UV/Vis Spectrometry as a Quantification Tool for Lignin Solubilized in Deep Eutectic Solvents

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In this short communication, UV/Vis spectrophotometry is described as an analytical tool for the quantification of lignin content in deep eutectic solutions. The lignin was solubilized with different deep eutectic solvent (DES). DESs were prepared as binary mixtures of choline chloride with lactic acid (1:9); (1:10); ethylene glycol (1:2); glycerol (1:2) and alanine:lactic acid (1:9), and betaine:lactic acid (1:2). The UV-Vis spectrometric quantification of the solubilized lignins was independent of the type of solubilized lignin. The approach consists of measuring the absorbance of a solution of lignins dissolved in the deep eutectic solvents at an absorbance of 440 nm.

Keywords: Deep eutectic solvents; Lignin; UV/Vis spectrophotometry; Absorbance

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INTRODUCTION

Valorisation is a key component of an economic and environmental lignocellulosic biorefinery (Jablonsky *et al.* 2015b; Surina *et al.* 2015). There are many new modes of pulp processing. Deep eutectic solvents (DESs) can be used to dissolve lignocellulosic biomass or its individual components such as lignins (Francisco *et al.* 2012). DESs have potential applications in the pulp, paper, and recycling industries. DESs have been used to delignify different biomasses such as wheat straw (de Dios 2013; Jablonsky *et al.* 2015b; Skulcova *et al*. 2016), rice straw (Kumar *et al.* 2015), pine wood (de Dios 2013), and thermomechanical pulp (Choi *et al.* 2016). Lignin is the second most abundant natural polymer after holocellulose. Lignin is a complex phenolic polymer found in biomass feedstocks and biomass-derived products. Lignin consists of three types of units called p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Boerjan *et al.* 2003; Davin and Lewis 2005). Lignin composition varies in different groups of vascular plants, with only G, G and S, and then H, G, and S lignins being characteristic of the combinations of lignin units for softwoods, hardwoods, and graminaceous plants, respectively (Sun *et al.* 2012).

It is difficult to determine lignin content in complex systems *via* extraction. Various separation (mainly high performance liquid chromatography) and optical techniques have been exploited to eliminate the interferences caused by other components (Lobbes *et al.* 1999).

UV/Vis spectroscopy is a user-friendly and adaptable analytical technique that provides the best results with regard to the structural differences between lignins. Several methods to determine lignin content use absorbance values at 205 or 280 nm for the quantitative and qualitative analysis of native lignins (Janshekar *et al.* 1981) and lignosulphonates and lignin precipitated with black liquor (Jablonsky *et al.* 2016). The lignin concentration can be determined in different solutions (Lee *et al.* 2013). However, the type and structure of lignin, solvent, and pH of the solution have a considerable influence on the UV/Vis spectra (Kallavus *et al.* 2015). Mongeau and Brooks (2001) found that using the acetyl bromide method overestimates the lignin concentration due to the interference of polysaccharides.

The aim of the present investigation was to develop a simple and fast spectral method for the evaluation of lignin content in the deep eutectic solvents.

EXPERIMENTAL

Materials

All chemicals were purchased from Sigma Aldrich (Bratislava, Slovakia). The solutions were stirred in a water bath to form homogeneous liquids. The DESs used were choline chloride and lactic acid (1:9) and (1:10), ethylene glycol (1:2), oxalic acid (1:1), malic acid (1:1), malonic acid (1:1), glycerol (1:2), and the systems alanine: lactic acid (1:9) and betaine : lactic acid (1:2) (Table 1).

