyzer (PDA 3000, Rank Brothers, UK) connected to a dynamic drainage jar (DDJ). First, 480 mL of deionized water was poured into the DDJ container fitted with a 70 mm mesh screen and circulated from the DDJ to the PDA through a 3 mm plastic tube. The DC voltage of pure water was recorded when the system reached a steady flow of 20 mL/min, which was regulated by a peristaltic pump throughout the experiment. Then, 20 mL of a 25 g/L kaolinite suspension was added to a DDJ container (to make a 1 g/L kaolinite concentration in DDJ), while circulated in the system continuously at a flow rate of 20 mL/min and stirred at 200 rpm. This caused a decrease in the initial DC voltage (DC0) to a new DC voltage (DCi) in PDA analysis. After reaching a steady state (100 s), the lignin-p(AM)-p(DADMAC) copolymers (5 g/L concentration) were added to the DDJ to induce the flocculation process. A steady state increase in DC voltage was represented as a final suspension DC voltage (DCf). The degree of flocculation was presented as relative turbidity, calculated from the variation in the DC voltage signals of the PDA analyzer before and after adding samples according to Equation (6).

$$\tau_r = \frac{\tau_f}{\tau_i} = \frac{\ln\left(\frac{DC_0}{DC_f}\right)}{\ln\left(\frac{DC_0}{DC_i}\right)} \qquad (6)$$

Where $\tau_i$ is the initial turbidity of kaolinite suspension; $\tau_f$ is the final turbidity of kaolinite suspension after adding the flocculant; $DC_0$ is the initial DC voltage of pure water; DCi is DC voltage of kaolinite suspension itself; and $DC_f$ is the DC voltage of kaolinite suspension after adding the flocculant.

## 4.3 Results and discussion

### 4.3.1 $^1$H NMR analysis

The $^1$H NMR spectra of kraft lignin are shown in Figure S4. The peak at 8.3 ppm is attributed to the unsubstituted phenolic protons. The board resonance is attributed to the substituted phenolic protons from 7 to 7.5 ppm and aromatic protons from 6 to 7 ppm. The board resonance between 3 and 4 ppm is associated with methyl protons widely present in methoxyl groups of the lignin macromolecule. (Gellerstedt, 2015)

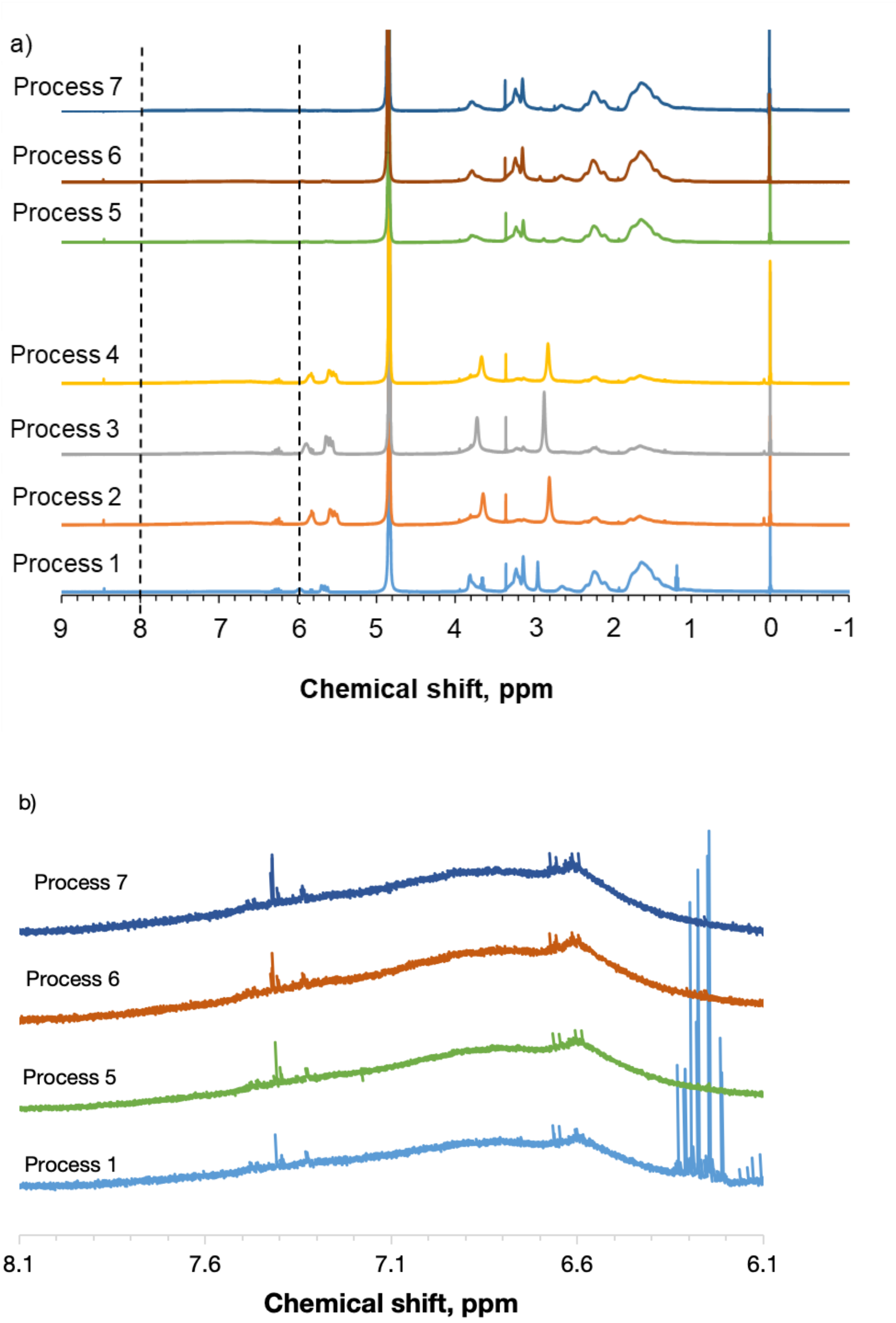
The $^1$H NMR spectra of lignin-p(AM)-p(DADMAC) purified by different methods are described in Figure 4.1. An enlarged view of the spectral range from 6 to 8 ppm is presented for a better comparison in that region. The weak bulges from 6 to 7.5 ppm are associated with the resonance in the lignin, indicating the presence of lignin in the copolymer(Wang et al., 2018). The 5-6.5 ppm peaks are related to allylic groups in the monomers of acrylamide and DADMAC.(Abdollahi et al., 2013). The resonance in the 3.6-3.8 ppm range and the 2.8 to 3.37 ppm peaks are associated with -CH2- groups and N-CH3 in DADMAC, respectively. The peaks at 2.65 ppm and 2.24 ppm are attributed to -CH- protons in DADMAC and AM, respectively. The resonance at 1.65 ppm is assigned to -CH2- protons at the main polymer backbone of P(AM) and P(DADMAC).

