# Optimisation of the process of abies ethanol lignin sulfation by sulfamic acid – urea mixture in 1,4-dioxane medium

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### Abstract

The new process of abies ethanol lignin sulfation by a low-toxic mixture of sulfamic acid and urea in 1,4-dioxane medium was optimized and the structure of sulfated ethanol lignin was studied. The process of lignin sulfation is described by a first-order equation in the temperature range 70-100˚C. The value of the rate constants are weakly dependent on the ratios of lignin and sulfating complex (sulfamic acid-urea mixture). The activation energy of the sulfation process decreaseses from 95 kJ / mol to 24 kJ / mol with an increase in the content of the sulfating complex (SC). The optimal conditions for sulfation of abies ethanol lignin with a high yield of water-soluble sulfated lignin ((100% of mass) with sulfur content of 7.9%) where found: temperature 95–100°C, L / SC ratio 1: 2,3- 1:2,9, time 2h. The composition and structure of water-soluble sulfated ethanol lignin were proven by elemental analysis, FTIR spectroscopy, two-dimensional NMR spectroscopy and gel permeation chromatography. It was shown that only alcoholic OH-groups of ethanol lignin react with sulfamic acid. Sulfated ethanol lignin has a higher molecular weight and a lower degree of polydispersity compared to the initial ethanol lignin.

Keywords: abies ethanol lignin , sulfation, sulfamic acid, urea, sulfated lignin, structure, FTIR, NMR, GPC.

### Hightlights

1. New process of abies ethanol lignin sulfation by a low-toxic sulfamic acid was optimized.

2. Kinetic studies of lignin sulfation process have been carried out in the temperature range 70-100˚C.

3. Composition and structure of sulphated ethanol lignin were proven by FTIR, 2D NMR, GPC, elemental analysis.

4. Sulfamic acid reacts only with alcoholic OH-groups of ethanol lignin.

5. Sulfated ethanol lignin has a higher molecular weight and a lower degree of polydespersity compsred to ethanol lignin.

### Introduction

Lignocellulosic biomass contains from 20 to 30 % mas of lignin, which is an aromatic biopolymer (Heitner C. et al 2010). Lignin macromolecules consist of three basic phenylpropane units, which are derived from aromatic alcohols, namely n-coumaryl, coniferyl and sinapyl alcohols.

Lignins isolated from lignocellulosic biomass by various methods differ in their composition and properties. In delignification processes, lignin undergoes significant structural changes and the reactivity of isolated lignins may differ significantly from that of native wood lignins (Calvo-Flores FG et al. 2015).

Due to its aromatic structure, lignin is a renewable source of raw materials for large-scale production of phenolic and aromatic chemicals, as well as liquid fuels (Zouohua S et al. 2018). The current ways of chemical processing of wood are mainly aimed at producing cellulose, while lignin remains a large-tonnage waste of the pulp

and paper industry, as well as hydrolysis industry. Till now the effective methods of lignin utilization are not available.

In recent years, there have been developed new methods of producing cellulose, based on the processes of organosolvent cooking in the medium of various organic solvents, such as methanol, ethanol, acetic and formic acids (Bajpai P 2010; Kangas H et al. 2015). One of the main advantages of organosolv delignification processes is their higher environmental safety as compared to sulfate and sulfite technologies. Organosolv lignins do not contain sulfur, have a rather narrow molecular weight distribution and a relatively small molecular mass (Torre MJ et al. 2013; Lindner A et al. 1988; Kuznetsov BN et al. 2016). They are chemically more active than technical lignins; they have high solubility in organic solvents, which facilitates their further chemical processing.

Nowadays, researchers are looking for new, more effective ways of utilizing lignins, as well as new areas for their application (Schutyser W et al. 2018; Agrawal A et al. 2014; Gilca IA et al. 2014). In many cases, the usefull biological activity of lignin derivatives is connected with their antioxidant activity, antiviral and antitumor potential (Vinardell MP, Mitjans M 2017)

One of the promising area of lignin processing is to obtain derivatives containing a sulfate groups, which increase the ability of lignin to dissolve in water and its biodegradability (Prinsen P et al. 2017). Sulfated lignin derivatives could not only replace widely used products of polysaccharides chemical modification, but also be used in pharmaceuticals as potential antiviral drugs and as a new class of anticoagulants (Raghuraman A  et al. 2007; Henry BL et al. 2014; Thakkar JN 2006; Raghuraman A et al. 2005).

The common methods of biopolymers sulfation use the aggressive and toxic sulfating reagents (sulfuric and chlorosulfonic acid, oleum, sulfuric anhydride and its complexes with pyridine and other toxic amines) (Chen G et al. 2013; Qin Z et al. 2014). A possible alternative to the abovementioned reagents is sulfamic acid, which is a stable, non-hygroscopic crystalline substance produced on an industrial scale by reaction of urea with oleum (Al-Horani RA, Desai UR 2010). Unlike traditional sulfating agents, sulfamic acid is low-toxic and non-corrosive compound.

Earlier, we have proposed a new method of abies ethanol lignin sulphating by low-toxic mixture sulfamic acid-urea (Vasilyeva NYu et al. 2018; Kuznetsov BN et al. 2018). In the present work this new process of abies ethanol lignin sulfation was optimized and the structure characteristics of the sulphated lignin were established using FTIR spectroscopy, two-dimensional NMR spectroscopy and gel permeation chromatography (GPC).

**Materials and methods**

Siberian abies wood (*Ábies sibírica*) containing 45.7% cellulose; 25.3% lignin; 17.7% hemicellulose; 6.2% extractives; 0.5% ash (in percentage, calculated based on the mass of absolutely dry wood) was used for ethanol lignin isolation. The wood was ground to particles with the size of less than 1 mm; after that the particles were deresinated by hexane (in accordance with the standard ANSI/ASTM D 1105 method) and then dried at 80°C up to moisture content of less than 1% of mass.

The deresinated air-dry sawdust was ground on a BP-2 shaker. Isolation of ethanol lignin  from wood was carried out by extraction with ethanol – water mixture at a temperature of 185°C and subsequent precipitation with cold water according to the method described in (Kuznetsov BN et al. 2018). The yield of ethanol lignin  was 9.5% of wood mass (30% of the initial lignin content in wood).

The sulfation of ethanol lignin was carried out in a three-neck flask (150 ml) equipped with a reflux condenser, a thermometer and a mechanical stirrer to add 1.25 g of ethanol lignin  to a mixture of sulfamic acid and urea (sulfating complex) in 15 ml of 1,4-dioxane heated to 80–100°C. The mixture was intensively stirred at a

temperature of 80–100°C for 1–3 hours. Then the reaction mixture was cooled to room temperature, the solvent was decanted, and the remaining solid product was dissolved in a small amount of water and neutralized with aqueous ammonia to pH 8. To remove the excess of reactants, the product was dialyzed against water in a plastic bag of MF-503-46 MFPI brand (USA) with a pore size of 3.5 kDa during for 8-10 hours. The water was changed every hour. After dialysis, an aqueous solution of sulfated lignin was evaporated in vacuum on a rotary evaporator to obtain a solid residue – sulfated lignin in the form of an ammonium salt.

