THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

Mass transfer challenges in wood decomposition

Investigation of lignin diffusion through confined pores

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Photography illustrating diffusion of liquid into a wood piece, Photo taken by Roujin Ghaffari.

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Mass transfer challenges during delignification

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Abstract

Even though the kraft cooking operation is more than 100 years old, the rate-determining step of this operation has not been fully elucidated. Recent studies point in the direction of mass transport of liberated lignin fragments from the fibers' walls into black liquor being influential in rate determination of kraft cooking. Thus, to further develop the kraft pulping operation, detailed knowledge of the mass transport events during pulping is of great importance. In this thesis, the diffusion of kraft lignin molecules through model cellulose membranes is studied by a diffusion cell methodology, where solubilized kraft lignin molecules diffuse from the donor chamber to the acceptor chamber through pores in the membrane. An advantage of using this method is that the influence of complex chemical reactions is eliminated while implementing various experimental parameters important for mass transport in the setup. Here we have investigated the effects of the membrane pore sizes, alkalinity of the solution, and size of the kraft lignin molecules on their diffusion through a porous cellulose membrane. Additionally, NMR spectroscopy, size exclusion chromatography, and UV/Vis spectroscopy techniques have been used to characterize the starting material and observe the differences between the starting material and the lignin molecules that have passed through the membrane. The average molecular weight of the species that diffused into the acceptor chamber after 168 hours was lower than that of the species in the donor chamber. The relative concentration of ionized conjugated kraft lignin molecules was higher in the acceptor chamber with the small pore membranes within the time span of the experiment. The results of this study show that the mass transport rate of lignin through a porous cellulose membrane is increased by increasing alkalinity and by decreasing molecular weight of the diffusing kraft lignin molecules. A possible explanation for the former is that the probability of forming associations in the solution is reduced at higher alkalinity levels. The latter can be explained by the higher diffusion coefficient of lower molecular weight molecules.

Keywords: lignin, lignin transport, kraft process, diffusion cells

"When you can't change the direction of the wind, just adjust your sails."

H. Jackson Brown Jr

List of Publications and Presentations

This thesis is based on the following appended papers:

I. Mass transport of lignin in confined pores

Roujin Ghaffari, Henrik Almqvist, Robin Nilsson, Gunnar Lidén, Anette Larsson

Polymers 2022, 14, 1993. https://doi.org/10.3390/polym14101993

II. Effect of alkalinity on diffusion of solvent fractionated lignin through cellulose membrane

Roujin Ghaffari*, Henrik Almqvist, Alexander Idström, Ioanna Sapouna, Lars Evenäs, Gunnar Lidén, Martin Lawoko, Anette Larsson

Manuscript

Contribution Report

The author of this thesis has made the following contributions to the publications included:

I. **First author.** Planned and performed the experimental work with support from Anette Larsson. Analyzed and interpreted the results in collaboration with all co-authors and wrote the first draft of the manuscript. Henrik Almqvist performed the SEC measurements and Robin Nilsson helped with the tritium diffusion experiments. Revision of the article was done in collaboration with all co-authors.

II. **First author.** Planned and performed the experimental work with support from Anette Larsson. Analyzed and interpreted the results in collaboration with all co-authors. Henrik Almqvist performed the SEC measurements, Alexander Idström did the diffusion NMR spectroscopy experiments, and Ioanna Sapnoa performed the ^{31}pNMR experiments. Revision of the article was done in collaboration with all co-authors.

Abbreviations and Symbols

C_a	Concentrations of species *i* in the acceptor at time t
C_{di}	Concentrations of species *i* in the donor at the start
D_i	Diffusion coefficient of species
H	Thickness of the membrane
KL	Kraft lignin
M_n	Number average molecular weight
M_W	Weight average molecular weight
MWCO	Molecular weight cut-off
NMR	Nuclear magnetic resonance
RC	Regenerated cellulose
R_T	Retention time
S	Surface area
SEC	Size exclusion chromatography
V	Volume of the donor and acceptor chambers
Đ	Poly dispersity index

Content

1. Introduction

The depletion of fossil-based resources and the increase in emission of greenhouse gases have increased interest in the replacement of fossil-based products with sustainable alternatives. Today, pulp fibers are the primary resource for paper and paperboard production, however, pulp fibers are also a promising feedstock for manufacturing a number of value-added sustainable products [1]. While advancements in the modern era have decreased the demand for various qualities of printing paper, other pulp and paper products, such as packaging materials and hygiene products, are still experiencing an increasing demand [2]. About 90% of pulp fibers are produced from wood through various pulping processes [3], making wood the most important raw material by far for the production of pulp fibers.

Wood is built up of various cells (mainly so-called fibers), which are connected to each other to form a porous three-dimensional matrix. The main building blocks in these cells are cellulose, various hemicelluloses, and lignin [4]. The principal aim of pulping processes is the liberation of fibers from the biomass matrix, which can be achieved either chemically or mechanically [3]. Pulp fibers produced from mechanical and chemical pulping processes have different properties. Chemical pulp fibers are less damaged and have higher strength, therefore, stronger paper can be made from these fibers [5]. Bleached chemical fibers are whiter and show less yellowing with time compared to mechanical pulp fibers [6]. The yield of mechanical pulping processes (85-95%) is generally higher than that of chemical pulping (45-50%). However, whilst chemical pulping can be designed to be self-sufficient in energy, mechanical pulping tends to consume more energy (at least 2 MWh t^{-1} for spruce) [4], [7]. Kraft pulping is the dominant chemical pulping process, accounting for about 80% of the total chemical pulping industry [8]. This method is based on digestion of wood chips, at elevated temperatures and pressure, in an aqueous mixture called white liquor, which

comprises active cooking chemicals (sodium hydroxide and sodium sulfide) together with some ballast chemicals. The liberation of fibers in kraft pulping processes is accomplished by degradation and dissolution of lignin from the fibers. For extraction of lignin from the wood fiber, several phenomena should be considered: (i) chemical reactions, (ii) mass transport events, (iii) solubility of degraded wood components in the liquor, and (iv) sorption of dissolved components on the solid material [9].

Mass transport events in pulping are important in two different stages: i) when cooking chemicals are transported into the wood chips and ii) when the liberated (degraded) lignin molecules diffuse through the fibers toward the free liquor. The diffusion of cooking chemicals into the wood chips has been vastly studied over the years [10]–[15]. However, surprisingly few studies have addressed the mass transfer of degraded and dissolved lignin through the fiber walls. Therefore, current understanding of these mass transfer events remains limited. In-depth knowledge of mass transport phenomena in pulping is hence important for improving current kraft pulping processes [4].

1.1. Aim and objectives

This study aims to provide insights into the diffusion of lignin molecules through wood structures after degradation and which properties of the lignin molecules affect their diffusion through the confinement of pores. The focus is on the following objectives:

1) To develop a diffusion cell methodology for studying lignin transport through model cellulose membranes.
2) To investigate how the mass transport of lignin through a model cellulose membrane is influenced by the molecular weight of lignin molecules and the size of the pores of the cellulose membrane.
3) To investigate the mass transport of different fractions of kraft lignin in the cellulose membrane at different sodium hydroxide concentrations.

The content of this thesis is based on two studies, which are referred to as Paper I and Paper II throughout the thesis. The first paper covers the development of the diffusion cell methods for investigating the diffusion of kraft lignin through model regenerated cellulose (RC) membranes with various pore sizes. The second paper applies the same methodology to investigate the influence of sodium hydroxide concentration on the diffusion of kraft lignin through small pores.

1.2. Wallenberg Wood Science Center (WWSC)

This work is associated with the Wallenberg Wood Science Center (WWSC), a multidisciplinary research center between Chalmers University of Technology, The Royal Institute of Technology (KTH), and Linköping University. The WWSC's main goals are to create new materials from trees and develop knowledge and competence as a basis for sustainable materials for the future. The studies presented in this thesis are part of the WWSC project 1.1.2a entitled "Mass transfer challenges during wood decomposition", which contributes to building knowledge around the importance and influence of mass transfer events in pulping methods.

2. Mass transport of lignin

In the kraft pulping method, lignin is removed. To remove the lignin from the fiber wall, both internal lignin bonds and bonds between lignin and other constituents break by chemical reactions. In the highly alkaline conditions of the cook, the released lignin molecules are solubilized in the cooking liquor and can be transported out from the fiber wall to the surrounding bulk liquor. To start the chemical reactions for fiber liberation, reactants (hydroxide and hydrogen sulfide ions) first need to be transported to the reaction sites in the wood chips. The mass transport of chemicals in wood during the initial part of the impregnation step is pressure-driven (advective). However, only a minor part of the necessary cooking chemicals is transported this way. During the later part of impregnation (when lumen capillaries are filled with cooking liquor) and later during the cooking step, the remaining cooking chemicals are transported into the wood chip by diffusion, which is concentration-driven [16]. The course of steps in delignification is as follows [17]:

1. Mass transfer of cooking chemicals from bulk liquor to the surface of the wood chip.
2. Mass transport of the cooking chemicals to the reaction sites inside the wood chip via the pores (lumen and pit pores) and cell walls.
3. Delignification reactions and dissolution of lignin and other wood components.
4. Mass transport of degraded, dissolved reaction products from reaction sites to the surface of the wood chip via the cell wall and the pores (lumen and pit pores).
5. Mass transfer of the degraded products from the surface of the wood chip to the bulk liquor.

Considering these steps, the rate of delignification can be controlled by either mass transfer events or the chemical reaction kinetics [18]. Chemical reactions during pulping together with their kinetics (step 3) have been studied extensively in the literature [19]–[23]. There are studies in the literature concerning the mass transfer events during pulping, which includes diffusion of cooking chemicals into the wood chips (steps 1 and 2) [10]–[15] and diffusion of degraded, solubilized lignin molecules out of the fiber walls into the free liquor (step 4) [18], [24]–[26]. However, the latter has been studied to a much lesser extent.