In the spectra of $^1$H NMR (Figure 4.1), processes 5 to 7 provided similar peaks, while processes 2 to 4 showed similar peaks. In Table 4.1, the concentrations of unreacted AM and DADMAC monomers in the samples were calculated by considering the integrated area of g, h, f, and a, b, respectively, which referred to backbones of AM and DADMAC in the $^1$H NMR, shown in Figure S1 in the appendix(Abdollahi et al., 2013). The peaks (at 1.65 ppm) related to the backbone of polymers are more evident in the dialyzed samples (processes 5, 6, 7) and the ethanol-extracted sample (process 1). It is considered that the component ratio of lignin-p(AM)-p(DADMAC) copolymers in these samples is much higher than that in the centrifuged (process 2) and acidified (process 3, 4) samples. The unreacted monomers in left in the products via processes 5 to 7 are much less than the other processes, demonstrating that dialysis was an efficient method to remove the monomers due to the low molecular weight of the monomers that could pass through the membrane pores. As these monomers are reactive and may be environmentally unfriendly (e.g., neurotoxicity, reproductive toxicity, and immunotoxicity of acrylamide) (Zamani et al., 2017), they should be removed from the copolymer product, and the membrane process showed an efficient way for this purpose. Processes 5 to 7 present negligible content of AM monomers and a low DADMAC content, while processes 1 to 4 show low AM but some DADMAC residues. AM monomer can easily self-assemble and form p(AM)

(Byard et al., 2017), and thus, its unreacted concentration would be generally lower than that of DADMAC in the reaction mixture after polymerization. Our previous study also confirmed that in the copolymerization of lignin, AM, and DADMAC, AM had a much higher conversion ratio (96%) than DADMAC (68%) because of the higher reactivity of AM (Gilarranz et al., 1998). Therefore, fewer AM monomers would be left in the reaction mixture after copolymerization, and the purification processes effectively removed it from the mixtures.

**Table 4.1** Concentrations of unreacted AM and DADMAC monomer left in the samples after purification processes.

| Sample | Unreacted AM, wt% | Unreacted DADMAC, wt% |
|---|---|---|
| Process 1 | 0.13 | 2.23 |
| Process 2 | 0.37 | 4.42 |
| Process 3 | 0.38 | 3.88 |
| Process 4 | 0.20 | 3.42 |
| Process 5 | 0.00 | 0.32 |
| Process 6 | 0.00 | 0.40 |
| Process 7 | 0.00 | 0.08 |


a)
Process 7
Process 6
Process 5
Process 4
Process 3
Process 2
Process 1
9
8
7
6
5
4
3
2
1
0
-1
Chemical shift, ppm
b)
Process 7
Process 6
Process 5
Process 1
8.1
7.6
7.1
6.6
6.1
Chemical shift, ppm

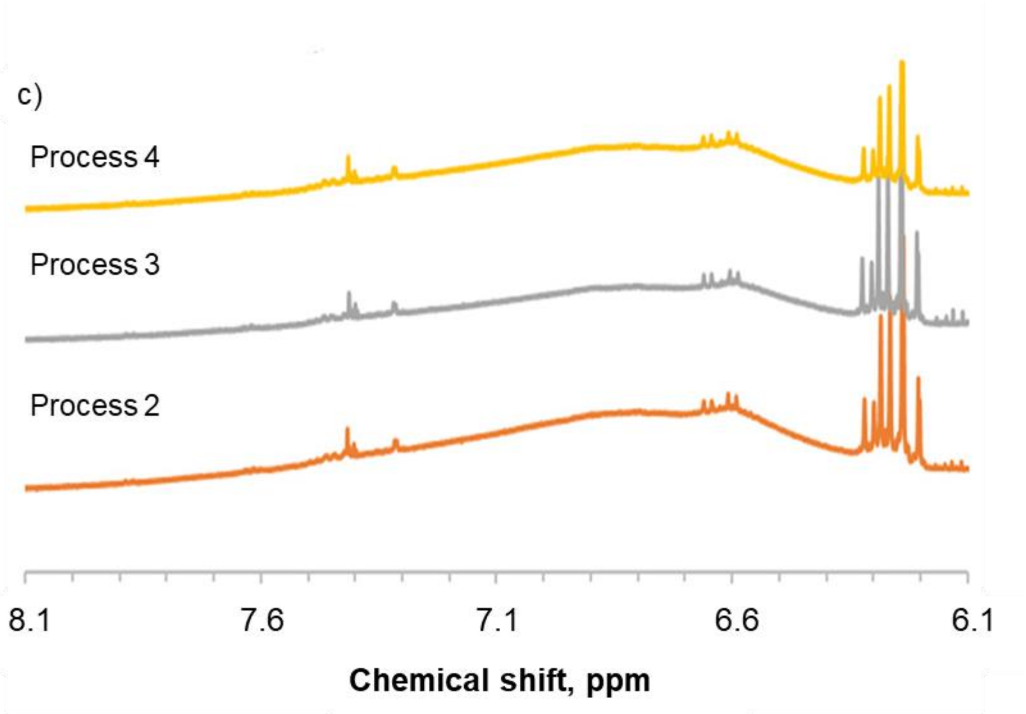


**Figure 4.1.** $^1$H NMR spectra of lignin-p(AM)-p(DADMAC) samples purified following different processes.

### 4.3.2 Yield, solubility, and charge density

Figure 4.2 describes the solubility and yield of lignin-p(AM)-p(DADMAC) purified following different methods. Prior to any purification process, the unpurified lignin-p(AM)-p(DADMAC) has a charge density of 1.43 mmol/g, a solubility of 80% at pH 7, a molecular weight of 6.53×105 g/mol. (Wang et al., 2023). The sample separated via ethanol precipitation (process 1) generated the copolymer with the highest solubility of 85 wt.% and yield of 63 wt.%. The samples purified by filtration (processes 5, 6, 7) had a similar solubility and yield to the ethanol-extracted sample (process 1). However, with the increase in the pore opening of filtration, the yield of the product decreased as more copolymer could pass through the filter pores and thus be wasted.

The acidification methods (processes 3, 4) seemed to generate products with a lower yield and solubility. The centrifugation itself induced 55 wt.% solubility (process 2), and its combination with acidification (processes 3 and 4) further reduced the solubility. The reason could be that acidification resulted in the precipitation of a large portion of unreacted lignin in the purified samples, which can be observed in the TGA profile (Figure 4.3) and will be discussed in section 3.5. According to Price et al. (2018), softwood kraft lignin is insoluble in water at acidic and neutral pH. Thus, unreacted lignin was probably precipitated and collected after adjusting pH to 1.5 and then centrifuging. In addition, due to the better solubility of lignin-p(AM)-p(DADMAC) (40 wt.% at pH 2 and 80% at pH 7), the lignin copolymers were partially dissolved at pH 1.5 and would not be fully collected at acidic pH. It is observable that all acidification and centrifugation methods had low effectiveness in separating lignin-p(AM)-p(DADMAC) (Figure 4.2).

After polymerization, in addition to lignin-p(AM)-p(DADMAC), the reaction mixture contained several by-products, including lignin-p(AM), p(AM)-p(DADMAC) and p(AM), unreacted lignin, and AM and DADMAC monomers. Lignin-p(DADMAC) and p(DADMAC) would not form under the studied reaction conditions(Wang et al., 2023). In the final products, lignin-p(AM)-p(DADMAC), p(AM)-p(DADMAC) and DADMAC monomers contributed to cationic charges, and the rest (i.e., lignin-p(AM), p(AM), unreacted lignin, and AM monomers) had anionic charges. The attachment of DADMAC to lignin or AM contributed to the cationic charge of the copolymer. Except for the centrifugation process (process 2), these results confirmed that the charge density of the samples was similar regardless of the purification process (Figure 4.2), demonstrating that the major component in the water-soluble part of final products was lignin-p(AM)-p(DADMAC) and p(AM)-p(DADMAC), which contributed to positive charges. It can be seen that the trend of charge density of the purified samples was consistent with the DADMAC contents shown in Table 4. 3. The lower charge density of the sample separated by process 2 may be due to fewer DADMAC-containing copolymers in the final product, which was proved in Table 4. 3.