Elemental analysis of sulfated lignin was performed on a FlashEA-1112 elemental analyzer (ThermoQuest, Italia).

The sulfur content in sulfated ethanol lignin was determined by burning the sample in a stream of oxygen at a temperature of 1000°C, followed by absorption of the combustion products by a 6% aqueous solution of hydrogen peroxide. The resulting sulfuric acid was titrated with 0.01 N sodium hydroxide solution with methyl red as an indicator.

The yield of water-soluble sulfated ethanol lignin (% of mass) was determined as the ratio of the mass of sulfated lignin obtained after purification to the mass of the initial lignin multiplied by 100%.

The FTIR spectra of initial lignin and sulfated lignin were recorded using a Tensor-27 FT-IR spectrometer (Bruker, Germany) within the wavelength range of 400–4000cm$^{-1}$. The spectral information was analysed using the OPUS program (version 5.0). Solid samples for analysis were prepared in the form of pills in a KBr matrix (2 mg sample/1000 mg KBr).

Two-dimensional HSQC NMR spectra were registered at 25°C on a Bruker AVANCE III spectrometer (600 MHz (1H) and 155 MHz (13C)). About 80 mg of lignin in 1.0 ml of deuterated dimethyl sulfoxide was set to dissolve for at least 12 hours. The number of spectrum accumulations was 16 and 128 time increments overall. The interscan relaxation delay was set to 5 s. A solvent peaks was used as an internal reference ($\delta$13C 40.1; $\delta$1H 2.5). The cross-signals of the HSQC spectra were interpreted according to literature data (Zeng J et al. 2013).

The average molecular weight (Mw), average molecular mass (Mn) and polydispersity of sulfated ethanol lignin samples were defined by gel permeation chromatography using an Agilent 1260 Infinity II Multi-Detector GPC/SEC System chromatograph with triple detection: by a refractometer (RI), by a viscometer (VS) and by a light scattering (LS). The separation was made on two Aquagel-OH Mixed-M columns using the solution $0.2MNaNO_3$ + $0,01MNaH_2PO_4$ in water (pH = 7) as the mobile phase. The column was calibrated using polydisperse polyethylene glycol standards (Agilent, USA). The feed rate of the eluent was 1 ml / min, the volume of the used sample was 100 μl. Before analysis, the samples were dissolved in the mobile phase (1 mg / ml) and filtered through a 0.45 μm PTFE membrane filter (Millipore). Data collection and data processing were performed using Agilent GPC / SEC MDS software.

Numerical optimization of the process of abies ethanol lignin sulfation by sulfamic acid was carried out with the use of Statgraphics Centurion XVI software (Design of Experiment) as described in (Sudakova IG et al. 2013).

**Results and discussion**

1. Impact of the conditions of abies ethanol lignin sulfation on the tield of sulphated lignin and sulphur content

We have suggested to use the low-toxic and non-corrosive mixture at sulfamic acid-urea for sulfation of abies ethanol lignin (Vasilyeva NYu et al. 2018; Kuznetsov BN et al. 2018). The sulfamic acid sulfates only alcohol

OH-groups (Al-Horani RA, Desai UR 2010). Whereas the use of traditional sulfating agents, like sulphuric anhydride or its complexes can result in sulphating also phenolic hydroxyl groups (Raghuraman A et al. 2005).

The reactivity of sulfamic acid is increased in the presence of basic organic catalysts, such as pyridine, urea, thiourea, acetamid and picoline (Al-Horani RA, Desai UR 2010; Levdansky AV, et al. 2014, Kuznetsov B, Levdansky V et al. 2018). The urea is the most effective catalyst. The increase in the reactivity of sulfamic acid in the presence of urea is explaned by the formation of a donor-acceptor complex.

The scheme of sulfation of lignin structure units by sulfamic acid urea mixture in 1,4-dioxane medium is shown in Fig. 1. Sulfated lignin was isolated as the ammonium salt.
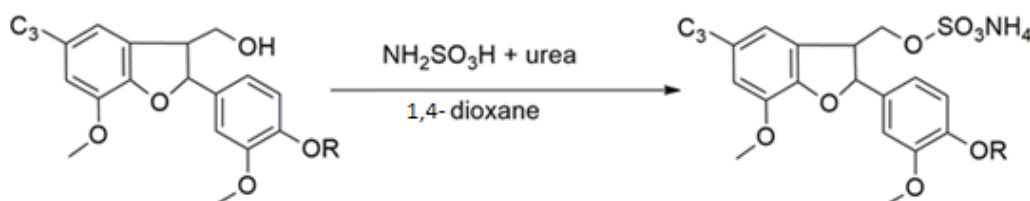


**Fig. 1** Scheme of the sulfation of a lignin structural unit (phenyl coumaran) by a mixture of sulfamic acid and urea

The impact of the conditions of abies ethanol lignin sulfation (temperature, time of the process, ratio of ethanol lignin: sulfating complex) on the yield of sulfated ethanol lignin and sulfur content was studied (Table 1, Fig. 2).

**Table 1** The effect of the conditions of abies ethanol lignin sulfation on the yield of sulfated lignin and sulfur content

| No | Ratio L:SC | Temperature, °C | Time, min | Yield, % mass | Sulfur content, % mass |
|----|-----------|-----------------|-----------|---------------|------------------------|
| 1  | 1:1.2 | 70  | 90  | 37.2  | 4.4 |
| 2  | 1:1.2 | 80  | 180 | 48.1  | 5.1 |
| 3  | 1:1.2 | 90  | 90  | 62.1  | 5.6 |
| 4  | 1:1.2 | 90  | 180 | 85.3  | 6.3 |
| 5  | 1:1.2 | 100 | 90  | 69.8  | 5.7 |
| 6  | 1:1.2 | 100 | 180 | 96.3  | 6.5 |
| 7  | 1:1.5 | 70  | 90  | 60.7  | 4.9 |
| 8  | 1:1.5 | 70  | 180 | 66.1  | 5.7 |
| 9  | 1:1.5 | 80  | 90  | 58.5  | 5.4 |
| 10 | 1:1.5 | 80  | 180 | 68.1  | 6.7 |
| 11 | 1:1.5 | 90  | 90  | 82.4  | 5.9 |
| 12 | 1:1.5 | 90  | 180 | 98.6  | 7.3 |
| 13 | 1:1.5 | 100 | 90  | 88.7  | 6.3 |
| 14 | 1:1.5 | 100 | 180 | 104.1 | 7.6 |
| 15 | 1:2.0 | 70  | 90  | 69.5  | 5.5 |
| 16 | 1:2.0 | 70  | 180 | 78.1  | 6.6 |
| 17 | 1:2.0 | 80  | 90  | 84.5  | 6.1 |
| 18 | 1:2.0 | 80  | 180 | 93.1  | 7.4 |
| 19 | 1:2.0 | 90  | 90  | 95.2  | 6.4 |
| 20 | 1:2.0 | 90  | 180 | 103.1 | 7.7 |
| 21 | 1:2.0 | 100 | 90  | 99.1  | 7.0 |
| 22 | 1:2.0 | 100 | 180 | 103.5 | 7.9 |