The dissolved lignin molecules may interact with the cellulose in the cell wall and have a low diffusion coefficient due to their large size. Furthermore, they need to diffuse through pores and between the cellulose fibrils in the cell wall, which slows down their diffusion due to confinements and tortuosity of the pores. Therefore, diffusion of the degraded, solubilized lignin macromolecules may affect the overall rate of delignification. Yet, our comprehension of these events is still limited. This thesis focuses on step 4 of the delignification events and attempts to widen our current knowledge of mass transfer events in the delignification of wood.

2.1. Previous studies on the mass transfer of lignin

As described above, delignification is a rather complicated process. It contains both chemical events, such as the reactions of heterogenous polymers and physical events, such as mass transport through fiber walls and dissolution of degraded molecules [27]. Bogren *et al.* studied the effect of inactive ions on delignification by adding various sodium salts to the cooking liquor at two different alkalinity levels [28]. They observed that the delignification rate could not only be explained by the fragmentation reaction rates but that the solubility of the lignin fragments is also important to consider.

Dang *et al.* studied the influence of the concentration of sodium ions ($[Na^+]$) on the molecular weight distribution of dissolved lignin during kraft cooking [29]. They observed that the average molecular weight (M_W) of the extracted lignin gradually increases with increasing cooking time and decreasing $[Na^+]$. The former may be

explained by the lower diffusion rate of larger molecular weight lignin molecules into the free liquor with time and the later was explained by the higher solubility of large lignin fragments in low [Na^+]. They showed that, during cooking, a change in [Na^+] would affect the molecular weight of dissolved lignin. This occurs gradually when [Na^+] is decreasing, which is possibly due to the enhanced solubility of large lignin molecules when [Na^+] is lowered. However, it takes some time for the solubilized large molecules to diffuse out of the cell wall resulting in a lag time before the increase in M_W of dissolved lignin in black liquor can be detected. When [Na^+] is increased, the effect of [Na^+] on lignin solubility is more rapidly detected since Na^+ ions can be transported into the fiber walls quickly.

The importance of lignin mass transfer in pulping was recently emphasized by Mattsson *et al.* [9]. They showed that most delignification reactions are completed within 20 min of the start of the cook. However, the total residence time during the cooking operation is about 3 h (depending on the temperature). This means that while the reactions are completed relatively early during the cook, the cook must continue for much longer to remove the dissolved lignin from fibers into the black liquor. This suggests that the mass transport events described as 4 (see above) are potentially rate-determining [9].

In another study, the lignin content of free and entrapped liquor in the lumen was investigated by Brännvall *et al.* [25]. The lignin content in free liquor was lower than the lignin content in the lumen liquor. They observed a slow mass transfer of the degraded and solubilized lignin fragments from the lumen liquor to the free liquor. They claimed that tortuosity of the wood chip is probably hindering the mass transfer of lignin from the lumen liquor to the free liquor.

These studies show the importance of solubility and mass transfer of degraded lignin during pulping. Maximizing the removal of solubilized lignin from the fiber products after cooking can reduce the need for bleaching [30]. Solubilized lignin remaining in the fiber products can be removed (leached out) during the washing step, therefore, mass transport of solubilized lignin from fibers is of importance in this step.

In 1981, Favis *et al.* studied the leaching of lignin from unbleached kraft fibers in aqueous suspension. They measured the diffusion coefficient of lignin transport through fibers experimentally and calculated the theoretically free diffusion coefficient for lignin. Based on the comparison of theoretical and experimental results, they concluded that the leaching rate of lignin from a fiber in aqueous solutions is controlled by the diffusion of lignin macromolecules [31].

Later, in 1993, Li *et al.* more comprehensively studied the effect of pH, electrolyte concentration, and temperature on alkaline lignin leaching from unbleached softwood kraft fibers. They observed that lignin removal is enhanced by increasing the alkalinity and the temperature and proposed that this higher leaching rate of lignin in caustic solutions is due to the dissociation of lignin complexes to smaller molecules, which would be able to diffuse more easily through the fibers [32]. In a later study, Li *et al.* experimentally determined the rate of lignin diffusion from kraft softwood fibers to bulk liquor under alkaline conditions [24]. They also studied the effect of ionic strength and pH. As previously observed by Li *et al.* in the earlier study, a higher pH led to faster diffusion. The effect of ionic strength was examined by comparing the diffusion rate in pH 12 and ionic strengths of 0.01 M (NaOH) and 0.1 M (0.01 M NaOH and 0.09 M NaCl). It was observed that a higher ionic strength increased the diffusion rate due to the screening of electrostatic interactions between charged lignin and charged pore walls. On this basis, they concluded that the diffusion of lignin could be affected by the tortuosity and size of the pores and molecular sizes, as well as the possible electrostatic interactions that can exist between the pore walls and the molecules [24].

From the results of the studies presented above, it is obvious that mass transfer events in delignification processes are of great importance. The diffusion of lignin molecules through cellulose fibers (*i.e.* in the lumen or the cell walls) can be affected by several factors: i) a decrease in molecular weight of lignin molecules increases the diffusion coefficient and, thus, the diffusion rate is increased, ii) an increase in the solution pH can reduce the association between lignin molecules, which implies that less

aggregates are present in the solution, therefore, the diffusion rate is increased, and iii) a reduction in attractive interactions between membrane walls and lignin molecules would increase their diffusion rate. At very high pH levels, both the cellulose and the lignin start getting negatively charged, which implies that there will be an electrostatic repulsion between these constituents. However, if the ionic strength is high enough, the ions in the solution can screen the electrostatic interactions between the membrane and lignin molecules in the solution.

2.2. Kraft lignin molecules in solution

Lignin is a branched, polydisperse, macromolecular substance that comprises three phenolic aromatic units called sinapyl alcohol (S), coniferyl alcohol (G), and p-coumaryl alcohol (H) units. Softwood contains approximately 26-32% lignin, mostly G units, and hardwood contains 20-28% lignin, mostly S and G units [4], [19]. The most important functional groups found in lignin are phenolic hydroxyl, benzylic hydroxyl, and carbonyl groups. During the kraft process, lignin molecules are degraded to smaller molecules but also partly condensed to larger molecules at longer cook times, contributing to the broad molecular weight distribution of kraft lignin [33], [34]. The degradation of lignin during the cook forms hydrophilic groups, mainly phenolic, which become charged at high pH levels. This is essential for solubilization and removal of lignin during defibrillation [35].

In Section 2.1., various factors affecting lignin diffusion were discussed, of which one was the interactions between lignin molecules, also called self-association. The association of kraft lignin molecules was studied in an aqueous solution by Rudatin *et al.* in 1989. They observed that the association of kraft lignin molecules is highly dependent on the pH of the solution [36]. The extent of the association differs for small (the ones which passed a 10000 MWCO membrane) and large molecules (the ones which did not pass a 10000 MWCO membrane), where small molecules did not associate above pH 13, whereas large molecules associated even at pH 13.5. The ionization of phenolic hydroxyl units affects lignin's association. As the molecular

weight of lignin increases, the number of phenolic hydroxyl groups per molecule increases. The pK_a value of the phenolic hydroxyls also increases by increasing the molecular weight, therefore, the phenolic hydroxyls require higher pH values to become charged [37]. This results in fewer charges on large molecules at the same pH levels compared to small molecules. Therefore, the electrostatic repulsion between large molecules is less at lower pH levels, which increases their association. Based on the literature, the association of lignin molecules is a reversible process [38] that is promoted by increasing lignin concentration [36], [39], increasing ionic strength [36], and decreasing alkalinity [36], [38], [40]–[42].

The diffusion behavior of kraft lignin molecules through the fiber walls is dependent on their size and their interactions either with each other or with the membrane. According to the Stokes-Einstein equation, the diffusion coefficient is inversely related to the hydrodynamic radius of the diffusing spherical species, therefore, larger molecular weight lignin molecules will have a lower diffusion coefficient.

2.3. Diffusion cells as a tool for lignin diffusion studies

Side-by-side standard diffusion cells have been used previously to study diffusion processes through films [43]. By using this device, the effect of the film structure, the pores, and the species diffusing through the films have been studied [44]. A diffusion cell consists of two chambers separated by a porous membrane, see Figure 1. The diffusing species is transported from the donor chamber to the acceptor chamber through the porous membrane. It is important to have sufficiently good mixing in the chambers in order to minimize the influence of mass transfer between the bulk and the surface of the membrane. The mass transport rate of diffusing molecules is calculated through analysis of the change in concentration in the acceptor chamber over time.

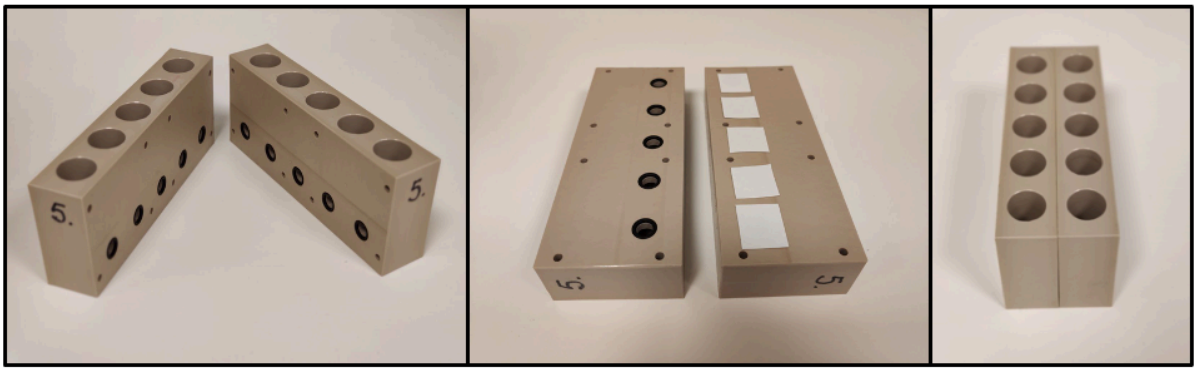

Figure 1 Photographs of standard side-by-side diffusion cells.