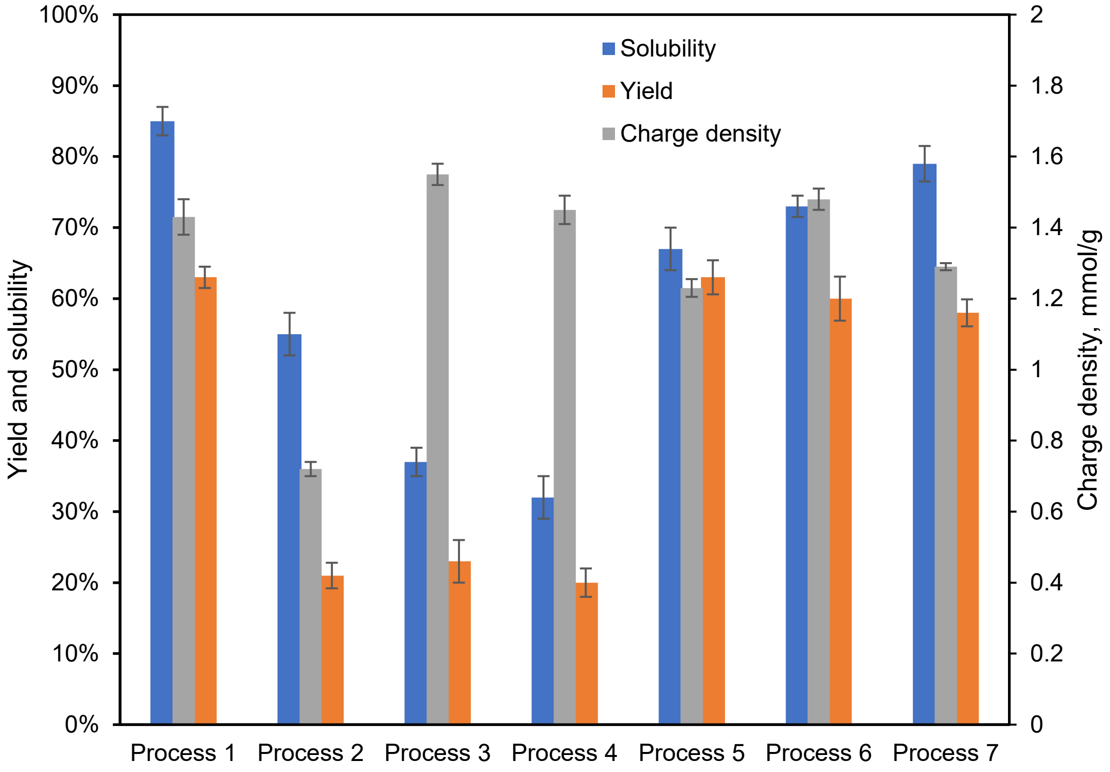


**Figure 4.2.** Solubility, yield, and charge density of lignin-p(AM)-p(DADMAC) purified by different methods.

### 4.3.3 Molecular weight analysis

Table 4. 2 lists the MW and radius of gyration (Rg) of lignin-p(AM)-p(DADMAC) separated by different methods. In the determination of MW by static light scattering (SLS) measurements, refractive index increment (dn/dc) is an important parameter (Han et al., 2020) in addition to the solvent, the wavelength of the light, and the temperature (which were kept consistent for the measurement), values for dn/dc depend on the chemical structure of the polymer and the average MW.(Kim et al., 2009) As can be seen in Table 4. 2, each MW of lignin copolymer sample corresponds to a specific value of dn/dc, which would be affected by the brownish color of the lignin-p(AM)-p(DADMAC) copolymer solutions due to the presence of lignin. It can be seen that a higher lignin (Table 4. 3) in the separated copolymer samples led to a higher dn/dc value (Table 4. 2). As higher lignin indicated a lower grafting ratio in the copolymer (Table 4. 3), the dn/dc value of lignin-p(AM)-p(DADMAC) copolymer increased with increased grafting ratio, which closely related to both the branching polymer structure and MW of the copolymer. This could be why no evident correlation between dn/dc and MW can

be found in this study, as it is the role of polymer structure (Table 4. 2). The centrifugation (process 2) provided the lowest molecular weight, 8.86×105 g/mol, while the membrane isolation using the highest MWCO (process 7) generated the highest MW value, 8.1×106 g/mol. As centrifugation collects copolymer based on gravitation force, it was the least discriminatory chemical process and thus isolated copolymers with different molecular weights, which induced the average MW of the collected copolymer lower than other samples. The MW of the samples collected via processes 3 and 4 were larger than that of process 2, indicating that acidification was somehow effective in collecting the copolymer with a higher MW. For the samples collected via processes 5 to 7, the larger MWCO allowed more effective removal of smaller molecules, resulting in the larger molecular weight of the separated samples.

The radius of gyration (Rg) of a polymer is a geometrical quantity defined as the average square distance of the chain segments from the center of the mass of the chain of a polymer segment. (Rouhi et al., 2015) The Rg is very sensitive to macromolecules' size, geometry, and structure and strongly depends on the degree of polymerization.(Petridis&Smith, 2016) A strong dependency of Rg on the molecular weight of synthetic polymers was observed in the literature.(Li et al., 2016) In Table 4. 2, larger Rg was determined for lignin-p(AM)-p(DADMAC) polymers with larger molecular weights, indicating longer chain length. It is noticed that the polymer generated from processes 4 and 5 had similar MW but different Rg. The large Rg of polymer from process 5 may indicate its more three-dimensional structure. The reason is that the higher yields of dialysis processes (processes 5-7) than that of the acidification processes (processes 3-4) (Figure 4.2) preserved more lignin-p(AM)-p(DADMAC) fractions with higher MW and chemical structure diversities, resulting in more three-dimensional structures of collected copolymers. Therefore, the relatively higher Rg for processes 5-7 than processes 1-4 indicated that membrane dialysis preserved more lignin-p(AM)-p(DADMAC) with three-dimensional structure than other purification methods (i.e., ethanol precipitation, centrifuge, and acidification).

**Table 4.2** Molecular weight of lignin and lignin-p(AM)-p(DADMAC).

| Sample | MW, g/mol | Rg, nm | dn/dc |
|---|---|---|---|
| Lignin | $(7.90\pm2.10)\times10^4$ | - | - |

|  |  |  |  |
|---|---|---|---|
| Lignin-p(AM)-p(DADMAC) |  |  |  |
| Process 1 | $(9.32\pm0.50)\times10^{5}$ | 68±3.8 | 0.14 |
| Process 2 | $(8.86\pm0.13)\times10^{5}$ | 50.2±1.2 | 0.18 |
| Process 3 | $(2.19\pm0.87)\times10^{6}$ | 99.3±5.8 | 0.16 |
| Process 4 | $(1.36\pm0.04)\times10^{6}$ | 56.4±2.4 | 0.17 |
| Process 5 | $(1.26\pm0.21)\times10^{6}$ | 76±10.0 | 0.15 |
| Process 6 | $(3.74\pm0.58)\times10^{6}$ | 101.3±9.3 | 0.16 |
| Process 7 | $(8.10\pm1.40)\times10^{6}$ | 127.1±9.9 | 0.15 |

### 4.3.4 Elemental analysis

Table 4. 3 indicates each sample's nitrogen content and the grafting ratio of AM and DADMAC. The sample produced via membrane isolation (processes 5-7) and ethanol extraction (process 1) had higher nitrogen content and AM than the other samples did. As explained in our previous study, the activation energy for AM (65.7 kJ/mol) was found to be slightly lower than that for DADMAC (69.3 kJ/mol) in the lignin-containing copolymerization reaction system(Wang et al., 2023). Thus, AM facilitated the production of lignin-p(AM)-p(DADMAC) and extended its MW (Table 4. 2) (Byard et al., 2017). Logically, the product generated via membrane dialysis with higher nitrogen contents and molecular weights possessed a higher AM and DADMAC grafting ratio than other purification processes. For purification via membrane filtration, the copolymers containing low molecular weights (and thus less AM) would probably pass through the pores, while only the higher molecular weight copolymer would be collected. The larger the pores of the membranes, the higher the molecular weight of the copolymer it would collect, and such copolymer would have a higher AM content.