| 23 | 1:3.0 | 70 | 90 | 74.9 | 6.4 |
|----|-------|-----|-----|-------|-----|
| 24 | 1:3.0 | 70 | 180 | 86.4 | 7.5 |
| 25 | 1:3.0 | 80 | 90 | 98.9 | 7.3 |
| 26 | 1:3.0 | 80 | 180 | 102.4 | 7.9 |
| 27 | 1:3.0 | 90 | 90 | 100.6 | 7.5 |
| 28 | 1:3.0 | 90 | 180 | 103.3 | 7.9 |
| 29 | 1:3.0 | 100 | 90 | 105.9 | 7.9 |
| 30 | 1:3.0 | 100 | 180 | 107.2 | 7.9 |

It was established that the yield of sulfated ethanol lignin and sulfur content are controlled by varying the temperature, composition of the reaction mixture and the duration of the sulfation process (Table 1 and Fig. 2). The maximum yield of sulfated ethanol lignin and the high sulfur content can be obtained at different combinations of the specified parameters of the sulfation process (temperature, L / SC ratio, time). So 100% yield of sulfated lignin with sulfur content of 7.6–7.9% of mass can be achieved at different temperatures: at 70°C – in 3 hours using the ratio L / SC = 1:3, at 80°C – in 2.5–3 hours using the ratio L / SC = 1: 2, at 90°C – in 3 hours with the ratio L / SC = 1: 1.5, at 100°C – in 2.5 hours with the ratio L / SC = 1: 1.5 (Table 1). The same yield of sulfated ethanol lignin and sulfur content are also obtained at different duration of the sulfation process: for 0.5 hour – at 100°C and L / SC = 1: 3, for 1 hour – at 90°C and L / SC = 1: 3, for 2 hours – at 90 ° C and L / SC = 1: 2, for 2.5 hours – at 80°C and L / CK = 1: 3, for 3 hours – at 80°C and L / SC = 1: 2. The same yield of sulphated lignin and sulphur content can be achived also at different ratios of L / SC: for L / SC = 1: 1.5 – at 90°C in 3 hours, for L / SC = 1: 2 at 80°C in 3 hours, for L / SC = 1: 3 at 70°C – in 3 hours.
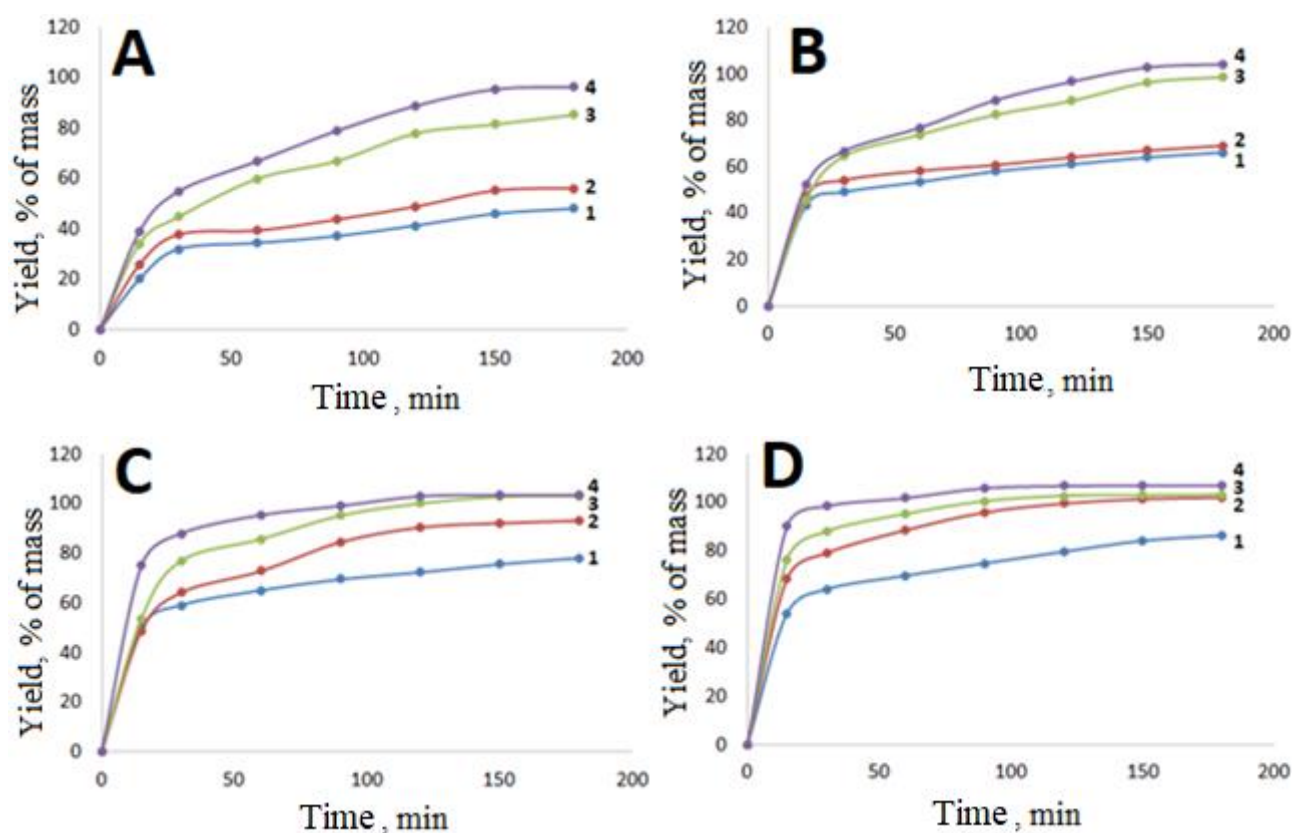


**Fig. 2** The effect of temperature and time of abies ethanol lignin sulfation at different ratio L:SC on the yield of sulfated ethanol lignin: A is the ratio L: SC = 1: 1.2; B is the ratio of L: SC = 1: 1.5; C is the ratio of L: SC = 1: 2; D is the ratio of L: SC = 1: 3 (1 – 70°C, 2 – 80°C, 3 – 90°C, 4 – 100°C).

The maximum possible content of sulfur in sulfated ethanol lignin was estimated to be 10.6% of mass for the hypothetical structure of Berkman spruce lignin (Heitner C et al. 2010), in which one phenylpropane unit has 0.9 mol of free OH groups capable of sulfation.

Data on the solubility of a sample of sulfated ethanol lignin with high sulfur content (7.4% of mass) in water and in a number of organic solvents are given in Table 2.