In this project, a series of experiments were designed to gain better understanding of lignin diffusion through confined pores: the effects of alkalinity, pore size, and molecular weight of kraft lignin molecules on diffusion rate was investigated. One of the advantages of the methodology used in this thesis is that the use of model cellulose membranes and the already degraded/solubilized kraft lignin eliminates the influence of complex chemical reactions and the rate of these, as well as the presence of polysaccharides during the cook, thereby focusing solely on studying the diffusion of lignin molecules through porous cellulose membranes.

3. Experimental procedures

In this section, diffusion cell methodologies, including sampling and concentration measurements, are first discussed. Thereafter, the characterization of the RC membranes, by diffusivity measurements using tritium, is described. Lastly, the kraft lignin fractionation procedure and subsequent characterization of the fractions, by ^{31}P-NMR and SEC, are presented.

3.1. Diffusion experiments

Tailor-made polytetrafluoroethylene (Teflon) diffusion cells, comprising two half-cells, were used for mass transfer experiments. The RC membranes of various pore sizes (100 and 200 nm for large pore membranes and 3.5 to 25 kDa molecular weight cut-off (MWCO) for small pore membranes, from Spectrum™ Labs Spectra/Por™, see Paper I) were placed between the cells. The half-cells were tightly screwed together by rods to prevent any leakages. After the diffusion cells were assembled, NaOH solutions containing lignin were prepared as donor solutions and blank solutions of the same NaOH concentration were prepared as acceptor solutions. The donor and acceptor solutions were simultaneously added to the diffusion cells, which were placed on an orbital shaker (shaking at 100 rpm) for the duration of the experiments. The agitation in the donor and acceptor solutions, induced by the orbital shaker, minimized the influence of mass transfer between the bulk liquid and the surface of the membrane. 1 mL samples from the acceptor side were withdrawn daily to be analyzed by the UV/Vis spectrophotometer. More detailed explanations about the diffusion cell methodology and the membranes can be found in Papers I and II. The effect of the pore sizes on the diffusion of kraft lignin molecules was studied in Paper I and the effects of solution alkalinity and lignin M_W on diffusion were studied in Paper II, see Table 1 for details.

Table 1 Summary of the studies presented in Paper I and Paper II.

	Paper I	**Paper II**
Purpose of the study	Study the effect of pore size on mass transfer	Study the effects of solution alkalinity and lignin molecular weight on mass transfer
RC membranes used	3.5, 15, and 25 kDa MWCO and 100 and 200 nm nominal pore size	50 kDa MWCO
Kraft lignin fraction	Unfractionated (KL)	Unfractionated (KL) and fractionated (KL 1-5)
Alkalinity	0.1 M NaOH	0.01 M, 0.1 M, and 1 M NaOH

3.2. Apparent diffusivity of water and lignin through the membranes

The apparent diffusivity (hereafter called diffusivity) of water through membranes was studied using the diffusion cell methodology and tritium labeled water. As described in Section 3.1., the membranes were placed between the diffusion cell compartments and 10 µL of 5 mCi (185 MBq) tritium-labeled water (PerkinElmer, Massachusetts, USA) was added to the donor side. The radioactivity of tritium, corresponding to the concentration, was monitored on the acceptor side by a liquid scintillation analyzer (PerkinElmer Tri-Carb 2810 TR, Massachusetts, USA). The water diffusivity, D (m^2 s^{-1}), of the membranes, was calculated from the increase in concentration of tritium in the acceptor chamber using a method proposed previously by van den Mooter *et al.* [45] and other researchers [43], [46]. According to van den Mooter [45], the transfer rate of a diffusing substance through a membrane can be determined by Fick's law:

$$\frac{dM}{dt} = -DS\left(\frac{dC}{dx}\right) \qquad \text{Equation (1)}$$

where dM/dt (g s^{-1}) is the mass transport rate, D (m^2 s^{-1}) is the apparent diffusion coefficient, S (m^2) is the surface area of the membrane from which diffusion occurs, and dC/dx (g m^{-2}) is the concentration gradient across the membrane. D is concentration dependent, therefore, only an average value can be calculated here. If it is assumed that it is a linear concentration profile through the membrane, the following expression can be used:

$$-\frac{dC}{dx} = \frac{C_{m0} - C_{mh}}{h} \qquad \text{Equation (2)}$$

where C_{m0} and C_{mh} (g L^{-1}) are the concentrations of the diffusing substance at the donor side and acceptor side of the membrane, respectively and h (m) is the thickness of the membrane. Equation 2 can be inserted into Equation 1 giving the following Equation 3:

$$\frac{dM}{dt} = DS\left(\frac{C_{m0} - C_{mh}}{h}\right) \qquad \text{Equation (3)}$$

where t (s) is time and S (m^2) is the surface area of the membrane. Here, it is assumed that the aqueous boundary layer on either side of the membrane does not affect the mass transport through the membrane. To relate the bulk concentrations in the donor/acceptor chamber to the concentrations at the membrane, a partition coefficient K is defined:

$$K = \frac{C_{m0}}{C_d} = \frac{C_{mh}}{C_a} \qquad \text{Equation (4)}$$

where C_a and C_d (g m^{-3}) are the concentrations of the diffusing substance in the bulk of the acceptor and donor chamber, respectively. It is assumed that the agitation of the solutions in the diffusion cells is high enough to create homogenous solutions

(*i.e.* mass transport resistance between the bulk and the membrane surface can be neglected) and, therefore, the concentration of lignin in the bulk and at the membrane boundaries is the same. Consequently, the partition coefficient can be assumed to be equal to one. If the amounts of the diffusing substance in the donor and acceptor chambers at time t = 0 s are denoted as M_d and M_a, respectively, considering that the amount of diffusing substance within the membrane at time t is negligible compared to M_d or M_a, the concentrations in the donor and acceptor chambers can be written as follows:

$$C_d = \frac{(M_d - M)}{V_d} \qquad \text{Equation (5)}$$

$$C_a = \frac{(M_a + M)}{V_a} \qquad \text{Equation (6)}$$

where M (g) is the mass change due to diffusion and V_a and V_d (m^3) are the volumes of solution in the acceptor and donor chambers. Considering that, at t = 0, there are no diffused substances in the acceptor chamber ($M_a = 0$) and the volume of the donor and acceptor chambers are the same (V), Equation 3 can be rewritten as Equation 7:

$$\frac{dM}{dt} = \frac{DKS}{h}\left(\frac{M_d - M}{V} - \frac{M}{V}\right) \qquad \text{Equation (7)}$$

Considering K =1 (due to high agitation), by integrating the above equation, the following Equation 8 is obtained:

$$\frac{2DS}{hV}t = -\ln\left(\frac{M_d - 2M}{M_d}\right) \qquad \text{Equation (8)}$$

Considering that the acceptor chamber was initially empty of any diffusing substances, the concentration in the acceptor chamber can be calculated as M/V. By

assuming that the decrease in lignin concentration in the donor chamber is negligible, the apparent diffusivity can be calculated from Equation 9:

$$\frac{2DS}{hV}t = -\ln\left(\frac{C_0 - 2C_a}{C_0}\right) \qquad \text{Equation (9)}$$

where C_0 and C_a (g m^{-3}) are concentrations of the donor solution at the start and the acceptor solution at time t, respectively. Since it was assumed that the decrease in lignin concentration in the donor chamber is negligible, it does not change the driving force. Equation 9 can therefore be used as far as this assumption is valid, which would be at the beginning of the experiments when the concentration in the donor chamber has not changed significantly.

To calculate the diffusivity of lignin or water through the membranes, $\ln\left(\frac{C_0-2C_a}{C_0}\right)$ was plotted against time, where C_a was measured at specific time intervals by sampling from the acceptor chamber. Figure 2 shows the diffusivity of water through membranes of various pore sizes and, for the 50 kDa membrane (RC50), at different alkalinities. The free self-diffusion coefficient of water at 25 ˚C is approximately 2.3 x 10^{-9} m^2 s^{-1} [47] but the diffusivity values obtained in this study are much smaller. This is due to the fact that water molecules need to pass through the pores of the membrane and a) the available pores are much smaller than the area in Equation 9 and b) the pores are not straight, so the real length is longer than the thickness in Equation 9. The general trend for the water diffusivity is an increase with increasing pore sizes and a slight increase with increasing alkalinity. The increase in water diffusivities through the 50 kDa membrane with increasing alkalinity levels is not fully understood. However, it may be explained by changes in swelling of the membrane [48], where higher alkalinities may cause structural changes in the membranes, leading to higher water diffusivities.

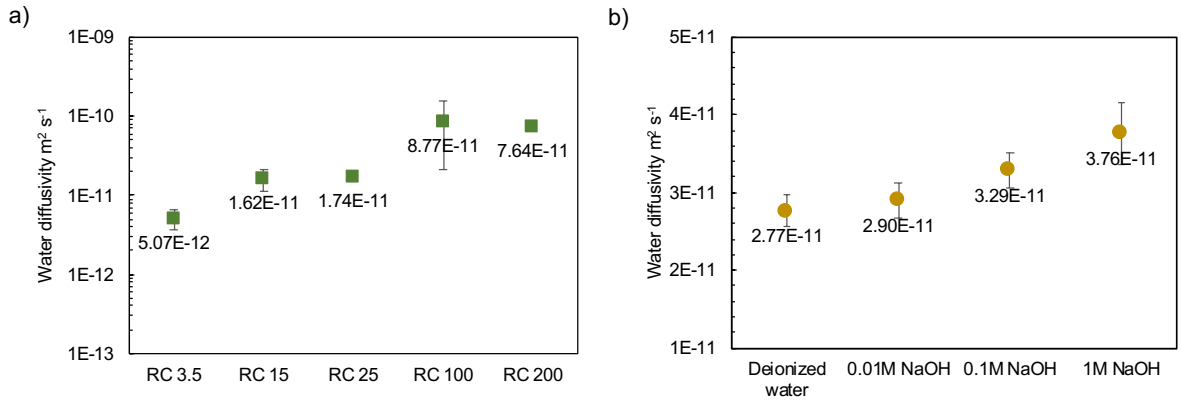

Figure 2 Water diffusivity through different membranes, measured as the diffusion of tritium oxide a) through pretreated RC membranes with various pore sizes (RC3.5, RC15, and RC25, representing MWCOs of 3.5, 15, and 25, respectively and RC100 and RC200 representing membranes with nominal pore sizes of 100 nm and 200 nm) in neutral conditions and b) through the 50 kDa MWCO membrane (RC50) in different alkalinities.

