Molecular Weight Distribution of Lignin

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Importance of Molecular Weight Distribution of Lignin

- Lignin is an important renewable resource and it is the second most abundant biopolymer after cellulose.
- Lignin is an amorphous biopolymer created by oxidative coupling of monolignol(s) (i.e., *p*-coumaryl alcohol, coniferyl alcohol, and/or sinapyl alcohol).[1]
- Due to their very complex structure, lignin has had historically limited industrial use so it is important to understand fundamental properties of lignin to find new value-added applications.
- Molecular weight distribution of lignin is one of the principle properties to study so as to understand the reactivity and physicochemical properties of lignin.[2]
- Gel Permeation Chromatography is a versatile tool used for determining molecular weight distribution of lignin.[3]

1. Nagy, M., *Ph.D. Thesis*, Georgia Institute of Technology, **2009**.; Brunow G, Lundquist K, Gellerstedt G. Lignin; Analytical Methods in Wood Chemistry. In: Pulping, and Papermaking. Sjostrom E (ed.), p. 77-124. Berlin: Springer, **1999**.
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3. Cao, S., Pu, Y., Studer, M., Wyman, C., Ragauskas, A.J. *RSC Adv*., **2012**, 2, 10925.; . Chen, F., Li, J., *J. Wood Chem. Technol*., **2000**, 20,3, 256.; Baumberger, S., Abaecherli, A., Fasching, M., Gellerstedt, G., Gosselink, R.,Hortling, B.,Li, J., Saake, B., de Jong, E. *Holzforschung*, **2007**, 61, 459.

Molecular Weight Distribution of Lignin

- The number average molecular weight (M_n) is defined by

$$M_n = \frac{\Sigma_i N_i M_i}{\Sigma_i N_i}$$

where N_i is the number of polymers chain, M_i is the molecular weight of a chain

- The weight average molecular weight (M_w) is defined by

$$M_w = \frac{\Sigma_i N_i M_i^2}{\Sigma_i N_i}$$

- Polydispersity (PD) is defined by

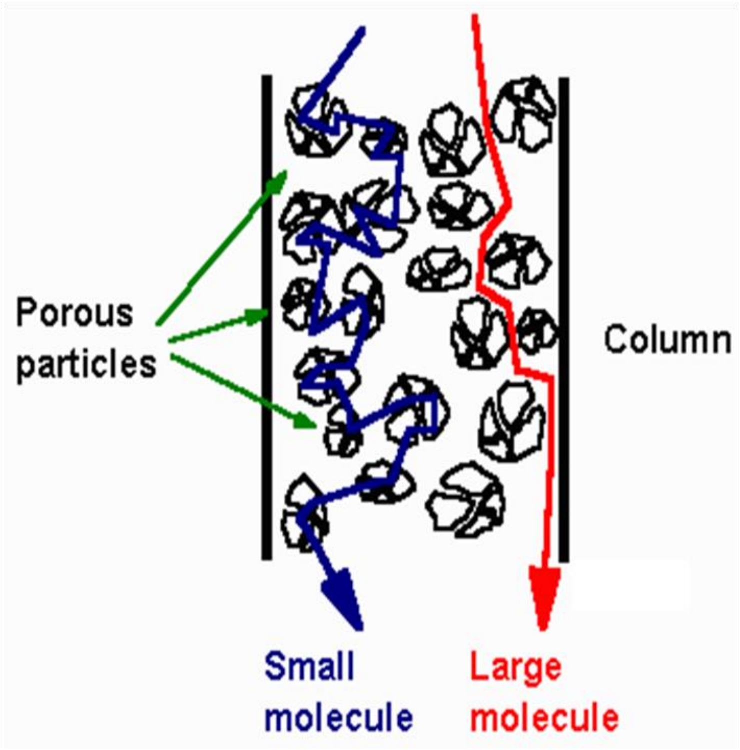
$$PD = \frac{M_w}{M_n}$$

Meunier D.M., *Handbook of Instrumental Techniques for Analytical Chemistry*, Molecular weight determinations, http://www.prenhall.com/settle/chapters/ch46.pdf

Principle of Gel Permeation Chromatography

- Gel Permeation Chromatography (GPC) is a type of Size-Exclusion Chromatography (SEC).
- Column filled with porous particles* which usually have pore size distribution close to the hydrodynamic volumes of polymers to be analyzed.
- Molecules in solution are separated by their size. Small molecule that can penetrate most pores will travel a longer distance and come out the column later, in contrast a large molecule that cannot penetrate into any pores will travel a shorter distance and come out earlier.