**Table 2** The elemental composition and solubility of samples of abies ethanol lignin and sulfated ethanol lignin

| Sample | Elemental composition, % of mass | | | Solubility | | | | |
|---|---|---|---|---|---|---|---|---|
| | C | H | S | Water | Ethanol | Dioxane | Dimethyl formamide | Dimethyl sulfoxide |
| Ethanol lignin | 66.6 | 6,6 | - | - | + | + | + | + |
| Sulfated ethanol lignin | 44,6 | 6,3 | 7,4 | + | Dilute solution | - | + | + |

Sulfated ethanol lignin is completely soluble in the wather, dimethylformamide, dimethylsulfoxide, limited soluble in ethanol and insoluble in dioxane.


2. Kinetics of abies ethanol lignin sulfation

The kinetics of abies ethanol lignin sulfation by sulfamic acid – urea mixture was studied in the temperature range of 70–100°C with variation in the mass ratio of ethanol lignin and sulfating complex (mixture of sulfamic acid and urea).

At all temperatures and L:SC ratios the process of abies ethanol lignin sulfation is described by a first-order equation (Fig. 3).
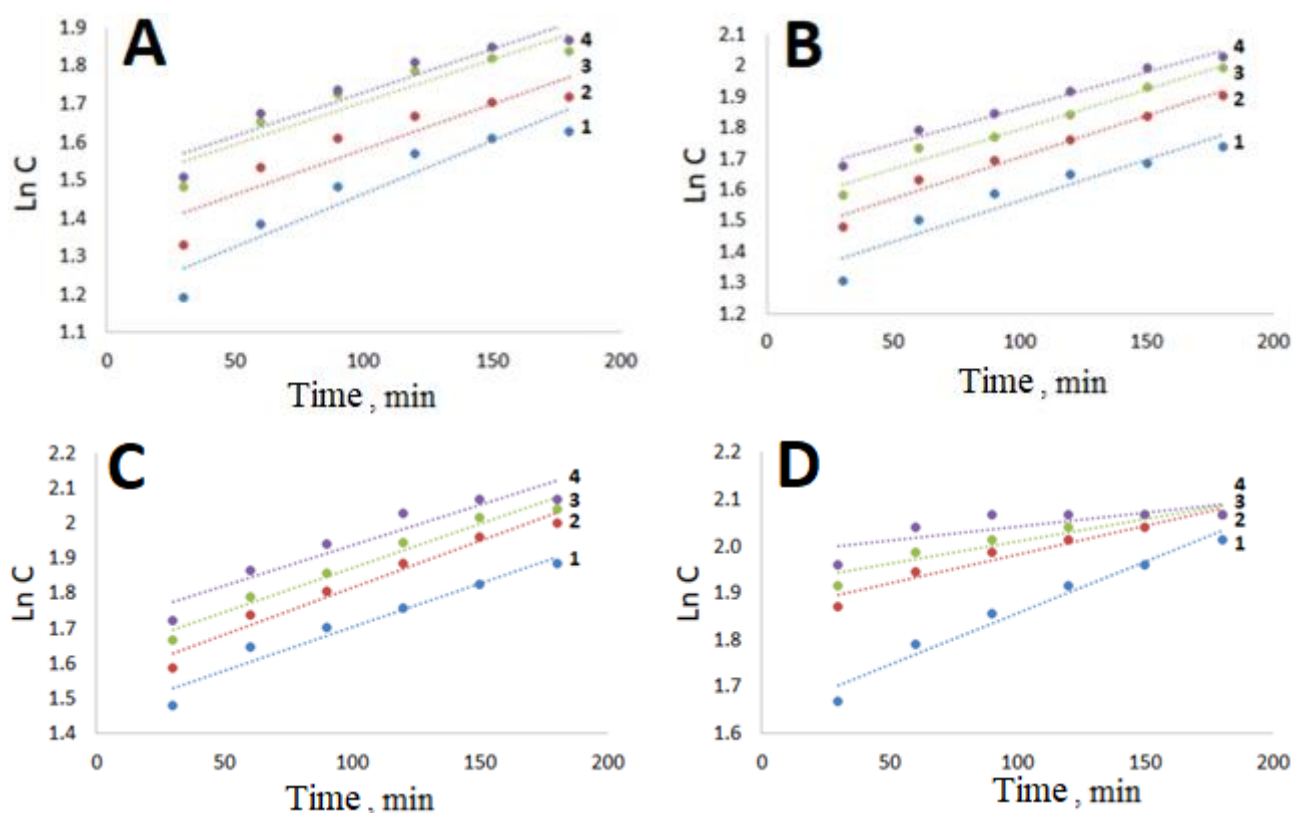


**Fig. 3** Time dependence of the sulfur content in sulfated ethanol lignin (Ln C) in the sulfation processes at different L:SC ratios and temperatures: A is the ratio L: SC = 1: 1.2; B is the ratio of L: SC = 1: 1.5; C is the ratio of L: SC = 1: 2; D is the ratio of L: SC = 1: 3 (1 – 70°C, 2 – 80°C, 3 –90°C, 4 – 100°C).

The rate constants of the sulfation process were calculated based on the change in the sulfur content in sulfated ethanol lignin. The value of activation energy of the sulfation process was determined using temperature dependence of rate constants in the Arrhenius coordinates (Fig. 4).
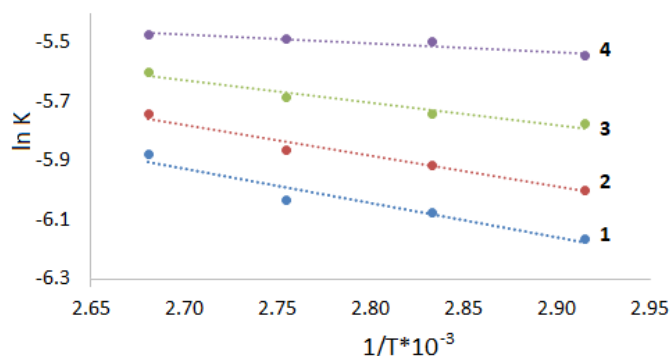


**Fig. 4** Temperature dependence of the rate constants of the ethanol lignin sulfation at different ratios L: SC (1 – L: SC = 1: 1.2; 2 – L: SC = 1: 1.5; 3 – L: SC = 1: 2.0; 4 – L: SC = 1: 3.0).

The calculated rate constants and activation energies of the process of ethanol lignin sulfation at different ratios of L: SK are given in Table 3.