3.3. Solvent fractionation of kraft lignin

Determination of the diffusion coefficient of kraft lignin is challenging due to its high polydispersity. The polydispersity of lignin molecules can be reduced through fractionation. There are several methods to fractionate technical lignin, such as pH control [49], solvent/water extraction [50], solvent extraction [51], [52], and ultrafiltration [53], [54]. In this thesis, organic solvents are used to fractionate lignin into five fractions for further diffusion studies [51].

Kraft lignin is a heterogeneous material and to better understand how its molecular weight influences diffusion, a stepwise solvent fractionation method was implemented. The sequential solvent fractionation method used here is based on a protocol introduced by Duval *et al.* [51]. At each step, the kraft lignin is dissolved in an organic solvent and filtered, the filtered solution is dried to produce a fraction of the lignin, and the insoluble residue is redissolved in the next solvent, see Figure 3. The effect of alkalinity on the mass transport for each fraction was determined.

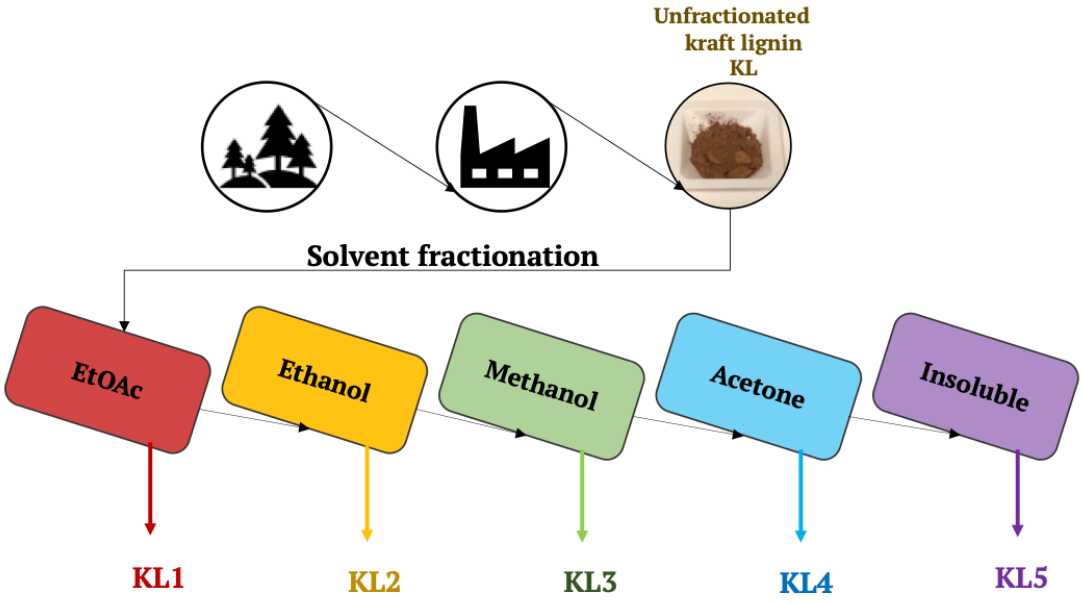

Figure 3 Sequential solvent fractionation method for softwood kraft lignin.

3.4. Characterization of the fractionated kraft lignin

The fractions produced by the solvent fractionation method were characterized by size exclusion chromatography (SEC) at high alkalinity (0.1 M NaOH) to study their molecular weights and ^{31}P-NMR to study their hydroxyl functionalities. For details on the methodology of the measurements, the reader is referred to the methods section of Papers I and II.

Table 2 summarizes the M_W values in three different alkalinities, calculated from the SEC measurements. The M_W increases from fraction 1 (KL1), which was soluble in ethyl acetate, to fraction 5 (KL5), which was insoluble in all the organic solvents used in the fractionation process. These M_W values differ from reported values for fractionation of softwood kraft lignin [51]. This could be due to the use of a water-based SEC system operating at high alkalinity, different experimental methodologies, including standards, or the different process conditions in the pulp mills. Nevertheless, the trend of increasing M_W from KL1 to KL5 has been observed both here and in the reported literature. When comparing the M_w values for 0.01 M NaOH

and 1 M NaOH, the apparent M_w values of the fractions are lower in 1 M NaOH, which is more clearly observed for large M_w fractions like KL3, KL4, and KL5.

Table 2 Summary of the average M_W and M_n values measured by SEC, using a UV detector at 280 nm.

	0.01 M NaOH			0.1 M NaOH			1 M NaOH		
	M_w (kDa)	**M_n (kDa)**	**PDI**	**M_w (kDa)**	**M_n (kDa)**	**PDI**	**M_w (kDa)**	**M_n (kDa)**	**PDI**
KL	4.4	1.3	3.4	4.6	1.3	3.5	3.8	1.2	3.1
KL1	1.9	0.9	2.2	1.8	0.9	1.9	1.8	0.8	2.1
KL2	3.4	1.4	2.4	3.6	1.5	2.4	2.9	1.3	2.3
KL3	3.9	1.6	2.4	4.0	1.6	2.4	3.1	1.3	2.4
KL4	6.1	2.0	3.0	6.0	2.0	3.1	4.6	1.6	2.9
KL5	10.8	2.6	4.2	10.9	2.9	3.8	8.4	2.2	3.9

Table 3 summarizes the results from the ^{31}P-NMR measurements. Generally, the total amount of phenolic hydroxyls decreases as M_W increases. This could be because the larger fractions are either less degraded or more condensed, corresponding to fewer end groups or phenolic hydroxyls, which is in agreement with previous reports [55]. Carboxylic functionalities show a similar trend and decrease as the M_w increases.

Table 3 Concentration of -OH functionalities of the fractionated kraft lignin measured by ^{31}P-NMR.

	-OH Functionalities				
	Aliphatic (mmol/g)	**C5-substituted (mmol/g)**	**G-units (non-condensed) (mmol/g)**	**H-units (mmol/g)**	**Carboxylic (mmol/g)**
KL	1.97	2.02	1.72	0.17	0.56
KL1	0.94	1.89	2.45	0.17	0.66
KL2	2.09	2.09	1.76	0.20	0.58
KL3	2.24	2.18	1.51	0.14	0.45
KL4	2.06	2.14	1.25	0.15	0.38
KL5	2.49	1.54	0.85	0.12	0.31

4. Influence of the membrane pore sizes on the diffusion rate of kraft lignin

The effect of membrane pore sizes on the diffusion of unfractionated kraft lignin molecules in alkaline conditions was studied. The methodology used in this chapter and the results presented are published in Paper I. Briefly, the diffusion cell methodology was used to study the diffusion of unfractionated kraft lignin molecules through RC membranes of various pore sizes (3.5, 15, 25 kDa MWCO and 100 and 200 nm nominal pore size) in an alkaline solution (0.1 M NaOH).

4.1. Diffusion rates through the membrane

The concentration of the lignin molecules, which passed through the membrane pores into the acceptor solution, was determined by UV/Vis absorption. Figure 4 a and b show a linear increase in concentration in the acceptor chamber with time, indicating that the diffusion process in the membranes is following a steady trend and does not present abrupt changes with time. The lignin concentration in the donor chamber at the start of the experiment was 0.4 g L^{-1}, therefore, to reach a concentration equilibrium on both sides, the concentration in the acceptor chamber must reach about 0.2 g L^{-1}. In large pore membranes, since diffusion was faster, the concentration in the acceptor chamber is closer to equilibrium and about 40% of the lignin that could have diffused, has done so already. However, in small pore membranes, only 2-5% of the lignin that could have passed through the membrane has passed through within 144 h from the start of the experiments. The concentration difference between donor and acceptor is the driving force for diffusion of lignin through the membrane. In the case of the small pore membranes, even after 144 h, the concentration difference between donor and acceptor is almost constant. Therefore, the driving force for diffusion is not changed much by time, which means that the linear approximation (see Equation 9) is acceptable.

For the large pore membranes, the situation is different: here the concentration difference between the donor and acceptor chambers are changed more. This means that the driving force for diffusion in large pore membranes becomes smaller, hence a small deviation from linearity is seen after 90 h. At the beginning of the experiments, however, a linear relation is observed, thus, the linear approximation can be used there.

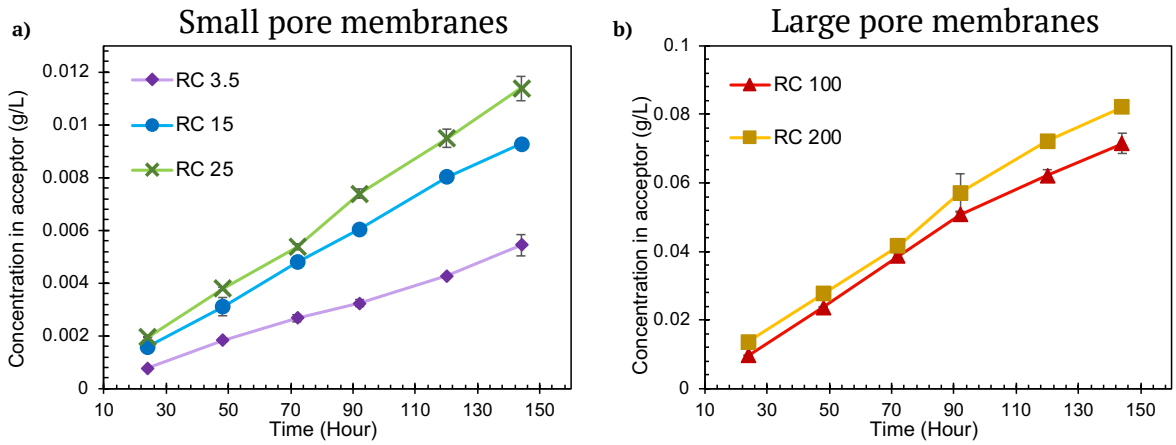

Figure 4 Concentrations of kraft lignin molecules that have passed through the membrane into the acceptor chamber versus time for different membranes for a) RC3.5, RC15, and RC25 (3.5, 15, and 25 MWCO respectively) and b) RC100 and RC200 (100 and 200 nm nominal pore sizes).