*Porous particle can be styrene-divinylbenzene copolymer gel, microgel, styragel etc.



Paul-Dauphin, S., Morgan,M-A, Herod, K. *Energy & fuels*, **2007**, *21, 6*, 3484.

Representative Lignin Sample Preparation for GPC

Lignin samples
(standard samples)

concentration(1mg/ mL),
≥5 mg,

Dissolve over night in solvent;
- Organic THF system
- Polar organic solvent system
- Aqueous system

25 mL vial

Filter

a hydrophobic PTFE membrane 45 μm filter

filter solution 20 μl

GPC sample vial

GPC

UV detector at 280 nm
Flow rate is ~1.00 mL/min.

Sannigrahi, P, Ragauskas, A. J., Miller, S. *Energy Fuels*, **2010**, 24, 683.

GPC System

- **Organic THF system** is applied for determining MW of derivatized lignin using THF as organic eluent. Lignin sample is require to be derivatization in order to increase the solubility in the eluent.[1]
- **Polar organic solvent system** is applied for determining MW of lignin by using polar organic solvent such as dimethysulfoxide (DMSO), dimethyformamide (DMF), and dimethylacetamide (DMAc). Lignin sample is not require to be derivatized. The association phenomena of lignin can be reduced by adding lithium salt.[2]
- **Aqueous system** is applied for strongly hydrophilic lignin. The sample is dissolved in an aqueous solvent. The aqueous media, pH and ionic strength strongly influence the elution exhibiting behaviors of polyelectrolyte.[3]

1. Gellerstedt, G, *Gel permeation chromatography*, in : C.W. Dence, S.Y. Lin (Eds.), Methods in Lignin Chemistry, Springer-Verlag, Heidelberg, **1992**, p.491.
2. Chum, H.L., Johnson, D.K., Agblevor, F.A., Evans, R.J., Hames, B.R., Milne, T.A., Overend, R.P. *Holsforschung*, **1987**, 41, 97.
3. Chen, F., Li, *J. Wood. Chem. Technol*., **2000**, 20, 265.

SEC-MALLS System

Size-exclusion chromatography and multi angle laser light scattering (SEC-MALLS) is a method applied for determining molecular weights distribution of lignosulfonate.[1,2] The intensity of the scattered light is proportional to the molecular weight. The weight average molecular weight (MW_w) can be calculated through the standard equations:

$$K_c/R_\theta = 1/MW_w + 2A_2c \quad (1)$$

$$K = 4\pi^2 n^2_0 (dn/dc)^2 N_A^{-1} \lambda_0^{-4} \quad (2)$$

where dn/dc is the refractive index increment

n_0 is the refractive index of the solvent

N_A is Avogadro's number

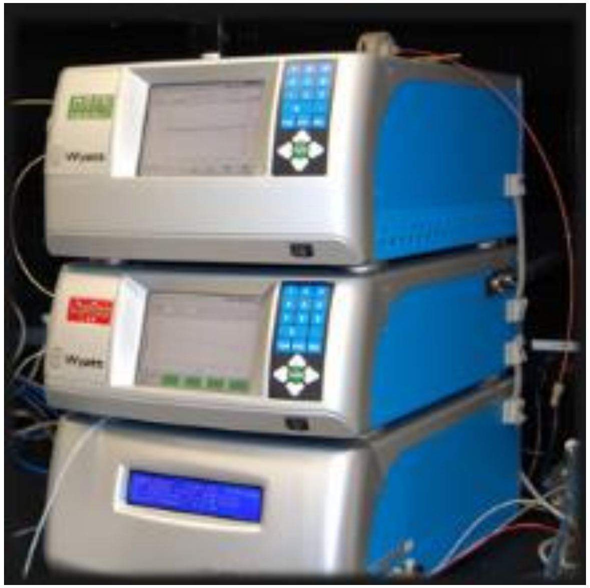
λ_0 is the wavelength of the incident light

A_2 is the second viral coefficient

c_i is the solute concentration

R_θ is Rayleigh excess ratio at an angle θ

K is an experimental constant



Source: http://www.york.ac.uk/media/biology/images/technologyfacility WyattDawnHELEOSII.jpg