The final products' lignin, AM, and DADMAC contents were estimated via the elemental analysis, and the results are tabulated in Table 4. 3. The grafting ratios were calculated based on the nitrogen content of AM and DADMAC samples. The lignin content in the copolymer was calculated by subtracting AM and DADMAC contents from 100%. The results confirmed that process 2 (centrifugation) would collect the copolymer with a lower DADMAC content. The major differences in components in the separated copolymers by different methods are AM and lignin contents. The results of AM and lignin are alike in the ethanol-extracted sample, and the dialyzed samples as the lignin contents of these samples are approximately 24-31 wt.%. The lower solubility of lignin would cause the precipitation of the copolymer with a higher lignin content (56-71 wt.%) for isolation by centrifugation (process 2) and acidification (process 3-4). As the process with higher pore size dialysis collected the copolymer with a larger molecular weight, it contained more AM than lignin (Table 4. 1).

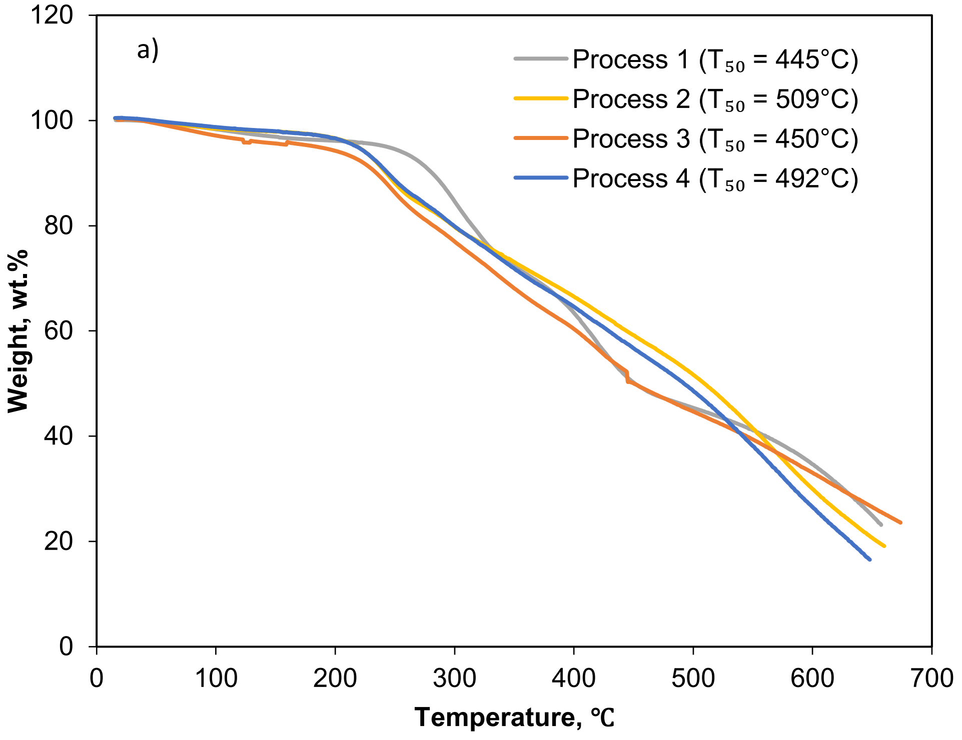
**Table 4.3** Lignin, AM, and DADMAC content in the final products.

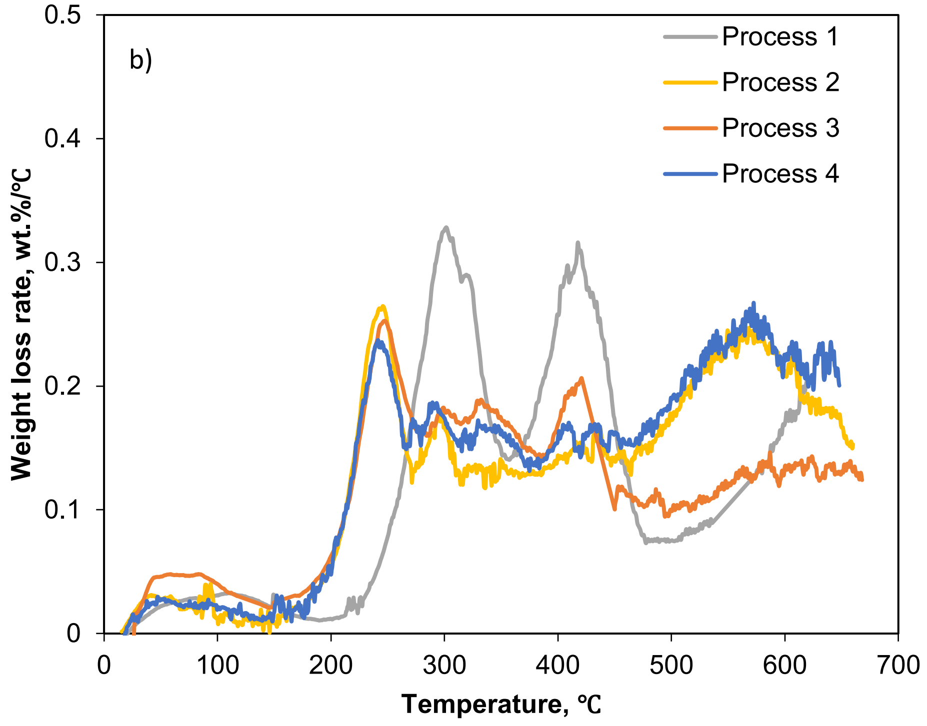
| Sample | N, wt. % | Grafting ratio of AM, wt. % | Grafting ratio of DADMAC, wt. % | NDADMAC, wt. % | NAM, wt. % | C, wt. % | DADMAC, wt. % | AM, wt. % | Lignin, wt. % |
|---|---|---|---|---|---|---|---|---|---|
| Lignin- p(AM)-p(DADMAC) | | | | | | | | | |
| Process 1 | 12.3 | 191 | 77 | 2 | 10.3 | 51.7 | 23 | 52 | 25 |
| Process 2 | 4.3 | 21 | 9 | 1 | 3.3 | 53.3 | 12 | 17 | 71 |
| Process 3 | 6 | 32 | 35 | 2.2 | 3.8 | 53.8 | 25 | 19 | 56 |
| Process 4 | 4.8 | 21 | 30 | 2 | 2.8 | 54.8 | 23 | 14 | 63 |
| Process 5 | 11.3 | 154 | 62 | 1.7 | 9.6 | 47.1 | 20 | 49 | 31 |
| Process 6 | 12.5 | 221 | 101 | 2.1 | 10.5 | 50.8 | 24 | 53 | 23 |
| Process 7 | 12.6 | 228 | 84 | 1.8 | 10.7 | 50.5 | 21 | 55 | 24 |