A question comes up about the molecules that passed through the membrane: do they differ depending on the pore size? To answer this question, SEC chromatograms of the acceptor samples were studied.

4.2. Molecular weight analysis of the acceptor samples

Determination of M_W using SEC analysis can be challenging. It has been reported that the M_W values obtained strongly depend on the details of SEC system used, such as the mobile phase, columns, and calibration standards [56]. SEC systems with refraction index detectors require standard samples to represent the desired molecule. In the case of kraft lignin molecules, various standard samples have been used, such as polyethylene glycol (PEG) [57], pullulan [58], or polystyrene (PS) [59]. However, none of these standards truly represent lignin molecules. Here we have used

low M_W PEG standards, however, for comparison purposes, we have focused on the SEC chromatograms and refer to the differences in the chromatograms using the retention times R_T.

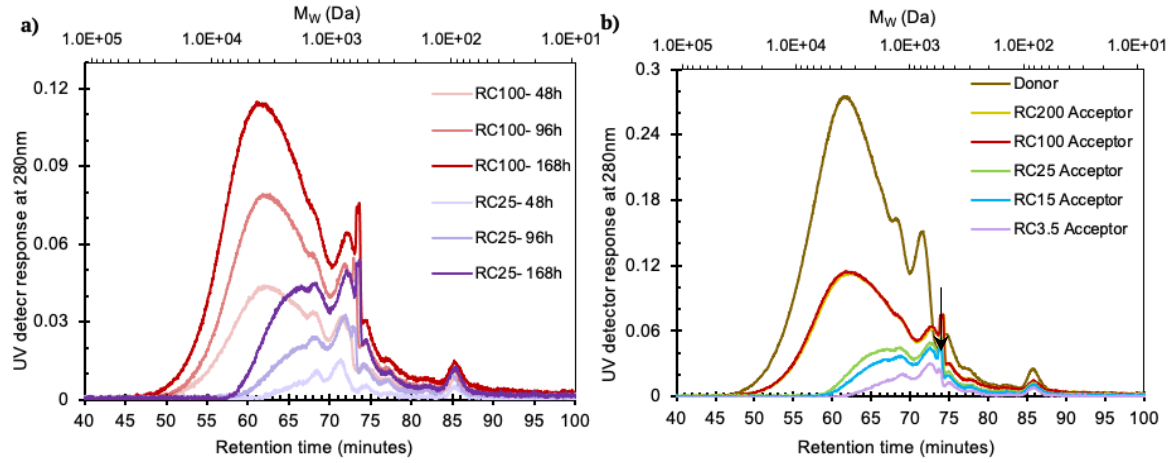

Figure 5 SEC chromatogram curves showing the UV detector response at 280 nm versus retention time for a) the acceptor samples that passed through RC100 and RC25 membranes at three diffusion times and b) the donor sample and acceptor samples that passed through various membranes after 168 h. The arrow refers to the peak at 73 min. The secondary x-axis above the graphs shows the M_W values calculated based on a calibration curve from PEG standards.

Figure 5a shows the SEC chromatogram for acceptor samples of RC100 and RC25 at different sampling times. The UV intensities increase for both RC100 and R25 with time, resembling the results in Figure 4. However, the onset of the peaks for RC25 starts at a retention time R_T of around 58 min (corresponding to a M_W of 4 kDa, according to the calibration), while the onset for RC100 starts at around 48 min (~ 34 kDa). This indicates that the small pore membrane retards larger molecules from passing through the membrane. Even though the M_W values, based on the calibration curves, are not a perfect representation of lignin molecules, they show that a remarkably large portion of the lignin molecules does not pass through the RC25 membrane (with a molecular weight cut-off of 25 kDa) within the time frame of the experiment.

Figure 5b shows the chromatograms of the samples from the donor solution at the start of the experiment and samples from the acceptor side of the diffusion cells after

168 h. The onset of the peaks for small pore membranes (RC3.5, RC15, and RC25) occur at much higher R_T values than that of the donor spectra and for RC200 and RC100. This confirms that all the membranes with small pore sizes limit the diffusion of larger molecular weight molecules with larger hydrodynamic radii (R_h). In the secondary cell wall, the mean sizes of the bundles of microfibrils (also referred to as cellulose aggregates) are 15-25 nm and size enlargements of the bundles of microfibrils are known to occur during pulping at the expense of maintaining the distance between the bundles of microfibrils [60], [61]. The distance between the bundles of microfibrils varies but it has been found to be in the range of 5 nm for softwood [62]. The transport of lignin in the secondary cell wall during pulping is likely to occur through the space between the bundles of microfibrils. According to the supplier's note [63], the pore sizes in RC3.5, RC15, and RC25 membranes are approximately 1-2 nm, 3-4 nm, and 4-5 nm, respectively. These are close distances between bundles of microfibrils. Our results indicate that diffusion through pores with similar pore sizes to the secondary cell wall is a slow process and can affect the rate of the delignification process.

The SEC chromatograms indicate a size difference between the molecules passing through the small pore membranes and the molecules passing through the large pore membranes. To further investigate other eventual chemical differences, UV/Vis spectra were recorded from samples collected from the donor and acceptor chambers of the diffusion cells.

4.3. Chemical differences of the lignin transported through membranes in diffusion cells

UV/Vis absorbance spectra (scaled at 300 nm) were used to elucidate the chemical differences between the lignin molecules both in the donor samples and those that passed through the membrane to the acceptor chamber (Figure 6). The spectra of acceptor samples from RC100 and RC200 are quite similar to the donor sample

spectrum, however, they show a higher absorbance at around 350 nm compared to the donor spectrum.

The acceptor spectra related to the RC3.5, RC15, and RC25 membranes differ from both the original lignin in the donor chamber and the lignin obtained in the acceptor cells with the RC100 and RC200 membranes, which are more similar to the original lignin spectrum. The first difference is at higher wavelengths (around 425 nm), where the donor spectrum and the acceptor samples from RC100 and RC200 membranes have a higher absorbance than the acceptor spectra for the small pore membranes (see the green arrow in Figure 6). This higher absorbance could be due to scattering by larger molecules and/or small, loose clusters of lignin molecules that can only pass through the larger pores. These loose clusters can form by associations between lignin molecules. At an alkalinity level of 1 M NaOH, such associations between kraft lignin molecules are unlikely, yet possible, especially for larger molecular weight molecules, as reported by Rudatin *et al.* [36]. These high molecular weight molecules have phenolic groups having a higher apparent pK_a value and a flatter dissociation curve due to their larger molecular weight [37]. This could result in the larger molecules only becoming partially charged, making them more likely to associate.

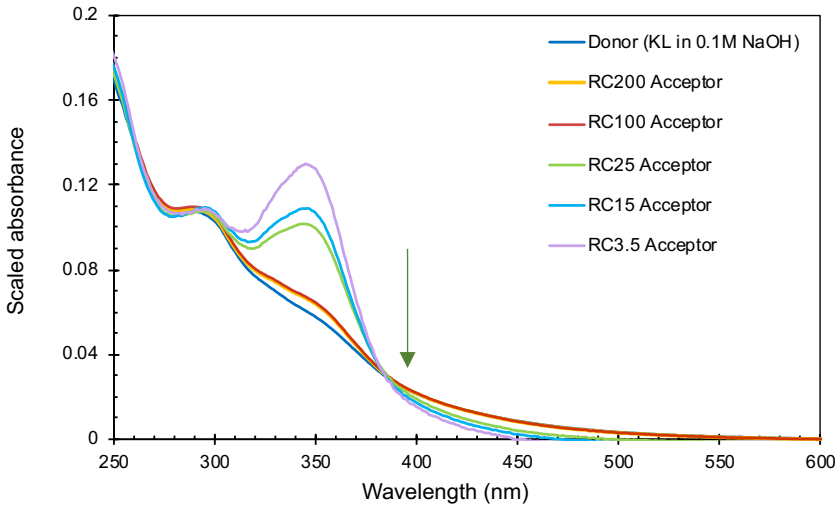

Figure 6 Scaled UV/Vis spectra of samples taken from the donor and acceptor sides of diffusion cells after 168 h. The green arrow represents the isosbestic point, above which more scattering is detected in the donor sample and the acceptor samples from compartments with large pore membranes (RC200 and RC100) compared to acceptor samples from compartments with small pore membranes (RC3.5, RC15, and RC25).

Another difference in the spectra is the appearance of a new peak at around 350 nm for acceptor samples from the small pore membranes, in addition to the conventional 300 nm peak for lignin observed for all samples. This could indicate a difference between the chemical structure of the molecules passing through the small pore membranes and that of the molecules hindered by the small pore membranes.

When phenolic hydroxyl groups become ionized in alkaline solutions, they can induce both bathochromic and hyperchromic shifts in the UV/Vis spectra [64]. In Paper I, we showed that, when the pH is reduced to neutral conditions, the 300 nm peak shifts to 280 nm and the 350 nm peak disappears (for more details, see Paper I), indicating that this peak originates from the ionization of phenolic groups. The difference in the UV/Vis spectra is interpreted as a chemical difference between the samples, where the 300 nm peak represents the totally ionized phenolic hydroxyls and the 350 nm peak represents the carbonyl or double bond conjugated phenols (which includes quinones and stilbenes), as well as carboxylate ions [65].

The 350 nm peak is only seen in the acceptor samples and the peaks are larger for samples related to the small pore membranes. The differences between the absorption, for the absorbance spectra in alkaline and neutral conditions, were calculated at 350 nm and 300 nm. The ratio between these differences has been shown to linearly correlate to the concentration of conjugated to non-conjugated phenols in the solution [66]–[68]. This ratio would be higher in the case of the small pore membranes, which shows that the relative number of fully ionized conjugated phenols is higher on the acceptor side of the small pore membrane (see Paper I for more details). Together, these findings indicate that possibly the smaller size molecules which are charged in alkaline solution (seen from the UV/Vis absorbance at 340 nm) have a higher mass transport rate through small pores compared to the partially charged non-conjugated molecules.