1. Fredheim, G., Braaten, S., Christensen, B. *J. Chromatogr. A*, **2002**, 942, 191.
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GPC/SEC System of Lignin Sample

GPC/SEC systems	Typical Eluent	Lignin sample
Organic solvent	• THF	• Milled-wood lignin*, Organosolv*, Kraft lignin*, Steam explosion*[1]
Organic polar solvent	• DMSO/H_2O/0.05 LiBr or DMAc/0.11 M LiCl • DMF/0.2 M LiCl	• Lignosulfonates, Kraft lignin, Alkali lignin, Steam explosion[2] • Alkaline, Organosolv[3]
Aqueous	• Phosphate/SDS/DMSO • 0.01 M EDTA/0.05 M Na_2SO_4/acetonitrile • 0.3 M NaOH	• Lignosulfonates[4] • Lignosulfonates[4] • Kraft lignin[5]

SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid

* Derivatized

1. Faix, O., Beinhoff, O. *Holzforschung*, **1992**, 46, 355.; Kubo, S., Uraki, Y., Sano, Y. *Holzforschung*, **1996**, 50, 144.;Tring, R.W., Vanderlaan, M.N., Griffin, S.L. *J. Wood Chem. Technol*., **1996**, 16, 139.; Kubo, S., Uraki, Y., Sano, Y. *Holzforschung*, **1996**, 50, 144.; Baumberger, S., Lapierre, C., Montries, B. *J. Agric. Food Chem*., **1998**, 46, 2234.
2. Ringena, O., Lebioda, S., Lehnen, R., Saake, B. *J. Chromatogr.A*, **2006**, 1102, 154.
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Acetylation of Lignin: Acetic anhydride/Pyridine

- Lignin, approximately 20 mg, was dissolved in a 1:1 acetic anhydride-pyridine mixture (1.00 mL) and stirred for 24 hours at room temperature.
- Add ethanol (25.00 mL) to the reaction mixture, stirred for 30 minutes, and then removed with a rotary evaporator.
- Repeat the addition and removal of ethanol ~7 times to completely remove acetic acid and pyridine from the sample.

Work up A[1,2]

- The acetylated lignin was dissolved in chloroform and added drop wise to diethyl ether followed by centrifugation.
- Precipitated acetylated lignin was washed 3 times with ether then dried under high vacuum for 24 hours at 40°C.

Work up B[3]

- The acetylated lignin was dissolved in chloroform, washed 3 times with cold deionized water then dried with anhydrous magnesium sulfate.
- Filtered the mixture and used rotary evaporator to remove chloroform.

1. Hallac,B. *Ph.D.thesis*, Georgia Institute of Technology, **2011**.
2. Gellerstedt, G, *Gel permeation chromatography*, in : C.W. Dence, S.Y. Lin (Eds.), Methods in Lignin Chemistry, Springer-Verlag, Heidelberg, **1992**, p. 491.
3. Pu, Y., Anderson, L., Lucia, L., Ragauskas, A.J. *Pulp Paper Sci.*, **2003**, 29, 401.

Acetobromination of Lignin: Acetic acid/Acetyl bromide I

- Lignin, approximately 10 mg, was dissolved in a 92:8 (v/v) glacial (anhydrous) acetic acid and acetyl bromide mixture (2.5 mL) and stirred for 2 hours at 50 °C.
- Evaporate acetic acid and excess of acetyl bromide with a rotary evaporator connected to a high vacuum pump and a cold trap.
- The acetylated lignin was immediately dissolved in THF and injected to SEC.[1]

Acetobromination of Lignin: Acetic acid/Acetyl bromide II

- Lignin, approximately 10 mg, was placed in 2.3 mL of glacial (anhydrous) acetic acid and stirred for 15 minutes to 20 hours.
- Add acetyl bromide (0.25 mL) into the reaction mixture, stirred for 20 hours at room temperature.
- Evaporate acetic acid and excess of acetyl bromide with a rotary evaporator.
- Dry under high vacuum for 30-45 minutes at 25-30°C.[2]

1. Guerra, A., Lucia, L.A., Argyropoulos., D.S. *Holzforschung*, **2008**, 62, 24.
2. Asikkala, J., Tamminen, T., Argyropoulos, D.S. *J. Agric.Food Chem*., **2012**, 60, 8968.