**Table 3** The rate constants and activation energies of the process of abies ethanol lignin sulfation at different L: SC ratios.

| L:SC ratio | T °C | $k*10^{-5}$ (s$^{-1}$) | $E_a$ (kJ/mol) |
|---|---|---|---|
| 1:1.2 | 70 | 2.1 | 95 |
| | 80 | 2.3 | |
| | 90 | 2.4 | |
| | 100 | 2.8 | |
| 1:1.5 | 70 | 2.4 | 83 |
| | 80 | 2.7 | |
| | 90 | 2.8 | |
| | 100 | 3.2 | |
| 1:2.0 | 70 | 3.1 | 58 |
| | 80 | 3.2 | |
| | 90 | 3.4 | |
| | 100 | 3.7 | |
| 1:3.0 | 70 | 3.9 | 24 |

As follows from the obtained data, the rate constants of the process of ethanol lignin sulfation are weakly dependent on the ratio L:SC, while the activation energy is reduced significantly (from 95 to 24 kJ / mol) with an increase of the ratio L:SC from 1: 1.2 to 1: 3.0. Since the sulfation of lignin by sulfamic acid in dioxane is carried out in a heterogeneous medium, it can be assumed that at a small content of sulfating complex (L: SC = 1: 1.2 ÷ 1.5 mol / mol) the sulfation proceeds in the kinetic region. With the increase of the content of sulfating complex the sulfation process movies from the kinetic to the diffusion region due to the increased viscosity of the reaction medium.

3. Mathematical optimization of the abies ethanol lignin sulfation process

The task of optimizing the sulfation process is to search for conditions ensuring the production of sulfated abies ethanol lignin with maximum yield and sulfur content.

The following factors were used as independent variables: the ratio L: SC ($X_1$) and the duration of the sulfation process ($X_2$). The result of the sulfation process was characterized by two output parameters: the sulfur content in sulfated ethanol lignin ($Y_1$) and the yield ($Y_2$) of sulfated ethanol lignin . The fixed parameter is the sulfation process temperature of 100°C.

Dispersion analysis and mathematical modelling were executed for each output parameter characterizing the process of ethanol lignin sulfation.

Figure 5 shows the surface response of the output parameter $Y_1$ (sulfur content) to the ratio L: SC ($X_1$) and the process duration ($X_2$) at a temperature of 100°C. The surface response was calculated using of the regression equation:

$$Y_1 = 1.02222 + 3.1667*X_1 + 0.03167*X_2 - 0.433*X_1^2 - 0.004167*X_1*X_2 - 0.0000648*X_2^2 \quad (1)$$
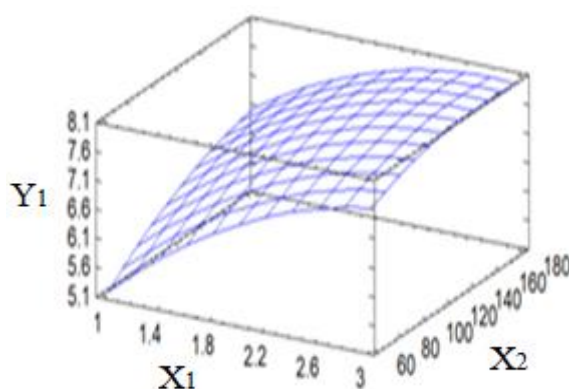


**Fig. 5** Surface response of the output parameter $Y_1$ (sulfur content, % mass) as depending on variable factors – $X_1$ (L: SC ratio) and $X_2$ (the process duration) at a temperature of 100°C

The optimized conditions for the process of lignin sulfation at a fixed temperature of 100°C, which allow to prowide the high sulfur content (7.9% of mass) in sulfated lignin, are the ratio L / SK = 1: 2.9 and the duration of 126 min.

Figure 6 shows the surface response of the output parameter $Y_2$ (output of sulfated ethanol lignin ) to variable factors $X_1$ and $X_2$. The surface response was calculated using the regression equation:

$$Y_2 = -29.6778 + 59.8*X_1 + 0.770833*X_3 - 7.78333*X_1^2 - 0.139583*X_1*X_3 - 0.00128704*X_3^2 \quad (2)$$
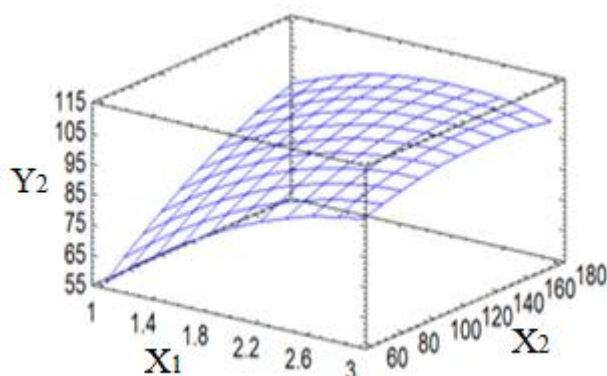
**Fig. 6** Surface response of the output parameter $Y_2$ (yield of sulfated ethanol lignin , % of mass) as depending on variable factors – $X_1$ (concentration of the sulfating complex) and $X_2$ (the process duration) at a stable temperature of 100°C

The maximum yield of sulfated ethanol lignin (100%) at a fixed temperature of 100°C is reached under the following conditions of the sulfation process: L / SC = 1: 2.3, duration 127 minutes.

Dispersion analysis showed that within the used experimental conditions to the total variance the both factors: the ratio L: SC – $X_1$ and the process duration – $X_2$ contribute significantly. The optimal conditions for the process of ethanol lignin sulfation at a temperature of 100°C, whith allow to provide both the high yield of sulfated lignin (100%) and high sulfur content (7.9% mass), are the ratio L / SC = 1: 2.3 and the duration 127 minutes

4. Study of the structure of sulfated abies ethanol lignin

Among the main monomeric and dimeric structural units of lignins (Heitner C, et al. 2010, Calvo-Flores FG, et al. 2015), presented in Fig. 7, structures A, B and C containing aliphatic hydroxyl groups subjected to sulfation.
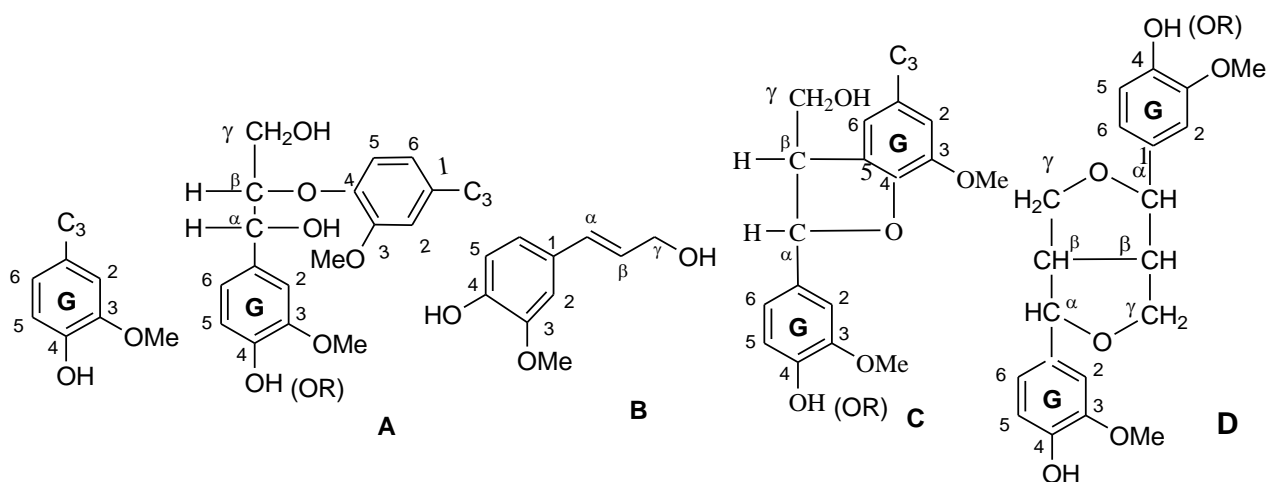


**Fig. 7** Monomeric and dimeric structure-forming units of lignins: G – guaiacyl, A – β-alkyl-aryl ether (β-O-4), B – coniferyl alcohol, C – phenyl coumaran, D – pinoresinol.