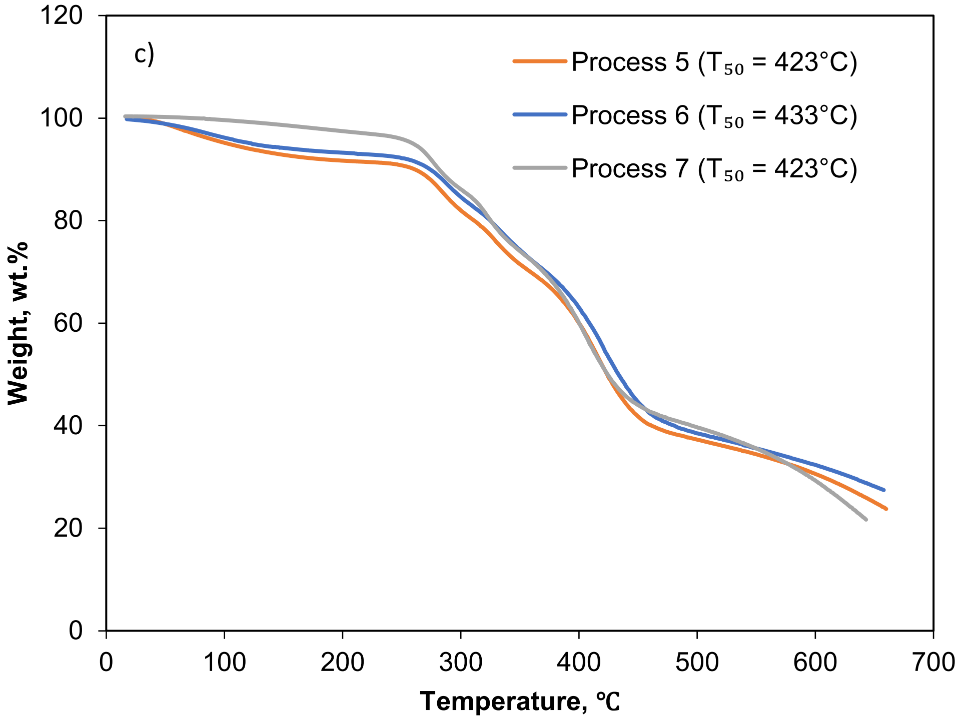
### 4.3.5 Thermogravimetric analysis

Thermogravimetric analysis (TGA) was carried out on all the samples of lignin-p(AM)-p(DADMAC) to study the thermal stability and decomposition of the samples. The thermal stability of polymers is essential as it depicts how the chemical structure of the copolymer would impact its thermal stability and its possible applications. The thermogravimetric (TG) (weight loss vs. temperature) and derivative thermogravimetric (DTG) curves (weight loss rate vs. temperature) of the samples are described in Figure 4.3. Figure 4.3a illustrates the weight loss of lignin-p(DADMAC)-p(AM) extracted via processes 1 to 4. All copolymer samples were stable < 160°C, which is related to the loss of moisture and impurities in the sample (Van Dyke & Asperski, 1993). The second stage (from 220 to 350°C) is the decomposition associated with amide and carboxyl groups (Wang et al., 2013). In this stage, quaternary ammonium salts in p(DADMAC) of the copolymer break up into ammonia and alkyl halide (Francis et al., 2007), while amide groups in p(AM) part of the copolymer break to release water, ammonia, and a small amount of carbon dioxide (Van Dyke & Asperski, 1993). The main p(AM) chains may remain interacted as their decomposition temperature is 340 - 440°C. (Van Dyke & Asperski, 1993). The last stage, occurring between 360 and 470°C, is attributed to the decomposition of p(DADMAC) chains and the breakdown of main p(AM) chains, causing the release of carbon dioxide, water, nitrile compounds, and imides (Van Dyke & Asperski, 1993; Wang et al., 2013).

The sample purified via ethanol extraction and dialysis started to degrade at 220°C (Figure 4.3b and 4.3d), consistent with their similar lignin contents and grafting ratios (Table 4. 3). Compared with the ethanol-extracted or dialyzed samples, all the acidified samples started to degrade at a lower temperature of 160°C (Figure 4.3b). They showed a higher 50% decomposition temperature (T50) (Figure 4.3) as they contained more lignin (Table 4.3). Additionally, T50 did not significantly change with molecular weight (Tables 4.2 and 4.4). According to the characteristics of TG and DTG curves, it could be concluded that the thermal degradation of lignin-p(AM)-p(DADMAC) occurred independent of molecular weight, while the lignin content influenced the degradation of investigated polymers differently.


a)
Process 1 ($T_{50}$ = 445°C)
Process 2 ($T_{50}$ = 509°C)
Process 3 ($T_{50}$ = 450°C)
Process 4 ($T_{50}$ = 492°C)
Weight, wt.%
0
20
40
60
80
100
120
0
100
200
300
400
500
600
700
Temperature, ℃


b)
Process 1
Process 2
Process 3
Process 4
Weight loss rate, wt.%/℃
0
0.1
0.2
0.3
0.4
0.5
0
100
200
300
400
500
600
700
Temperature, ℃



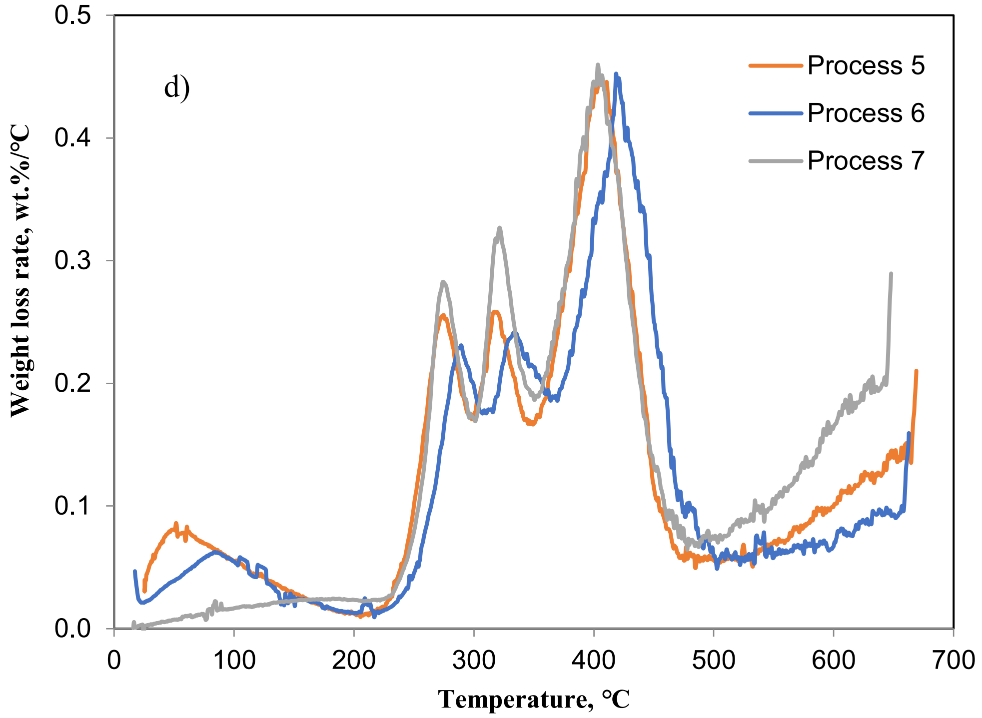
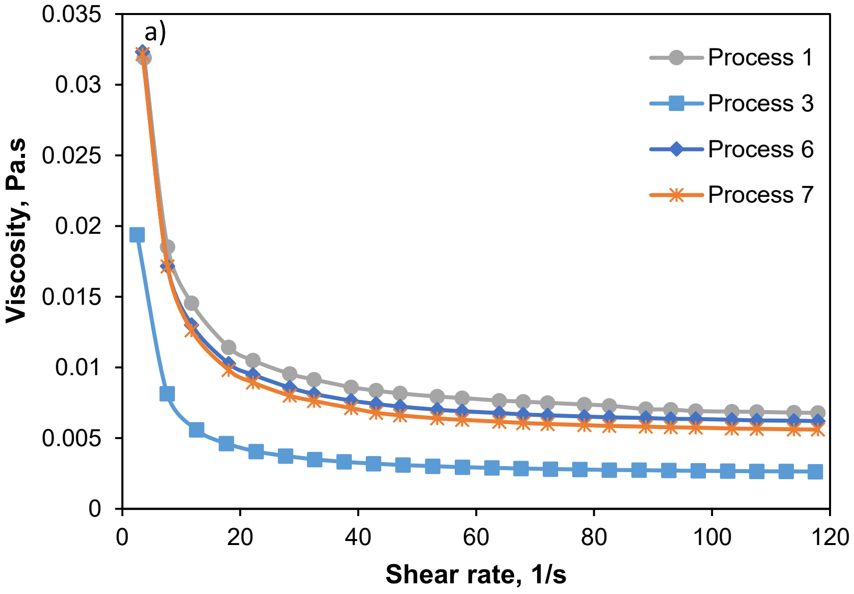


**Figure 4.3.** Weight loss (a) and weight loss rate (b) of lignin-p(AM)-p(DADMAC) purified via processes 1-4. Weight loss (c) and weight loss rate (d) of lignin-p(AM)-p(DADMAC) purified via processes 5-7.