Silylation

- Lignin, approximately 15 mg, was placed in THF (0.40 mL).
- Added distilled pyridine (0.04 mL) and N,O-bis(trifluorotrimethylsilyl)-acetamide (0.40 mL) into the reaction mixture.
- Stirred the reaction mixture for 24 hours at 21 °C under nitrogen atmosphere.
- Afterward, dry the sample by a rotary evaporator.

Pellinen, J., Salkinoja-Salonen, M. *J. Chromatogr*., **1985**, 328, 299.

Molecular Weight Distribution of Softwood Kraft Lignin

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Pine (Indulin AT)	ac: Ac_2O:Py, THF SEC	1600	6500[1]	4.06
Pine (Indulin AT)	ab: AcOH:AcBr, THF SEC	1700	8000[1]	4.71
Curan 100*	0.5 M NaOH, Aqueous SEC	799	11119	13.9[2]
Curan 100*	ac: Ac_2O:Py, THF SEC	1097	7643	7.0[2]
Pine+Spruce/ LignoBoost process	ac: Ac_2O:Py, THF SEC	1000	4500	4.5[3]
Pine	sil: BSA:Py, THF, HPSEC	1120	7480	6.69[4]
Pine	ac: Ac_2O:Py: THF, HPSEC	1010	7590	7.54[4]

*Trade name of 100% softwood kraft lignin pellets.

ac, acetylation; ab, acetobromination; Ac_2O, acetic anhydride; Py, pyridine; AcOH, acetic acid; AcBr, acetyl bromide; sil, silylation; BSA,N,O-bis(trifluorotrimethylsilyl)-acetamide; HPSEC, High-performance size-exclusion chromatography

1. Asikkala, J., Tamminen, T., Argyropoulos, D.S. *J. Agric.Food Chem*., **2012**, 60, 8968.
2. Baumberger, S., Abaecherli, A., Fasching, M., Gellerstedt, G., Gosselink, R.,Hortling, B.,Li, J., Saake, B., de Jong, E. *Holzforschung*, **2007**, 61, 459.
3. Brodin, I. *Licentiate Thesi*s, KTH Royal Institute of Technology, **2009,** 22**.**
4. Pellinen, J., Salkinoja-Salonen, M. *J. Chromatogr*., **1985**, 328, 299.

Molecular Weight Distribution of Softwood Kraft Lignin

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Spruce+Pine/ LignoBoost process	ac: Ac_2O:Py, THF SEC	1300	6400	5.0[5]
Spruce+Pine/ LignoBoost process	ac: Ac_2O:Py, THF SEC	1000	4470	4.5[6]
Curan 100*	DMSO/H_2O/0.05 M LiBr, Organic polar solvent SEC	1300	9900	7.6[7]
Curan 100*	DMAc/0.11 M LiCl, Organic polar solvent SEC	2000	11000	5.5[7]
Douglas Fir/ Acidification, dissc. in 0.1 M NaOH, concn. 1.0 g/L, 0.8 h	0.1 M NaOH, Aqueous SEC	3490	9680[8]	2.77
Douglas Fir/ Acidification, dissc. in 0.1 M NaOH, concn. 1.0 g/L, 388 h	0.1 M NaOH, Aqueous SEC	2930	6990[8]	2.39
Douglas Fir/ Acidification, dissc. in 0.1 M NaOH, concn. 1 g/L, 122 h then concn. 0.5 g/L, 1612 h	0.1 M NaOH, Aqueous SEC	2600	5390[8]	2.07

concn., concentration, dissc, dissociation

5. Norberg, I., Nordström, Y., Drougge, R., Gellerstedt, G., Sjöholm, E. *J. Appl. Polym. Sci.*, **2013**, DOI: 10.1002/APP.38588.
6. Brodin, I, Sjöholm, E., Gellerstedt, G. *Holzforschung*, **2009**, 63, 290.
7. Ringena, O., Lebioda, S., Lehnen, R., Saake, B. *J. Chromatogr.A*, **2006**, 1102, 154.
8. Sarkanen, S., Teller, D.C., Stevens, C.R., McCarthy, J.L. *Macromolecules*, **1984**, 17, 12, 2588.