The structure of sulfated abies ethanol lignin was studied by FTIR, 2D NMR spectroscopy and GPC methods.

In contrast to the initial ethanol lignin, in the FTIR spectrum of sulfated ethanol lignin the new intensive absorption bands are present in the region the peaks of 803–861 cm$^{-1}$, corresponding to C–O–S-stretching vibrations of the $SO_3$ group, the wide absorption bands in the region of 1270-1200 cm$^{-1}$, corresponding to asymmetric stretching vibrations of the O=S=O group and skeletal vibrations in the guaiacyl ring (Roeges NPG 1995).
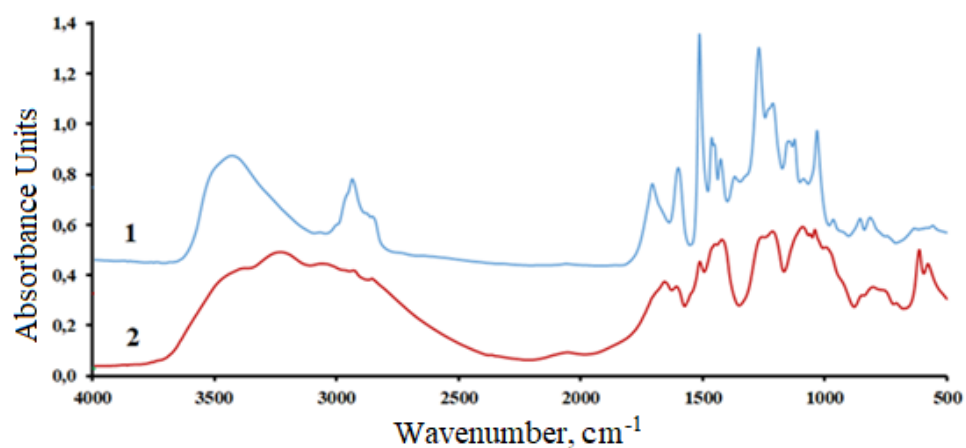
**Fig. 8** FTIR spectra of abies ethanol lignin (1) and ammonium salt of sulfated ethanol lignin (2) with sulfur content 7.4% mass.

More detailed information on the structure of sulfated abies ethanol lignin was received with the use of two-dimensional NMR spectroscopy. This method is the most promising in the structural analysis of lignins and it has been widely used in the study of the structure of lignin and other complex systems of natural origin (Zeng J, et al. 2013; Kalabin GA, et al. 2000, Fulmer GR, et al. 2010).

2D NMR HSQC spectra of the initial and sulphated abies ethanol lignin are presented in Fig. 9. The attribution of the peaks of $^1$H-$^{13}$C in the HSQC spectra is given in Table. 4 (Fulmer GR, et al. 2010).
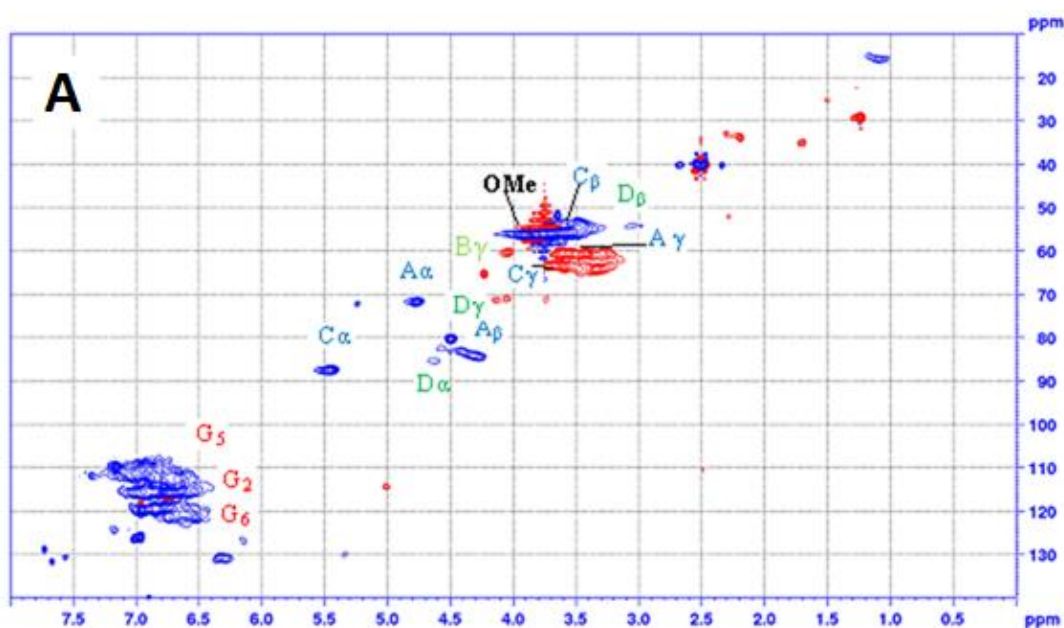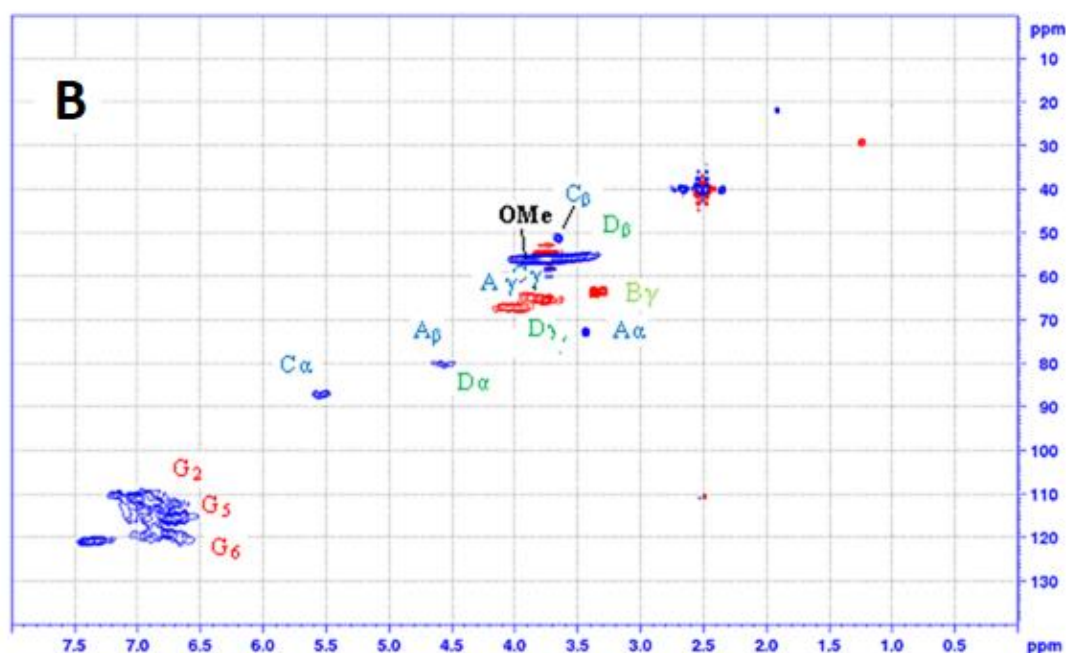
**Fig. 9** Signal regions of aromatic and aliphatic atoms in HSQC spectra: A – abies ethanol lignin ; B – sulphated abies ethanol lignin.