The presence of highly ionized and conjugated structures in the acceptor solution may be due to various reasons: The transported molecules are the smaller molecular weight fragments of lignin (monomers and oligomers) formed during the kraft

process. β-O-4' bonds are the bonds that are mainly broken during pulping through quinone methide intermediates, which can form conjugated bonds on α or β carbons and result in the formation of conjugated phenolic end groups that will be ionized in the alkaline solutions [4], [69]. Another possible explanation for finding highly charged, small molecules on the acceptor side could be that these molecules tend to participate less in the loose associations in solution. The charges on the fully ionized molecules would induce repulsion between them, preventing them from participating in the associations. This repulsive force between the negative charges on the molecules could also prevent them from associating with the membrane, therefore, passing through the pores with fewer interactions.

4.4. Effects on the delignification process

In the pulping process, diffusion of liberated lignin molecules occurs mainly through the fiber cell wall, lumen, and pits. Adjacent lumens in the wood structure are connected by holes, also known as pits, which allow communication between them. The pits have a membrane with pores on the nanometer length scale [70]. The wood cell wall can be described as parallel cellulose bundles in a matrix of lignin and hemicelluloses. The cellulose bundles or aggregates are 20-25 nm in diameter and the distances between them are of nm scales [60]. The pores in the RC3.5, RC15, and RC25 membranes used here are of similar size to the spaces between the bundles of microfibrils in the secondary cell wall [71]. Based on the findings of the current study, this size of pores can significantly limit mass transport. The various functional groups present in both membranes and lignin molecules can be charged at high pH levels, making electrostatic interactions between them possible.

When lignin is liberated and solubilized in the solution, it passes through the fiber wall into the lumen, where it is transferred into the bulk liquor through the large pits in the cell wall. Parts of the pit membranes dissolve during pulping since it is high in hemicelluloses. These pits are represented here by the large pore membranes (RC100 and RC200). Since the diffusion rate through the RC100 and RC200 membranes were

similar, we suggest that the diameters of the pores are much larger than the lignin molecules (or the possible clusters), therefore, they are not expected to hinder diffusion as significantly as the small pores.

5. Influence of molecular weight and alkalinity on kraft lignin diffusion

In Chapter 2, a short review of the effect of alkalinity on diffusion was provided. This chapter presents a more detailed investigation into the diffusion of lignin molecules, within a cellulose membrane, at different alkalinity levels. Specifically, fractionated kraft lignin was used to facilitate the study of diffusion of various lignin fractions through 50 kDa RC membranes.

5.1. Diffusion rates of the fractionated kraft lignin in different alkalinities

Diffusion cells were used to investigate the effect of alkalinity on the transport behavior of different lignin fractions. The concentration of the donor solution at the start of the experiment was 0.4 g L^{-1}. The concentration of the transported molecules in the acceptor chamber was quantified by UV/Vis absorbance. Figure 7 a, b, and c show the lignin concentration in the acceptor chamber versus time for fractionated lignin at three different alkalinity levels, 0.01 M, 0.1 M, and 1 M NaOH. Alkalinity and M_W had a significant effect on the diffusion rates, where the transport rate increased when the alkalinities in solutions increased and the M_W decreased. The percentage of transported lignin molecules across the membrane varies from less than 1% for the large M_W fraction (KL4 and KL5) to almost 10% for KL1 in 1 M NaOH. Therefore, the concentration difference between donor and acceptor, which is the driving force for diffusion, can be approximated to be constant. Equation 9 can be used to estimate the diffusion coefficients for the different cases, the results of which is found in Figure 7d. Here it can be seen that the diffusion coefficients of all fractions increase with higher concentrations of NaOH. Figure 7d also demonstrates that smaller molecular weight fractions have higher diffusion coefficients and a similar trend was seen in the diffusion coefficients obtained from diffusion NMR experiments. The diffusion NMR spectroscopy experiments support the increase in transport rate with a decrease of

M_W, where an increased M_W (thus increased hydrodynamic radius R_h), decreases the diffusion coefficient (see DOSY NMR spectroscopy results in Paper II).

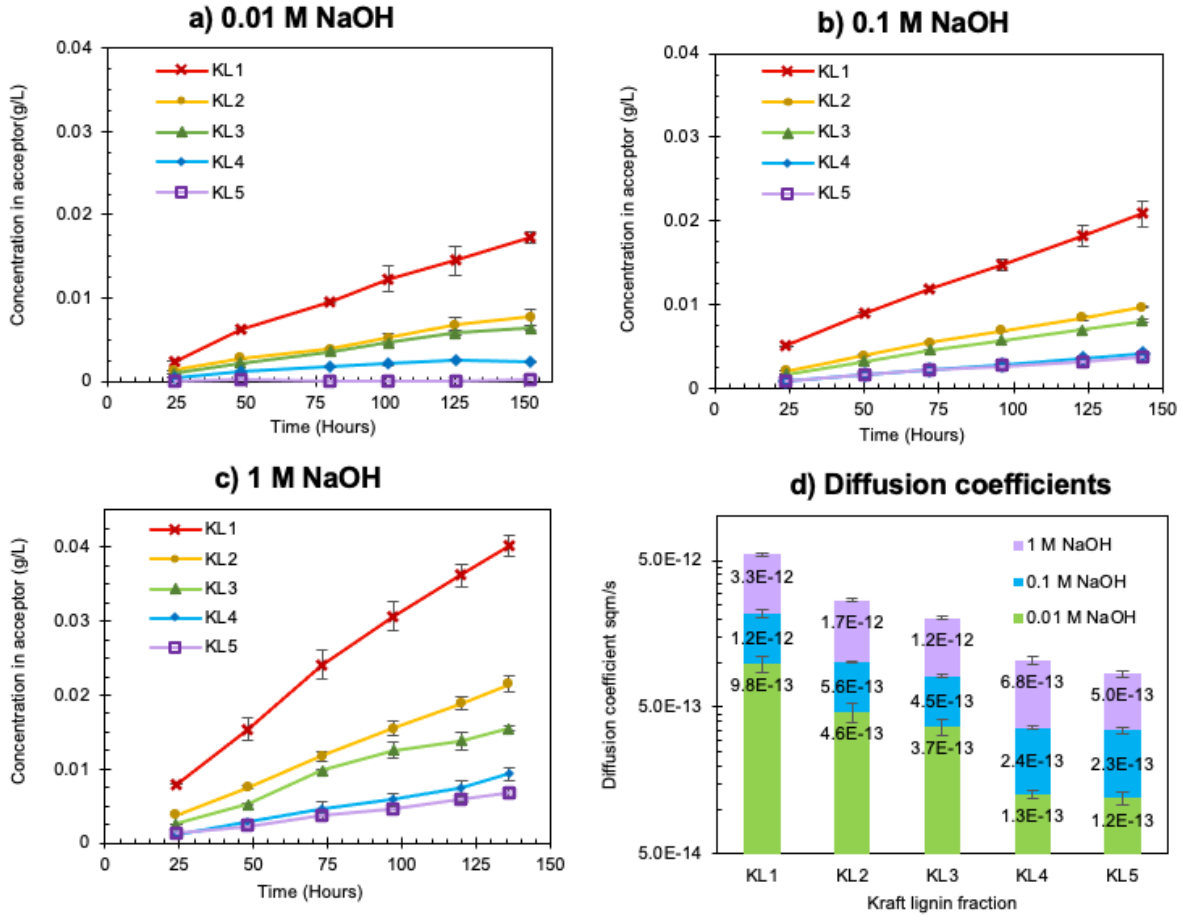

Figure 7 Concentration of lignin in the acceptor chamber versus time in the a) 0.01 M NaOH solution, b) 0.1 M NaOH solution, and c) 1 M NaOH solution, together with d) a summary of the fractionated diffusion coefficients of lignin measured from the diffusion cells. The concentrations were determined by UV/Vis absorbance according to a calibration curve for kraft softwood lignin in alkaline solutions.

It is slightly more challenging to understand why higher alkalinity levels increase the diffusion rates. Increasing the NaOH concentrations in the solution can affect the transport of different fractions through the membrane in several possible ways, one of which is changing the pore sizes of the membrane by affecting the swelling behavior of the cellulose. It is known that cellulose swells in aqueous solutions and the degree of alkalinity and ionic strength can affect its swelling [72]. The possible effect of changes in the swelling of cellulose on transport rates was examined by water

diffusivity measurements. The water diffusivities through 50 kDa MWCO membranes in different alkaline solutions were presented in Chapter 3, Section 3.2. The water diffusivity of the membranes increases about 35% by increasing the concentration of NaOH from 0.01 M to 1 M (see Figure 2). However, depending on the kraft lignin fraction, the diffusion increased between 300-500% when alkalinity increased from 0.01 M to 1 M (Figure 7). This suggests that the increase in diffusivity of the membranes due to alkalinity cannot solely be responsible for the significant increase in the rate of lignin transport through the membrane. The question of whether the changed NaOH concentration can affect the lignin molecules and their interactions, either with each other or with the membrane, remains to be answered. To investigate this, we studied the SEC chromatograms of acceptor samples in different alkalinities as well as their UV/Vis absorbance spectra.

5.2. Molecular weight analysis of the acceptor samples

Samples taken from the acceptor side of the diffusion cells were analyzed by SEC in highly alkaline solutions. For calibration of the SEC system, low M_w PEG standards were used. As mentioned before, the determination of the M_W values of lignin molecules is challenging since the available standard samples for calibration are not true representations of lignin molecules [73]. Similar to Section 4.2., the discussions in this section are based on the shape of the chromatograms instead of the M_W values. Figure 8 a, c, and e show the SEC chromatograms of the donor samples and Figure 8 b, d, and f show the SEC chromatograms of the acceptor samples of different fractions at three levels of alkalinity. The overall shapes of the chromatograms change significantly by increasing the alkalinity from 0.01 M NaOH to 1 M NaOH.