Molecular Weight Distribution of Softwood Kraft Lignin

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Pinus sylvestris/ ECF bleaching system, ultrafiltration, O	0.1 M NaOH, Aqueous SEC	1740	9756[9]	5.61
Pinus sylvestris/ ECF bleaching system, ultrafiltration, EOP	0.1 M NaOH, Aqueous SEC	2091	7210[9]	3.45
Pinus sylvestris/ ECF bleaching system, ultrafiltratio, D_1	0.1 M NaOH, Aqueous SEC	1573	5033[9]	3.20
Pinus sylvestris/ ECF bleaching system, ultrafiltration, ED	0.1 M NaOH, Aqueous SEC	1068	5541[9]	5.19
Pinus sylvestris/ TCF bleaching system, ultrafiltration, O	0.1 M NaOH, Aqueous SEC	1780	10347[9]	5.81
Pinus sylvestris/ TCF bleaching system, ultrafiltration, Z_1	0.1 M NaOH, Aqueous SEC	593	1828[9]	3.08
Pinus sylvestris/ TCF bleaching system, ultrafiltration, Q_1	0.1 M NaOH, Aqueous SEC	5473	16648[9]	3.04
Pinus sylvestris/ TCF bleaching system, ultrafiltration, P_3	0.1 M NaOH, Aqueous SEC	3337	10149[9]	3.04

ECF, elemental chlorine-free; TCF, totally chlorine-free; O, oxygen delignification; EOP, pressurized alkaline extraction stage with addition of peroxide; D_1 last chlorine dioxide stage; ED, alkaline chlorine dioxide stage; Z_1, first ozone stage; Q_1, first chelating stage; P_3, third peroxide stage

9. Kukkola, J., Knuutinen, J., Paasivirta, J., Herve, S., Pessala, P., Schultz, E. *Environ Sci Pollut Res*, **2011**, 18, 1049.

Molecular Weight Distribution of Hardwood Kraft Lignin

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
PC-1369*	ac: Ac_2O:Py, THF SEC	1000	3300[1]	3.30
PC-1369*	ab: AcOH:AcBr, THF SEC	1000	3900[1]	3.90
Birch	0.1 M $NaNO_3$, Aqueous GPC	7523	19650	2.69[2]
Eucalyptus globulus/ LignoBoost process	ac: Ac_2O:Py, THF SEC	530	2300	4.3[3]
Birch/ LignoBoost process	ac: Ac_2O:Py, THF SEC	440	1600	3.6[3]
Eucalyptus grandis, kappa number 20/ Acidification	ac: Ac_2O:Py, THF HPSEC	1193	4980	3.7[4]
Eucalyptus grandis, kappa number 15/ Acidification	ac: Ac_2O:Py, THF HPSEC	1311	2865	2.2[4]

*Commercial hardwood kraft lignin

1. Asikkala, J., Tamminen, T., Argyropoulos, D.S. *J. Agric.Food Chem*., **2012**, 60, 8968.
2. Chen, F., Li, *J. Wood. Chem. Technol*., **2000**, 20, 3, 265.
3. Brodin, I. *LicentiateThesis*, KTH Royal Institute of Technology, **2009**, 22.
4. Nascimento, E., Morais, S., Machado, A. *J. Braz.Chem.Sec*., **1992**, 3, 3, 61.

Molecular Weight Distribution of Hardwood Kraft Lignin

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Birch+ Aspen/ LignoBoost process, Ultrafiltration	ac: Ac_2O:Py, THF SEC	780	1700	2.2[5]
Birch/ LignoBoost process	ac: Ac_2O:Py, THF SEC	440	1600	3.6[6]
Birch/Filtration (5 kDa), LignoBoost process	ac: Ac_2O:Py, THF SEC	320	980	3.1[6]
Birch/Filtration (15 kDa), LignoBoost process	ac: Ac_2O:Py, THF SEC	360	1100	3.1[6]
Eucalyptus globulus/ LignoBoost process	ac: Ac_2O:Py, THF SEC	530	2300	4.3[6]
Eucalyptus globulus/ Filtration (5 kDa), LignoBoost process	ac: Ac_2O:Py, THF SEC	440	1300	3.0[6]
Eucalyptus globulus/ Filtration (15 kDa), LignoBoost process	ac: Ac_2O:Py, THF SEC	550	1700	3.1[6]

5. Norberg, I., Nordström, Y., Drougge, R., Gellerstedt, G., Sjöholm, E. *J. Appl. Polym. Sci.*, **2013**, DOI: 10.1002/APP.38588.
6. Brodin, I, Sjöholm, E., Gellerstedt, G. *Holzforschung*, **2009**, 63, 290.