**Table 4** Attribution of $^1$H-$^{13}$C signals in HSQC-spectra of the samples of initial and sulfated abies ethanol lignin

| Attribution | $\delta^1_H/\delta^{13}_C$ lignin, ppm | $\delta^1_H/\delta^{13}_C$ sulfated lignin, ppm |
|---|---|---|
| (CH)2 in guaiacyl fragments (G$_2$) | 6,90/111,0 | 7,0/111,1 0 |
| (CH)5 in guaiacyl fragments G$_5$ | 6,70/115,9 | 6,70/115,8 |
| (CH)6 in guaiacyl fragments G$_6$ | 6,76/119,2 | 6,77/119,1 |
| CHα in pinoresinol fragments (Dα) | 4,61/85,3 | 4,56/83,8 |
| CH$_\beta$ in pinoresinol fragments (D$_\beta$) | 3,06/54,3 | 3,20/54,8 |
| CH$_2$γ in pinoresinol fragments (Dγ) | 4,14, 3,89/71,8 | 4,12, 3,82/ 70,1 |
| CHα in phenyl coumaran fragments (Cα) | 5,45/87,8 | 5,54/87,9 |
| CH$_\beta$ in phenyl coumaran fragments (C$_\beta$) | 3,61/52,2 | 3,70/51,6 |
| CH$_2$γ in phenyl coumaran fragments (Cγ) | 3,66/63,5 | 4,04/67,5 |
| CHα in β-aryl ether fragments (β-O-4) (Aα) | 4,77/71,5 | 3,44/73,2 |
| CHβ in β-aryl ether fragments (β-O-4) (A$_\beta$) | 4,30/84,2 | 4,59/80,5 |
| CH$_2$γ in β-aryl ether fragments (β-O-4) (A γ) | 3,50/60,9 | 4,02/67,3 |
| CH$_2$γ in coniferyl alcohol fragments (Bγ) | 4,09/61,9 | 3,33/64,6 |

The most intensive signals in the aromatic region of the HSQC spectra ($\delta^1$H/$\delta^{13}$C 6,7-6,5/111-119 ppm), which are shown in Fig. 9, correspond to the aromatic guaiacyl structural units (G). The peaks attributed to the CH

groups at positions 2, 5, 6 of the guaiacyl ring (Table 4) in the initial ethanol lignin  and sulfated ethanol lignin are practically unaltered, which confirms the absence of sulfation of phenolic hydroxyls.

The signals of pinoresinol fragments (D) were registered both in the spectra of ethanol lignin  and of its sulfated derivative. Correlation signals of pinoresinol fragments of abies ethanol lignin  and sulfated ethanol lignin have similar values, these include the following δH / δC signals: 4,61 / 85,3 ppm, 3,06 / 54,9 ppm, 4.14, 3.89 / 71.5 ppm and 4,56 / 83,8 ppm; 3,20 / 54,8 ppm; 4,12, 3,82 / 70,1 ppm, emitted by α-, β- and γ-position of these fragments respectively.

Abies ethanol lignin  and sulfated ethanol lignin  contain phenyl coumaran fragments (C), for which the peaks identified in the HSQC spectra of ethanol lignin  and sulfated ethanol lignin  appertain to α-, β- and γ-positions of these fragments: δH / δC – 5,45 / 87,82 ppm, 3,61 / 52,20 ppm, 3,66 / 63,51 ppm; δH / δC in the ethanol lignin  spectrum; and δH / δC – 5,54 / 87,9 ppm, 3,70 / 51,61 ppm, 4,04 / 67,51 ppm in the sulfated ethanol lignin spectrum. Based on these data, it can be expected that OH-groups of CHγ in the phenyl coumaran fragments undergo sulfation, as the signals of the corresponding carbons in the sulfated lignin in the HSQC spectra are shifted to a weak field for the initial lignin, besides, the signal of the adjacent carbon $CH_\beta$ in these fragments is also shifted to a strong field (Fig. 9, Table 4).

Comparing the values of the correlation signals emitted by α-, β- and γ-atoms of the β-aryl ether (β-O-4) of ethanol lignin and sulfated lignin structures, we noticed that the signal values of the corresponding atoms in the HSQC spectra are different in all indicated positions of these structures. Moreover, the signals of CHα and CH2γ carbon atoms in sulfated lignin are shifted to a weak field by 1.7 and 6.4 ppm respectively as compared to the signals of the initial lignin. Also, the signals of $CH_\beta$ carbon atoms are shifted to a strong field by 3.7 ppm. Such a shift of signals in the spectra is evidence of the sulfation of OH-groups connected with the CHα and CH2γ structures of the β-aryl ether (β-O-4) of lignin macromolecule. A similar shift of signals in the HSQC spectrum of sulfated lignin (δH/δC – 3,33/64,6 ppm) in comparison with the initial lignin (δH/δC – 4,09/61,9 ppm) was found in signals belonging to CH2γ terminal groups of coniferyl alcohol (Bγ), which additionally indicates the sulfation of OH-groups connected with CH2γ of this lignin fragment.

The molecular mass distribution of the initial and sulfated abies ethanol lignin samples was examined by gel permeation chromatography (Table 5, Fig. 10, 11).