The shape of the chromatograms of the lignin in the acceptor chambers show some similarities but the intensity of different peaks varies with alkalinity and size fraction. Furthermore, for the membrane used in these experiments, the onset of the peaks of the lignin in the acceptor chambers starts between approximately 55 and 60 min, depending on size fraction and alkalinity. If this is compared with the onset of the

peaks of the lignin in the donor chamber, it can be concluded that the largest lignin molecules are not passing through the cellulose membrane in a measurable content. For KL1 (the fraction with the smallest molecules), it is only a small fraction that does not pass through the membrane in measurable contents but, for KL5, it may be 40 - 50% of the size fraction that does not pass through the membrane in measurable contents.

One difference in the chromatograms of different alkalinities is the peak at R_T ~74 min, which is present for all fractions in 0.1 and 1 M NaOH but not in 0.01 M NaOH samples. This peak represents small molecules of about 0.5 kDa, according to the PEG standards. The 74 min peak is more pronounced for KL 5 in 1 M NaOH (Figure 8c) and can possibly be the result of some degradation products due to a long storage time. However, since the diffusion experiments are performed at room temperature, the rate of degradation of lignin in high alkalinity should be low, therefore, the formation of small molecules due to breakage of the native bonds in KL5 is not probable. The reason for the appearance of the large 74 min peak is, therefore, not yet fully understood.

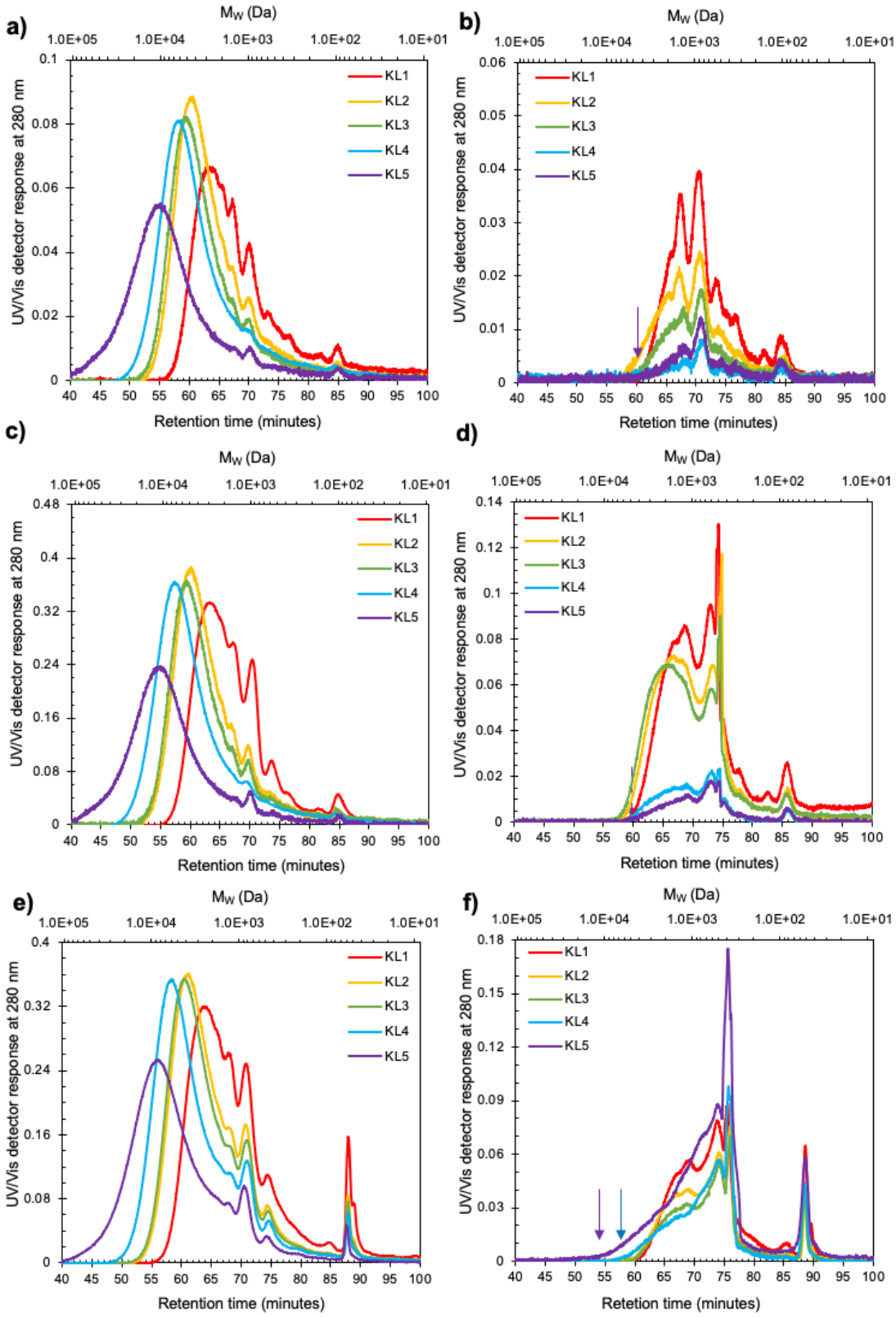

Figure 8 SEC chromatograms of the acceptor samples of different fractions after 168 h in a) 0.01 M NaOH donor, b) 0.01 M NaOH acceptor, c) 0.1 M NaOH donor, d) 0.1 M NaOH acceptor, e) 1 M NaOH donor, and f) 1 M NaOH acceptor. The secondary x-axis above the graphs shows the M_W values calculated based on a calibration curve from PEG standards.

For KL1, the onset of the peak is around 60 min R_T for all alkalinities, however, the intensity of the peak is much lower in the 0.01 M NaOH. KL2, another low M_W fraction, shows a similar trend. However, for the large M_W fractions, such as KL4 and KL5, the onset of the peaks in the chromatograms is shifted toward lower R_T values (meaning higher M_W values) when alkalinity is increased (see Figure 9 for a better illustration). For example, the onset of the peaks for KL5 in 0.01 M NaOH is at around 60 min, while in 1 M NaOH it is around 54 min. This indicates that higher alkalinity levels facilitate the transport of high molecular weight molecules.

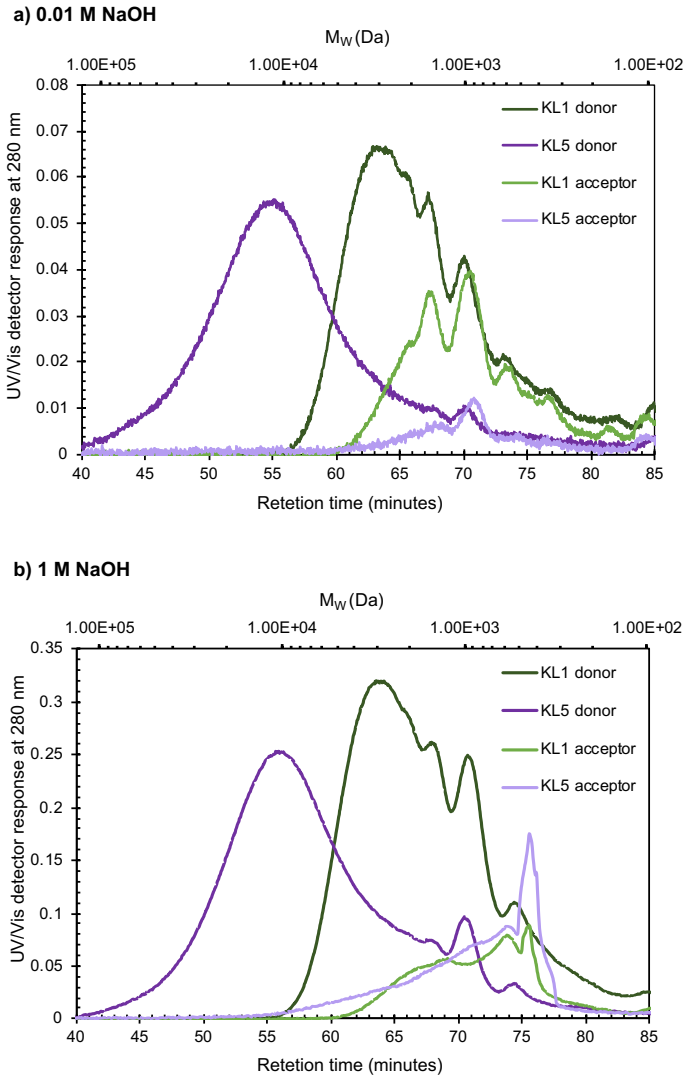

Figure 9 Comparison between SEC chromatograms of donor and acceptor samples for KL1 and KL5 in the a) 0.01 M NaOH solution and b) 1 M NaOH solution. The secondary x-axis above the graphs shows the M_W values calculated based on a calibration curve from PEG standards.

How can high alkalinity affect the transport of larger molecules over the membrane? One possible answer lies in the dissociation of phenolic hydroxyls at high pH levels. When the phenols get charged in high alkalinity, they can have negative charges on their surfaces, inducing repulsive forces between them and the other negatively charged molecules. In other words, high alkalinity can reduce the association between the kraft lignin molecules and prevent clusters from forming, as explained in Chapter 2. This is supported by the lower apparent average M_W values of the fractionated softwood kraft lignin molecules in 1 M NaOH (the donor samples), as presented in Table 2.