Molecular Weight Distribution of Softwood Lignosulfonate

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Spruce/ Ethanol-water Fraction, F40	Phosphate/DMSO/SDS, pH 10.5, SEC-MALLS	115000	398000	3.5[1]
Spruce/ Ethanol-water Fraction, F50	Phosphate/DMSO/SDS, pH 10.5, SEC-MALLS	18000	34000	1.9[1]
Spruce/ Ethanol-water Fraction, F70	Phosphate/DMSO/SDS, pH 10.5, SEC-MALLS	3200	4600	1.5[1]
Spruce/ Phenolation, Run 1	0.5 M NaOH, Aqueous GPC	88563	302000	3.41[2]
Spruce/ Phenolation, Run 9	0.5 M NaOH, Aqueous GPC	113693	578700	5.09[2]
Spruce/ Phenolation, Run 16	0.5 M NaOH, Aqueous GPC	129649	627500	4.84[2]

1. Fredheim, G., Braaten, S., Christensen, B. *J. Chromatogr. A*, **2002**, 942, 191.
2. Alonso, M.V., Oliet, M., Rodŕiquez, F, Garćia, J., Gilarranz, M.A., Rodŕiquez, J.J. *Bioresour. Technol.*, **2005**, 96, 1013.

Molecular Weight Distribution of Softwood Lignosulfonate

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Spruce/ Filtration, Ion exchange	Phosphate/DMSO/SDS buffer, pH 10.5, SEC-MALLS	3200	36000	11.2[3]
Spruce/ Ultrafiltration, Ion Exchange	Phosphate/DMSO/SDS buffer, pH 10.5, SEC-MALLS	5000	61000	12.3[3]
Spruce/ Ultrafiltration, Ion Exchange, pH 12-13, heat treat	Phosphate/DMSO/SDS buffer, pH 10.5, SEC-MALLS	5100	41000	8.2[3]
Pinus massion	0.1 M $NaNO_{3,}$ Aqueous GPC	8691	29038	3.36[4]
Pinus massion	0.1 M $NaNO_{3,}$ Aqueous GPC	3441	7082	2.05[4]

3. Braaten, S.M., Christensen, B. E., Fredheim, G.E. *J. Wood Chem. Technol*., **2003**, 23, 2, 197.
4. Chen, F., Li, J., *J. Wood Chem. Technol*., **2000**, 20, 3, 256.

Molecular Weight Distribution of Hardwood Lignosulfonate

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Mix hardwood	0.5 M NaOH, Aqueous SEC	786	3927	5.0[1]
Sulfurized birch kraft	0.1 M $NaNO_3$, Aqueous GPC	5455	9914	1.82[2]
Eucalyptus globulus	Phosphate/DMSO/SDS buffer, pH 10.5, SEC-MALLS	2200	6300	3.0[3]
Eucalyptus grandis	Phosphate/DMSO/SDS buffer, pH 10.5, SEC-MALLS	1900	5700	3.0[3]
Aspen	Phosphate/DMSO/SDS buffer, pH 10.5, SEC-MALLS	2200	12000	5.3[3]
Poplar	0.10 M $NaNO_3$, pH 8, Aqueous GPC	2400	9100	3.79[4]
Sulfonated kraft poplar	0.10 M $NaNO_3$, pH 8, Aqueous GPC	1000	2500	2.50[4]

1. Baumberger, S., Abaecherli, A., Fasching, M., Gellerstedt, G., Gosselink, R., Hortling, B.,Li, J., Saake, B., de Jong, E. *Holzforschung*, **2007**, 61, 459.
2. Chen, F., Li, *J. Wood. Chem. Technol*., **2000**, 20, 3, 265.
3. Braaten, S., Christensen, B., Fredheim, G. *J. Wood Chem. Technol*, **2003**, 23, 2, 197.
4. Zhou, H., Lou, H., Yang, D., Zhu, J.Y., Qui, X. *Ind. Eng. Chem. Res.,* **2013**, 52, 8464.