**Table 5** Average molecular weights (Mn, Mw) and polydispersity (D) of initial and sulfated abies ethanol lignin samples

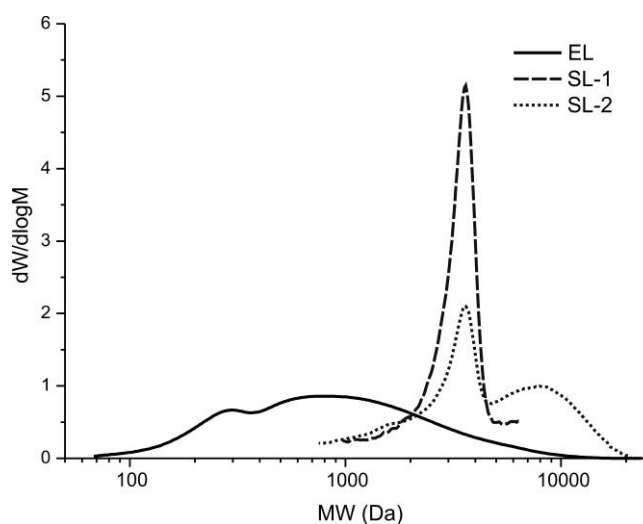| Sample | Mn (Da) | Mw (Da) | D |
|---|---|---|---|
| Abies ethanol lignin | 950 | 1830 | 1,95 |
| Sulfated ethanol lignin 1 (80°C, 180 min; 5.5 % mass. S) | 2923 | 3291 | 1,13 |
| Sulfated ethanol lignin-2 (90°C, 240 min; 7.1 % mass. S) | 3276 | 5353 | 1,63 |
| Sulfated ethanol lignin-3 (70°C, 30 min; 4.7% mass. S) | 1994 | 2274 | 1,14 |
| Sulfated ethanol lignin-4 (70°C, 180 min; 5.5 % mass. S) | 2237 | 2592 | 1,16 |

**Fig.10** Molecular weight distribution curves of lignin samples: EL – abies ethanol lignin; SL-1 – sulfated ethanol lignin (80°C, 180 min; 5.5 % mass. S); SL-2 – sulfated ethanol lignin (90°C, 240 min; 7.1 % mass. S)

Compared to the initial ethanol lignin, the average molecular weigths of sulfated ethanol lignin increases significantly due to the introduction of sulfo groups, while the degree of polydispersity falls from 2 to ~ 1.2 (Table 5). As shown by Fig. 10, the sample with low sulfur content (SL-1) obtained at a temperature of 80°C has practically no region of high (about 10 kDa) molecular weight. This indicates a low degree of sulfation of high-molecular lignin fractions which have at diminished reactivity sulfation temperature 80°C. The sample with higher sulfur content (SL-2) obtained at a temperature of 90°C has the another profile of molecular weight distribution: lignin fractions with a molecular weight of about 10 kDa increases and the polydispersity rises to 1.63. This result can be explained by an increase in the degree of sulfation of lignin fractions with high molecular mass at temperature 90°C.

Fig. 11 illustrates the impact of sulfation time on molecular weight distribution curves of sulfated ethanol lignin samples.
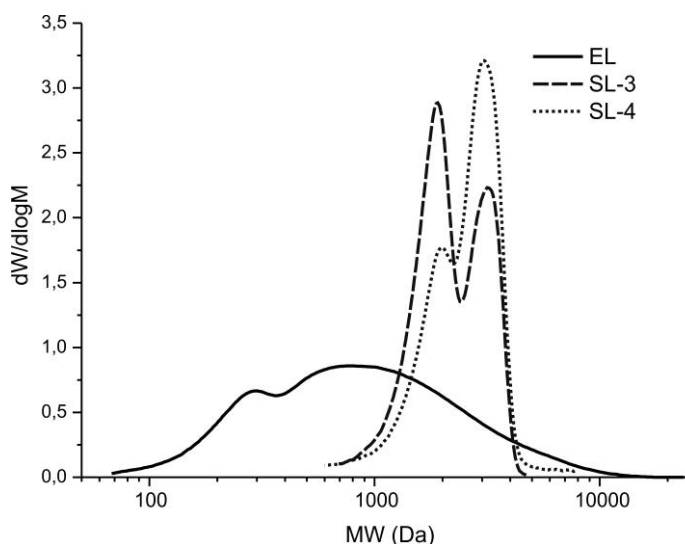


**Fig. 11** Molecular weight distribution curves of ethanol lignin samples: EL – abies ethanol lignin; SL-3 – sulfated ethanol lignin (70°C, 30 min; 4.7 % mass. S); SL-4 – sulfated ethanol lignin (70°C, 180 min; 5.5 % mass. S)

The curve of molecular weight distribution of the sulfated lignin samples obtained at a temperature of 70°C within 30 minutes (SL-3) has two main fractions with a weight of ~ 1.5kDa and ~ 3.5kDa. With the increace in the duration of the sulfation processes from 30 min to 180 min a decreace content of the fraction ~ 1.5kDa is observed. Probably this fraction consist of partially sulfated low molecular weight lignin and of the mixture of sulfamic acid

and urea. Simultaneously there is an increase in the sulfur content to 5.5% and in the content of fraction of sulfated lignin with a mass of ~ 3.5kDa

All sulfated ethanol lignin samples do not contain the products with a molecular weight less than 1 kDa. Obviously the sulfated lignins with low molecular weight are removed through 3.5 kDa pores during dialysis process.

### Conclusion

The optimal process parameters were determined for the sulfation of abies ethanol lignin by a sulfamic acid – urea mixture in 1,4-dioxane: temperature 90–100°C, ratio L:SC 1:1.5 ÷ 2.0 (mol: mol), process duration 1.5–3 hours. This conditions allow to produce the sulfated ethanol lignin with the yield about 100% mass and with sulfur content up to 7.9% mass.

For the first time a kinetic study of the process of abies ethanol lignin sulfation by sulfamic acid-urea mixture in 1,4-dioxane was accomplished at the temperature range of 70–100°C. The processes of lignin sulfation are described by a first-order equation over the entire temperature range. The rate constants are weekly depend on the ratio of lignin: sulfating complex (SC). However, the activation energy is reduced from 95 kJ / mol to 24 kJ / mol with the increase of SC content from 1:1,2 to 1:3. Obviously, with the increase of the content of SC the sulfation process moves from the kinetic to the diffusion region due to the increasing viscosity of the reaction mixture. Data on the composition and structure of sulfated abies ethanol lignin were obtained using elemental analysis, FTIR, 2D NMR and GPC methods.

According to 2D NMR data only alcoholic OH-groups react with sulfamic acid, namely OH-groups at CHα and CH$_2$γ in fragments of β-aryl ether (β-O-4), OH-groups at CHγ in phenyl coumaran fragments and OH-group at CH$_2$γ in terminal fragments of coniferyl alcohol.

According to gel permeation chromatography results, water-soluble sulfated abies ethanol lignin has a higher molecular mass and a lower degree of polydispersity as compared to the original ethanol lignin. Reduction of polydispersity in the sulfation process occurs from one side due to removing of low-MW products in dialysis process, from other side high-MW lignins has low degree of sulfation and low solubility and removed from product after dissolution in water

### Acknowledgements

### Conflict of interest

There is no conflict of interest.

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