### 4.3.6 Rheological studies

As samples generated via processes 1, 3, 6, and 7 generated better results of charge density, solubility, and yield (Figure 4. 2), they were chosen for further analysis. The rheological properties of the chosen samples analyzed under flow ramp experiments and dynamic frequency-sweep experiments is shown in Figure 4. 4. The viscosity and shear stress of the samples were recorded at a shear rate range of 0 and 120 1/s in 120 seconds. The results in Figure 4. 4 indicate a decrease in viscosity following the shear rate reduction, reflecting the samples' shear-thinning behavior (Muller & Davidson, 1994). Generally, viscosity is directly proportional to the molecular weight of the polymer in solution; however, this trend was not seen in Figure 4. 4 as these samples were not soluble. The most important factor affecting the viscosity developed by water-soluble polymers in solution is the solution concentration of the polymer (Guo et al., 1998). Process 1, with the lowest MW, induced the highest dynamic viscosities, attributed to its higher solubility of 85% (Figure 4. 2), while process 3 showed the lowest viscosities resulting from its lowest solubility of only 37% (Figure 4. 2).



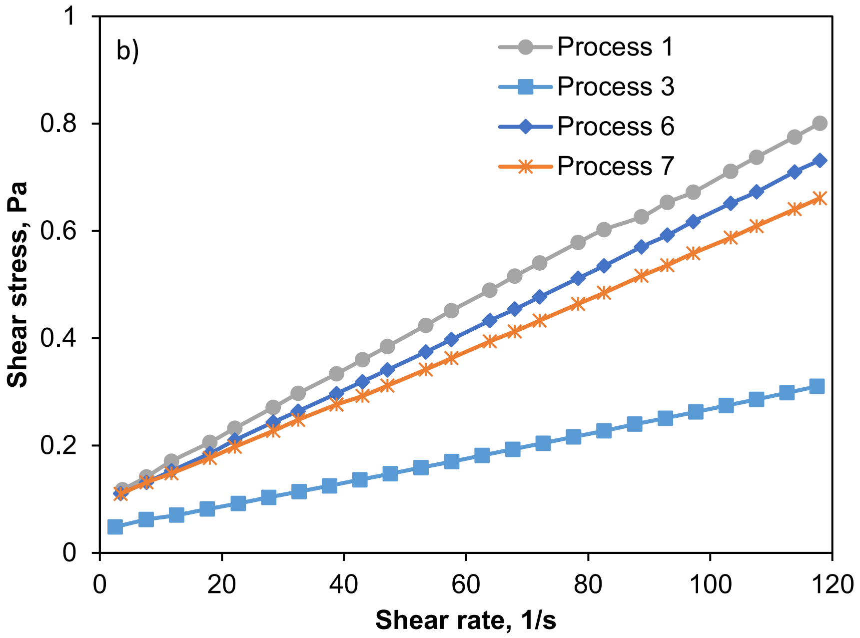


**Figure 4.4.** (a) Viscosity and (b) shear stress as a function of shear rate for 3 wt.% different separated samples in deionized water at 25°C.

### 4.3.7 Flocculation performance

The application of the selected samples as flocculants for kaolinite suspensions was evaluated, and the results are available in Figure 4. 5. The results confirmed that all samples, except for sample 3, purified via acidification, had a similar flocculation efficiency. For these samples, the optimum flocculation dosage was 0.5 mg/g, which reduced the turbidity removal of kaolinite by 97.7%, 96.2%, and 97.3% for process 1, process 6, and process 7, respectively. The acidified sample had an optimal dosage of 0.8 mg/g, generating a turbidity removal of 95.2%. The flocculation mechanisms have been reported to involve charge neutralization, bridging, electrostatic patch, and hydrophobic interaction depending on the properties of the flocculants used, the interactions between the flocculants and the wastewater components, and the operating conditions (Price et al., 2018; Lee et al., 2014). For instance, in one study, the dominant flocculation mechanism of using a cationic flocculant (dodecyl poly(glucoside)-AM-DADMAC with a cationic degree of 30 mmol/g and dosage of 8.5-9 mg/L) for treating oil-containing wastewater was charge neutralization and hydrophobic association due to hydrophobic character of both DPL and oil (Ma et al., 2018). Another research using AM-DADMAC stated that charge neutralization led to flocculation of kaolin-humic acid wastewater in acidic conditions, while bridging and adsorption mainly affected flocculation in alkaline conditions(Ma et al., 2017). In this study, lignin-p(AM)-p(DADMAC) flocculants with high molecular weight could generate bridging with their cationic charges, contributing to charge neutralization in the flocculation process (Guo et al., 2018). Also, the dialyzed samples with a higher MW and lower charge density (produced via process 7) obtained a higher turbidity removal than the sample generated via process 6.

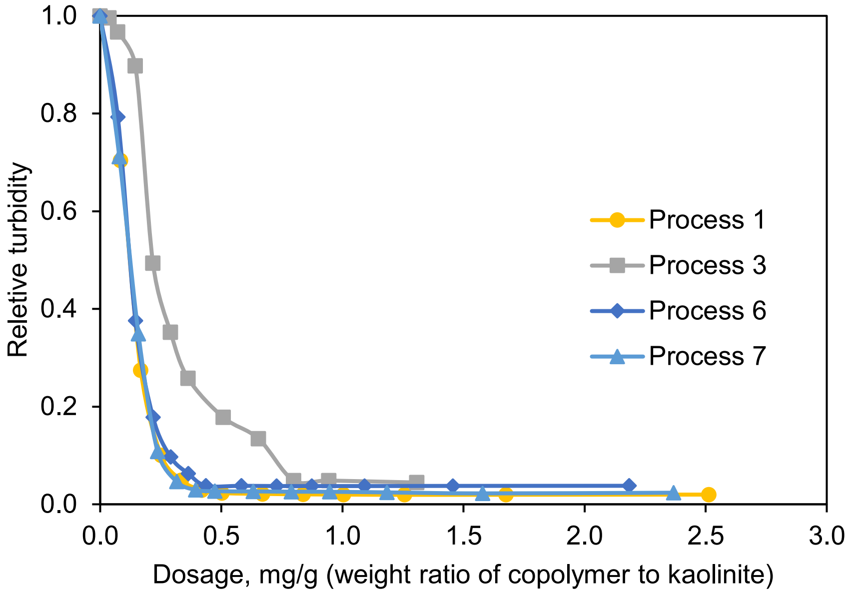


**Figure 4.5.** Relative turbidity as a function of lignin-p(AM)-p(DADMAC) dosage (mg/g) in kaolinite suspension.

### 4.3.8 Purification discussion

Among all seven purification processes, centrifugation (process 2) was the most convenient but least favorable process as it generated lignin-p(AM)-p(DADMAC) with the lowest charge density (Figure 4. 2b), molecular weight (Table 4. 2), and grafting ratios (Table 4. 3).

Acidification with or without aeration (processes 3 and 4) is relatively complex compared to other purification methods. The acidified samples showed a relatively higher charge density (Fig 2b) and molecular weights (Table 4. 2). However, the solubility and yield of the acidification processes were relatively low (Figure 4. 2a). The acidification process resulted in the precipitation of products with unreacted lignin (Table 4. 3), leading to their higher degradation temperature (Figure 4. 3b). Additionally, the higher amount of unreacted

AM and DADMAC monomers (Table 4. 1) in the acidified samples may limit their direct applications due to the toxic effects of these monomers, as mentioned previously. The aeration (process 4) increased the process complexity for acidification (process 3) and reduced the solubility, yield, charge density, and molecular weight of the acidified samples. Therefore, aeration is not recommended for separating lignin-p(AM)-p(DADMAC) by acidification.