Molecular Weight Distribution of Hardwood Lignosulfonate

Lignin sample/ Isolation	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Beech/ Ultrafiltration Fraction, F1	DMSO/H_2O/0.05 M LiBr, Organic polar solvent SEC	15000	33000	2.2[5]
Beech/ Ultrafiltration Fraction, F3	DMSO/H_2O/0.05 M LiBr, Organic polar solvent SEC	5900	7700	1.3[5]
Beech/ Ultrafiltration Fraction, F5	DMSO/H_2O/0.05 M LiBr, Organic polar solvent SEC	650	1500	2.4[5]
Beech/ Ultrafiltration Fraction, F1	DMAc/0.11 M LiCl, Organic polar solvent SEC	9300	27000	2.9[5]
Beech/ Ultrafiltration Fraction, F3	DMAc/0.11 M LiCl, Organic polar solvent SEC	4800	6700	1.4[5]
Beech/ Ultrafiltration Fraction, F5	DMAc/0.11 M LiCl, Organic polar solvent SEC	490	970	2.0[5]

5. Ringena, O., Lebioda, S., Lehnen, R., Saake, B. *J. Chromatogr.A*, **2006**, 1102, 154.

Molecular Weight Distribution of Native Lignin

Lignin sample/ Isolation	Method	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Spruce/ Björkman process	Milled- wood	ac: Ac_2O:Py, THF SEC	1500	20000[1]	13.33
Spruce/ Björkman process	Milled- wood	ab: AcOH:AcBr, THF SEC	3000	10000[1]	3.33
Eucalyptus grandis	Milled-wood	ac: Ac_2O:Py, THF HPSEC	1960	8400	4.3[2]
Loblolly Pine/ Acidification	Wiley milled -wood	ac: Ac_2O:Py, THF GPC	7590	13500	1.77[3]
Spruce/ Enzymatic mild acidolysis	Ball milled-wood, day 1	ab: AcOH:AcBr, THF SEC	9260	55150	5.9[4]
Spruce/ Enzymatic mild acidolysis	Ball milled-wood, day 7	ab: AcOH:AcBr, THF SEC	9424	54500	5.7[4]
Spruce/ Enzymatic mild acidolysis	Ball milled-wood, day 25	ab: AcOH:AcBr, THF SEC	11120	97500	8.8[4]

1. Asikkala, J., Tamminen, T., Argyropoulos, D.S. *J. Agric.Food Chem*., **2012**, 60, 8968.
2. Nascimento, E., Morais, S., Machado, A. *J. Braz.Chem.Sec*., **1992**, 3, 3, 61.
3. Sannigrahi, P, Ragauskas, A. J., Miller, S.J. *Energy Fuels*., **2010**, 24, 683.
4. Guerra, A., Filpponen, I., Lucia, L.A., Saquing, C., Baumberger, S., Argyropoulos., D.S., *J. Agric. Food Chem*., **2006,** 54, 5939.

Molecular Weight Distribution of Native Lignin

Lignin sample/ Isolation	Method	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Spruce/ Cellulolytic enzyme	Vibratory milled- wood	ab: AcOH:AcBr, THF SEC	9450	53850	5.7[4]
Spruce/ Enzymatic mild acidolysis	Vibratory milled- wood, 2 h	ab: AcOH:AcBr, THF SEC	11000	95600	8.7[4]
Spruce/ Enzymatic mild acidolysis	Vibratory milled- wood, 28 h	ab: AcOH:AcBr, THF SEC	9500	71600	7.5[4]
Spruce/ Enzymatic mild acidolysis	Vibratory milled- wood, 96 h	ab: AcOH:AcBr, THF SEC	8600	56500	6.6[4]
Eucalyptus globulus/ Björkman process	Ball milled -wood	ab: AcOH:AcBr, THF SEC	2600	6700	2.6[5]
Eucalyptus globulus/ Cellulolytic enzyme	Ball milled -wood	ab: AcOH:AcBr, THF SEC	5500	17200	3.1[5]
Eucalyptus globulus/ Enzymatic mild acidolysis	Ball milled -wood	ab: AcOH:AcBr, THF SEC	8700	32000	3.7[5]

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Molecular Weight Distribution of Native Lignin