The number of charges on the molecules of different fractions can be different, due to both the number of available phenolic groups and the different pK_a of the phenolic groups that changes with M_W. As the M_W increases, the pK_a value for phenolic groups also increases [37], [74]. For example, the pK_a value for coniferyl alcohol was reported as 10.25 at room temperature, while for a fraction of lignin with a M_W of about 20 kDa, the pK_a is about 11. Furthermore, dissociation curves for larger M_W fractions are less steep, which is a consequence of the available phenolic groups and how they can get charged. This means that the fraction of ionized molecules increases less sharply with increasing pH. The dissociation degree at pH 12 for the higher M_W fraction (M_W = 8 kDa) was reported at about 0.85, while for coniferyl alcohol it is close to 1 [37]. Therefore, at lower alkalinity levels (0.01 M NaOH), lignin would have fewer charges compared to at higher alkalinity levels (*e.g.* 1 M NaOH). This effect is more pronounced in larger molecules, which depends on the number of available phenolic groups and their pK_a values. The partially charged kraft lignin molecules may form loose associations, which can lower their diffusion rates, while at high levels of alkalinity the association of the kraft lignin molecules would be less probable. This effect can be more pronounced for large molecular weight fractions, such as KL4 and KL5.

Another possible explanation why alkalinity could increase the transport rate through the membranes is a change in the membrane-lignin interactions. The RC membranes

contain hydroxyl functional groups. In highly alkaline conditions, these groups can be negatively charged, however, the pK_a value for the hydroxyl groups in cellulose is high (above 13.5) [75]. This indicates that in 1 M NaOH solution, some of these hydroxyl groups can be negatively charged and repulsive forces can arise between the membrane and the negatively charged molecules. However, the effect of high pH on the membrane and its influence on the diffusion of lignin molecules through the pores is not fully understood.

So far, the data from the SEC analysis has indicated a significant difference between samples from the acceptor side and donor side. More information about the differences between donor and acceptor chromatograms is provided in Paper II.

5.3. Chemical differences in the fractionated lignin transported through the membrane of diffusion cells

To examine the chemical differences between the lignin molecules that passed through the membrane and the donor samples, UV/Vis absorbance spectra were compared at different alkalinities. Figure 10 shows scaled (at 300 nm) UV/Vis absorbance spectra for the donor and acceptor samples of KL1 and KL5 samples collected from the diffusion cells. For a more straightforward interpretation, only KL1 and KL5 are presented here since these are the fractions that are the most different from each other. More details on the UV/Vis spectra for all fractions can be found in Paper II. There is a significant difference between the UV/Vis spectra for the acceptor and donor samples. There is a particularly prominent peak at around 350 nm in all acceptor samples, while the donor spectra display only slightly higher absorbance around 350 nm. The 350 nm peak resembled the corresponding peak seen in the small pore membrane acceptor samples in Chapter 4 and Paper I. In acceptor samples, a shoulder at around 320 nm is also seen. At the two highest NaOH concentrations, *i.e.*, 0.1 and 1 M NaOH, the peak in the donor spectra moves from 280 nm to 300 nm, which is more pronounced for the higher M_W fractions, such as KL5. As previously mentioned in Chapter 4, the conjugated phenolic and dissociated aromatic carboxylic acid groups

absorb at around 350 nm [65]. Aromatic carboxylic groups and α-carbonyl groups can absorb at 320 nm, which can account for the observed shoulder at 320 nm [65].

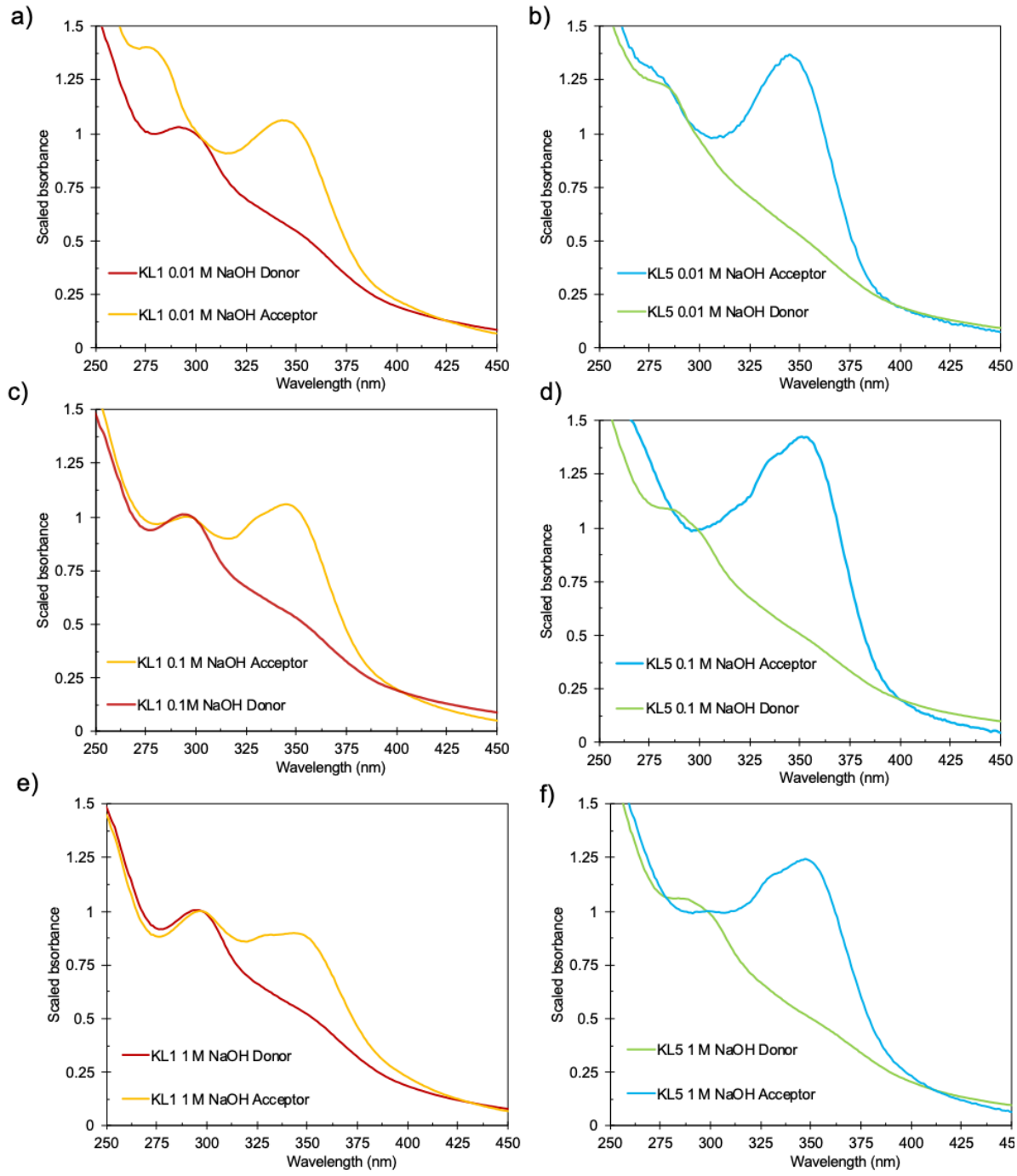

Figure 10 Scaled UV/Vis absorbance spectra of the donor (at the start) and acceptor (after 168 h) samples of a) KL1 in 0.01 M NaOH, b) KL5 in 0.01 M NaOH, c) KL1 in 0.1 M NaOH, d) KL5 in 0.1 M NaOH, e) KL1 in 1 M NaOH, and f) KL5 in 1 M NaOH.

The simultaneous increase in absorbance at 320 nm and 350 nm is indicative of the formation of quinone from ionization of the carbonyl-conjugated phenolates, which is seen when the alkalinity is 0.1 or 1 M NaOH.

The 350 nm peak in the acceptor solution indicated a higher concentration of conjugated structures than unconjugated ones, in contrast to the spectrum for the donor solution. One of the potential reasons is that the conjugated structures are common in smaller molecules, such as lignin monomers or oligomers. Naturally, since these molecules have higher diffusion coefficients, they can diffuse faster through the membrane than larger molecules. As a result, they are found in higher amounts in the acceptor solution than in the donor solution. The SEC results showed that, in the case of the low molecular weight fractions, KL1 and KL2, the molecular weight distribution of species in the acceptor samples were more similar to the donor samples. For KL5 and KL4, the SEC chromatograms of the acceptor sample instead showed that many of the large molecular weight molecules could not pass through the membrane. However, the UV/Vis spectra of both KL1 and KL5 donor and acceptor samples showed a significant differences, indicating that perhaps the chemical difference between the donor and acceptor is not solely a consequence of a size difference between donor and acceptor. The chemical difference between donor and acceptor could be a result of sorption of lignin on cellulose, for example. However, with the current results, this is only speculation and more experiments are needed to get more information on this.

6. Conclusion and future remarks

In this thesis, the diffusion cell methodology was shown to be a promising method to study the diffusion of solubilized lignin fragments through cellulose membranes. The studies on diffusion of lignin through membranes with various pore sizes showed that, when membrane pores were of a few nanometers in size, only lignin molecules with lower molecular weight were allowed to pass through, whereas large pore membranes allowed most of the lignin molecules/clusters to pass through. The influence of alkalinity of the solution on diffusion of lignin was also studied. The results showed that a higher alkalinity in solution can significantly increase the transport rate of all molecules, for which there may be several reasons: i) changes in the structure of the membrane, due to an increased concentration of NaOH, can affect the pore sizes of the membranes, also indicated by the membrane water diffusivity experiments, ii) association between lignin molecules can occur, which is affected by the alkalinity of the solution. More molecules can pass through the membrane as a result of lower association amongst them, which would increase the diffusion rates, and iii) smaller molecules, which can diffuse faster through the membrane, may form due to slow degradation of kraft lignin molecules at high alkalinity.

This study shows that the diffusion of lignin molecules through cellulose membranes is highly dependent on alkalinity, and pore sizes. However, several other interesting aspects, pertaining to the diffusion of kraft lignin through cellulose membranes, are worth investigating. For example, the diffusion of lignin through cellulose pores can also be affected by sorption of lignin onto cellulose fibers. It would be of interest to perform surface plasmon resonance experiments to investigate sorption of kraft lignin onto the cellulose membranes. Mass transfer of lignin through cellulose fibers may also be affected by temperature, ionic strength of the solution, and presence of hemicelluloses in the solution. These experimental conditions can be implemented into the diffusion cell methodology used here and their effects on diffusion of lignin can thereby be investigated.

7. Acknowledgments

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