Although ethanol extracted sample exhibited comparably high solubility, yield, and charge density (Figure 4. 2), grafting ratios (Table 4. 3), thermal degradation temperature (Figure 4. 3b), and flocculation efficiency (Figure 4. 5); its lower molecular weight (Table 4. 2) may limit its performance for other flocculation applications due to lowered bridging effect. Also, this process consumes a large quantity of organic solvent, and its products contain relatively higher amounts of unreacted AM and DADMAC monomers (Table 4. 1), which are not environmentally friendly.

The membrane dialysis processes (processes 5 to 7) are indicated to have the highest effectiveness for separation of lignin-p(AM)-p(DADMAC) in terms of their extremely low unreacted AM and DADMAC monomers (Table 4. 1), high solubility, yield, and charge density (Figure 4. 2), high molecular weight (Table 4. 2), higher grafting ratio with low lignin content (Table 4. 3), high thermal degradation temperature (Figure 4. 3d), and high flocculation efficiency (Figure 4. 5). The membrane with a higher MWCO generated products with higher solubility (Figure 4. 2a) and molecular weight (Table 4. 2), which would improve the flocculation performance through bridging. Also, membrane dialysis would be more environmentally friendly compared to other methods.

## 4.4 Conclusions

The different purification methods of lignin-p(AM)-p(DADMAC) were studied in the work, including ethanol extraction, centrifuging, acidification, acidification with aeration, and membrane dialysis with different MWCO (processes 1 to 7). The ethanol-extracted or dialyzed samples had higher solubility and yield than the acidified samples, while all the samples had a similar cationic charge density. The membrane dialysis (processes 5 to 7) presents negligible content of AM monomers and a low DADMAC content, while processes 1 to 4 show much higher AM and DADMAC residues in the products. All the samples had degradation peaks in the range of 220 to 350°C, and this temperature range would reflect the decomposition of quaternary ammonia groups. The dialyzed samples started to degrade at 220°C, which was higher than that (160°C) for acidified samples because acidified samples contained a great number of unreacted lignin. The samples' rheological properties indicated that the polymer solutions' viscosities were independent of molecular weights due to the significant impact of the solubility of the investigated polymers. The flocculation studies showed that the ethanol-extracted and dialyzed samples obtained higher turbidity removal at a lower optimum dosage (0.5 mg/g) than the acidified sample (0.8 mg/g). Therefore, membrane dialysis with a higher MWCO was considered the most favorable method due to its highest effectiveness for separating lignin-p(AM)-p(DADMAC), best product properties and flocculation performance, and environmentally friendliness. This study discussed the effectiveness of different polymer purification methods, which are crucial for selecting the purification process and controlling the desired properties of the lignin-based polymeric products.

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# Chapter 5: Overall conclusions and recommendations for future work

## 5.1 Overall conclusions

The present work mainly focused on the kinetics and mechanism of copolymerization process of lignin with AM and DADMAC as well as the different purification methods of lignin-p(AM)-p(DADMAC). Through the in-situ rheological and FBRM studies, the mechanism of particle size changes was studied. Also, the purified lignin-p(AM)-p(DADMAC) following different purification methods was systematically analyzed as cationic flocculants.

Kraft lignin was synthesized with AM and DADMAC in an acidic environment by free radical polymerization. In the lignin-AM-DADMAC polymerization system, the conversion ratio of AM (96%) was significantly higher than that of DADMAC (68%) causing by the higher reactivity of AM. The conversion rates of both AM and DADMAC monomers increased by the temperature elevation, with AM seeing a more pronounced shift than DADMAC. The results showed that lignin inhibited the copolymerization and had a higher effect on the conversion ratios of DADMAC monomers than of AM monomers. DADMAC neither generated homopolymers nor reacted directly with lignin, as evidenced by its 0% monomer conversion ratio in the lignin-DADMAC system and DADMAC homopolymerization system. Compared to other temperatures, the reaction at 75 °C exhibited a generally higher viscosity. The reduced viscosity of the reaction system or the breakdown of copolymers at a higher temperature may be the cause of the slower rate of viscosity gains at 85 °C than 75 °C during the reaction process. The growing chain of polymers in the reaction was confirmed by the elastic and viscous moduli, and this growth persisted even after the monomer conversion rate peaked. The solubility of the reaction mixtures improved due to the formation of the copolymers, as evidenced by the decrease in coarse particles (greater than 300 μm) and rise in fine particles (<10 and 10−50 μm) in the onsite particle size analysis. Particle size and viscosity measurements showed that the reaction mixture changed from a suspension to a colloidal system after 5 minutes because the particle size ranges shifted from coarse to fine.

The work also examined various lignin-p(AM)-p(DADMAC) purification techniques including ethanol extraction, centrifuging, acidification, acidification with aeration, and membrane dialysis with different molecular weight cut off for purifying the produced lignin derived polymers. All purified samples had a similar cationic charge density, but the ethanol-extracted or dialyzed samples had higher solubility and yield than the acid purified samples. The membrane dialysis method exhibits negligible content of AM monomers and DADMAC in the purified sample. The acidified samples began to degrade at a lower temperature (160°C) than the dialyzed samples (220°C) due to a greater amount of unreacted lignin contained in the acidified samples. According to the flocculation studies, the ethanol-extracted and dialyzed samples achieved greater turbidity reduction at a lower optimal dosage (0.5 mg/g) than the acidified sample (0.8 mg/g). Based on all the results, membrane dialysis with a higher molecular weight cut off was therefore deemed to be the most advantageous purification method for lignin-p(AM)-p(DADMAC) due to its highest separating effectiveness, superior product qualities, flocculation performance, and environmental friendliness.

## 5.2 Future work

This thesis studied the mechanism of cationic polymerization of lignin, but anionic polymerization of lignin is also effective for generating flocculants. Therefore, the mechanism of anionic polymerization of lignin can be studied in future. Also, the flocculation mechanism of more wastewater samples can be studied to widen the use of cationic and anionic flocculants in wastewater systems.