Lignin sample/ Isolation	Method	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Douglas Fir/ Björkman process	Ball milled -wood	ab: AcOH:AcBr, THF SEC	2500	7400	3.0[5]
Douglas Fir/ Cellulolytic enzyme	Ball milled -wood	ab: AcOH:AcBr, THF SEC	5500	21800	4.0[5]
Douglas Fir/ Enzymatic mild acidolysis	Ball milled -wood	ab: AcOH:AcBr, THF SEC	7600	38000	5.0[5]
White Fir/ Enzymatic mild acidolysis, Fresh	Ball milled -wood	ab: AcOH:AcBr, THF SEC	7700	57000	7.4[6]
White Fir/ Enzymatic mild acidolysis, Dissociated	Ball milled -wood	ab: AcOH:AcBr, THF SEC	2800	7500	2.7[6]
Eucalyptus globules/ Enzymatic mild acidolysis, Fresh	Ball milled -wood	ab: AcOH:AcBr, THF SEC	6500	23400	3.6[6]
Eucalyptus globules/ Enzymatic mild acidolysis, Dissociated	Ball milled -wood	ab: AcOH:AcBr, THF SEC	2890	8100	2.8[6]

6. Guerra, A., Lucia, L.A., Argyropoulos., D.S. *Holzforschung*, **2008**, 62, 24.

Molecular Weight Distribution of Lignin from Pretreatment Biomass

Lignin sample/ Isolation	Pretreatment	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Aspen	Steam explosion	0.5 M NaOH, Aqueous SEC	1028	14179	13.8[1]
Aspen	Steam explosion	0.1 M NaOH, Aqueous SEC	100	2200	22[1]
Alcell	Organosolv	0.5 M NaOH, Aqueous SEC	511	3959	7.7[1]
Alcell	Organosolv	ac: Ac_2O:Py, THF SEC	642	1582	2.5[1]
Aspen	Steam explosion	DMSO/H_2O/0.05 M LiBr Organic polar solvent SEC	1430	9830[1]	6.87
Olea Europaea	Autohydrolysis	DMF/ 0.1% LiBr, HPSEC	2120	3662	1.73[2]
Olea Europaea	Organosolv	DMF/ 0.1% LiBr, HPSEC	2252	12919	5.74[2]
Olea Europaea	Alkaline	DMF/ 0.1% LiBr, HPSEC	4925	10875	2.21[2]

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Molecular Weight Distribution of Lignin from Pretreatment Biomass

Lignin sample/ Isolation	Pretreatment	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Poplar/ Acidification	Autohydrolysis, 200 °C, 30 min residence time	ac: Ac_2O:Py, THF GPC	448	868	1.9[3]
	Autohydrolysis, 180 °C, zero residence time	ac: Ac_2O:Py, THF GPC	530	1421	2.7[3]
	Autohydrolysis, 150 °C, zero residence time	ac: Ac_2O:Py, THF GPC	1594	3507	2.1[3]
Aspen	Steam explosion	DMSO/H_2O/0.05 M LiBr, Organic polar solvent SEC	1500	43000	28.7[4]
Aspen	Steam explosion	DMAc/0.11 M LiCl, Organic polar solvent SEC	2200	35000	15.9[4]
Eucalyptus grandis	Organosolv	ac: Ac_2O:Py, THF HPSEC	1523	5600	3.7[5]
Loblolly Pine/ Acidification	Organosolv	ac: Ac_2O:Py, THF GPC	3070	5410	1.77[6]

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Molecular Weight Distribution of Lignin from Pretreatment Biomass

Lignin sample/ Isolation	Pretreatment	GPC/SEC Conditions	M_n (g/mol)	M_w (g/mol	PD
Miscanthus x giganteus	Mild formosolv	ab: AcOH:AcBr, THF SEC	1301	3643	2.8[7]
Miscanthus x giganteus	Basic organosolv	ab: AcOH:AcBr, THF SEC	753	2051	2.7[7]
Miscanthus x giganteus	Cellulolytic enzyme	ab: AcOH:AcBr, THF SEC	751	1959	2.6[7]
Birch	Explosion	sil: BSA:Py, THF, HPSEC	2160	12110	5.60[8]
Birch	Explosion	ac: Ac_2O:Py, THF HPSEC	1440	9210	6.38[8]
Poplar	Explosion	sil: BSA:Py, THF, HPSEC	1340	5200	3.88[8]
Poplar	Explosion	ac: Ac_2O:Py, THF HPSEC	850	3300	3.89[8]

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