# Appendix

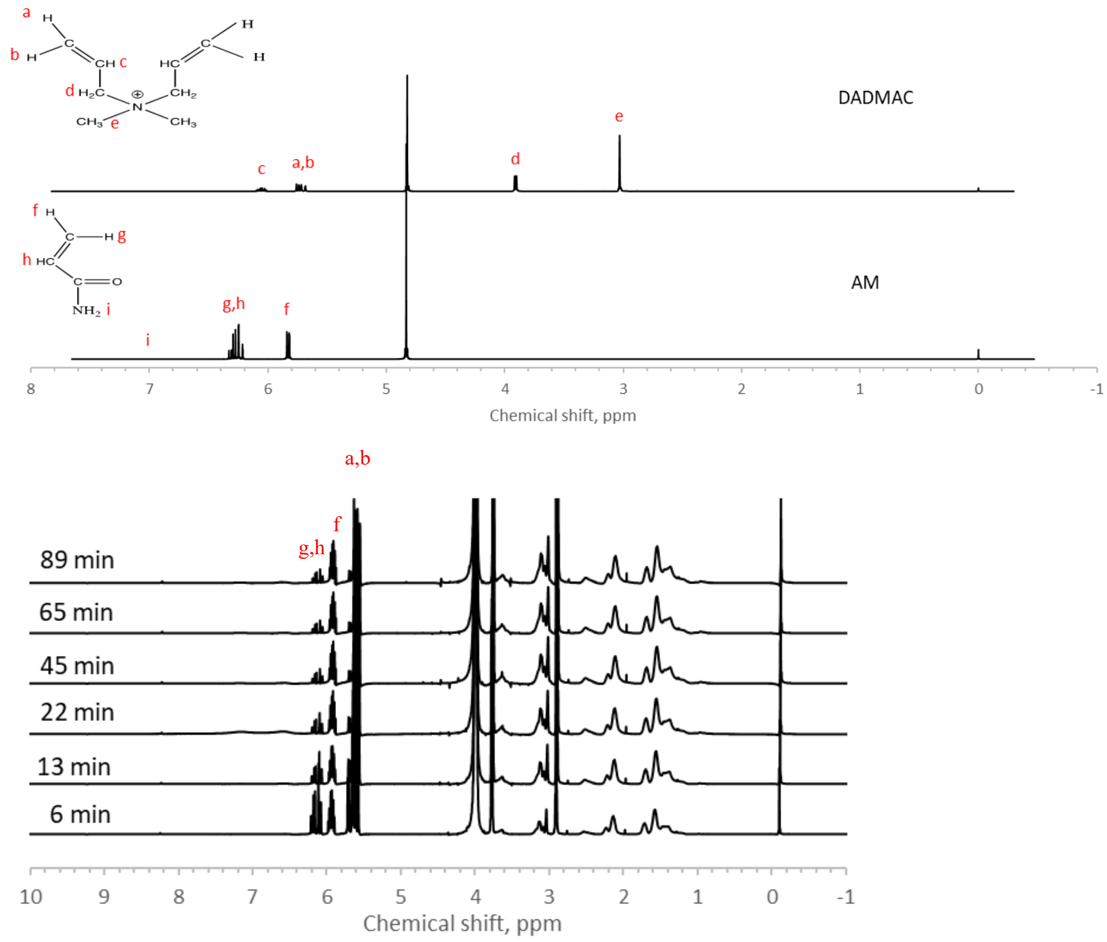

**Figure S1** a)$^1$H NMR of the AM and DADMAC monomers (Referred to Chapter 3&4); b) Progress of the copolymerization for lignin-p(AM)-p(DADMAC) at 85℃(Referred to Chapter 3).

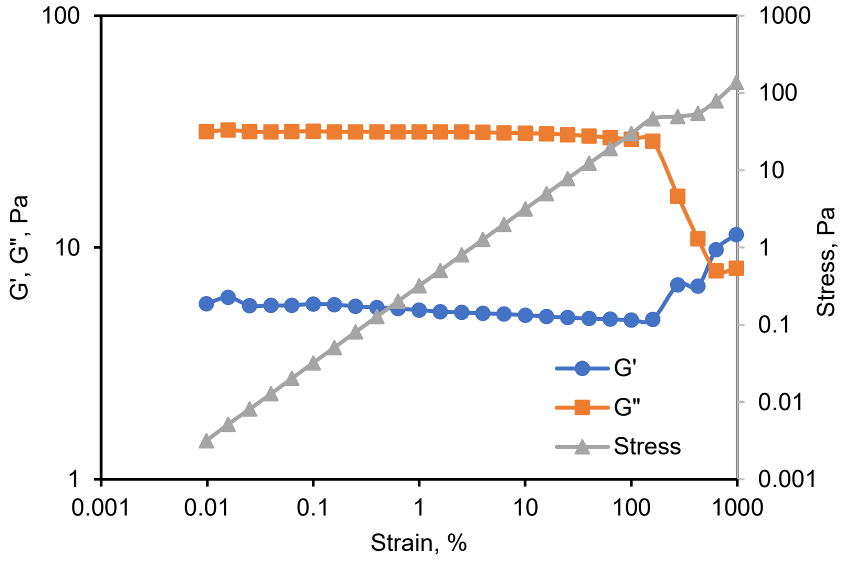

**Figure S2** Linear viscoelasticity region (LVR) of lignin-p(AM)-p(DADMAC) (Referred to Chapter 3).

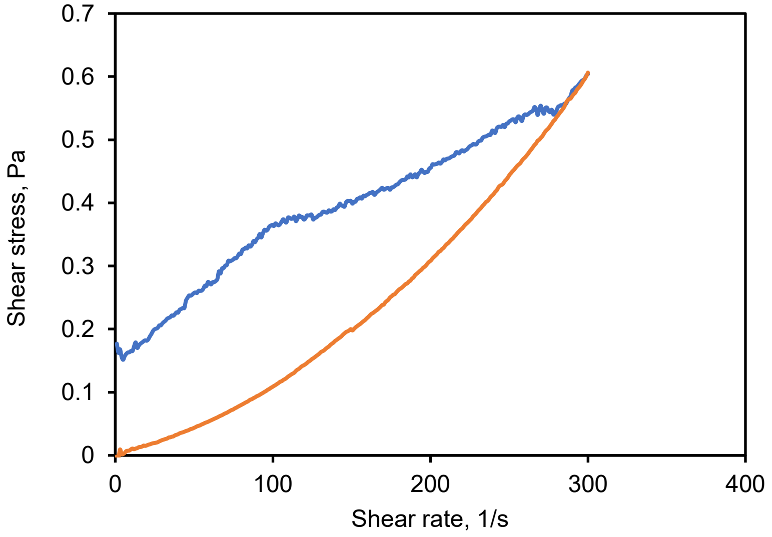

**Figure S3** A loop test of the shear stress flow curve of thixotropy phenomenon with the increase in the shear rate from zero to 300 /s (blue curve) and then a decrease to zero (orange curve) in the same way for the system containing only lignin at pH 2 and 85˚C (Referred to Chapter 3).

The reaction of the system containing lignin experienced a small viscosity drop within 5 min of reaction in Figure 6a. To understand this change, the behavior of a suspension containing only lignin (at pH 2 and temp of 90 °C) was studied by the rheometer at different shear rates, and the results are presented in Figure S3. It is evident that, the shear stress increased because of the shear rate increase. A similar behavior was observed when the shear rate was reduced. However, the rate of shear stress increase and shear stress decrease were different creating a gap between the shear stress-shear rate curves for the scenarios for accelerating of decelerating shear rates. This phenomenon is called thixotropy, and is defined as the breakage of lignin agglomerates caused by the applied shears. (Barnes, 1997) Therefore, the small drop in the viscosity of the system containing lignin in Figure 6a is perhaps associated with the breakage of lignin particles as a result of applied shear at the beginning of the reaction. (Mewis &Wagner, 2009) The thixotropy behavior of sodium lignosulfonate was studied by Tang's research (2015), in which the concentrated sodium lignosulfonate solutions from pH 2.89 to 10.34 all displayed different degrees of thixotropy (Tang et al., 2015).

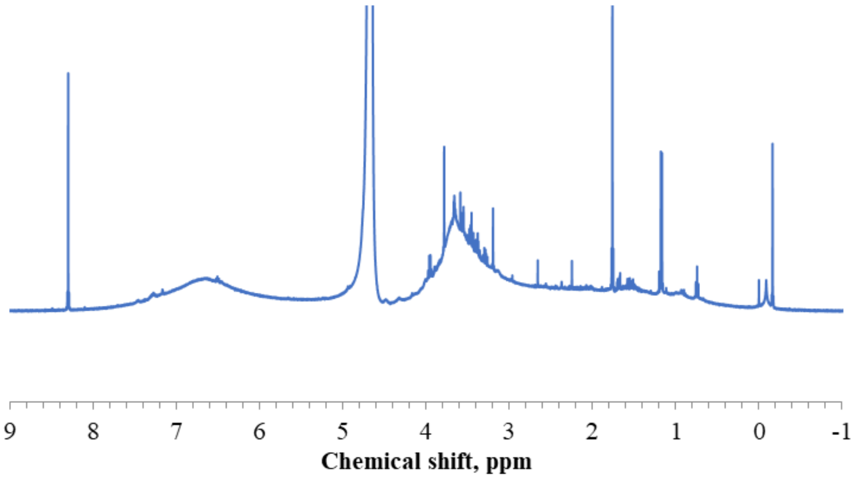

**Figure S4** $^1$H NMR for softwood kraft lignin used in the study (Referred to Chapter 4).

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