Table 1. Properties of DESs

Sample	System DES	Solubility of Lignin	Molar Ratio	Density (kg/m^3) at 25 °C	Refractive Index at 25 °C	Viscosity (mPa·s) at 28 °C
DES1	*ChCl : lactic acid	Yes	1:10	1241	1.4407	71.1
DES2	*ChCl : lactic acid	Yes	1:9	1217	1.4432	70.9
DES3	*ChCl : ethylene glycol	Yes	1:2	1141	-	38.7
DES4	Alanine : lactic acid	Yes	1:9	1230	1.4397	168.0
DES5	Betaine : lactic acid	Yes	1:2	1195	1.4620	-
DES6	*ChCl : glycerol	Yes	1:2	1197	1.4781	292.3
DES7	*ChCl : oxalic acid	No**	1:1	1280	1.4662	126.1
DES8	*ChCl : malic acid	Partially**	1:1	1299	1.4790	-
DES9	*ChCl: malonic acid	Partially**	1:1	-	1.4864	-
*ChCl – Choline chloride, ** Not used for UV/Vis analysis						

Lignin Samples

The annual plants, hemp, and flax, used for obtaining black liquor were kindly supplied by OP Papírna Ltd. (Olšany, Czech Republic). The cooking conditions were as follows: active alkali sodium hydroxide and the presence of anthraquinone (AQ). The black liquor obtained had the following characteristics: pH of 12.9 ±0.3 (determined by a digital Jenway (3510 pH-meter, UK) and density 1.242 g/mL (determined by measuring the mass with the known volume of the black liquor), ash 45.75 wt%, and dry matter 36.80 wt%. The precipitation of lignin from black liquor was used as a single step process in which a dilute solution of sulphuric acid (5 wt% (1.05 N)) was added to the black liquor with the pH adjusted to the desired value. 100 mL of the black liquor was treated with diluted sulphuric acid to obtain a final pH value 3. The precipitated straw lignin was washed twice with hot water (total volume 400 mL, pH= 6.8) to remove impurities. The lignin was then dried at 25 °C under a pressure of 0.5 mbar using lyophilisation equipment (LYOVAC (GT2, Germany) until reaching a constant weight.

Lignoboost lignin was purchased from Innventia AB (Stockholm, Sweden), ash content 0.6 wt%. Softwood kraft lignin was isolated from the original black liquor using the LignoBoost process by Innventia AB. The lignin was then dried at 25°C under a pressure of 0.5 mbar using lyophilisation equipment (LYOVAC (GT2, Germany) until reaching a constant weight.

Absolutely dry lignin samples (lignoboost lignin or straw lignin; see Table 2) were added to individual DESs, and the mixture in a closed flask was stirred in a water bath at 80 °C for 20 min. Table 2 shows the lignin properties, and Fig. 1 shows the solubilized lignin in cuvettes.

Table 2. Analysis of Lignin Properties

Sample	C (%)	H (%)	N (%)	S (%)	Ash (%)	OCH_3* (%)	HHV** (MJ/kg)
Lignoboost lignin	65.00	5.44	0.12	1.14	0.42 ± 0.02	13.17	26.8 ± 0.5
Straw lignin	65.54	6.17	1.20	0.04	0.37 ± 0.04	15.73	23.61 ± 0.3

*Calculated according to Jablonsky *et al.* (2015a)
**Higher heating value

Fig. 1. Pure DES 1, solubilized lignin with different concentration of lignins, and straw lignin

UV/Vis spectrometry analysis of solubilized lignin in DES

Lignin content was determined using UV/VIS spectrophotometry with a UV-1600 series spectrophotometer (VWR, Leuven, Belgium). Absorbance within a 200 to 800 nm spectral range was measured at 1 nm spectral resolution. Samples were referenced by pure individual DESs.

RESULTS AND DISCUSSION

The UV/Vis absorbance spectra of various lignins at different concentrations (lignin lignoboost; 0.09 to 0.78 mg/g DES; straw lignin 0.08 mg/g to 0.86 mg/g DES; (Table 3)) solubilized in choline chloride : lactic acid (1:9) are depicted in Fig. 2. Given the presence of lignins, a spectral maximum centered at about 280 nm was expected, given that absorption bands centered at 280 nm and/or 205 nm are used for lignin determination (Sun *et al.* 2001; Kline *et al.* 2010).

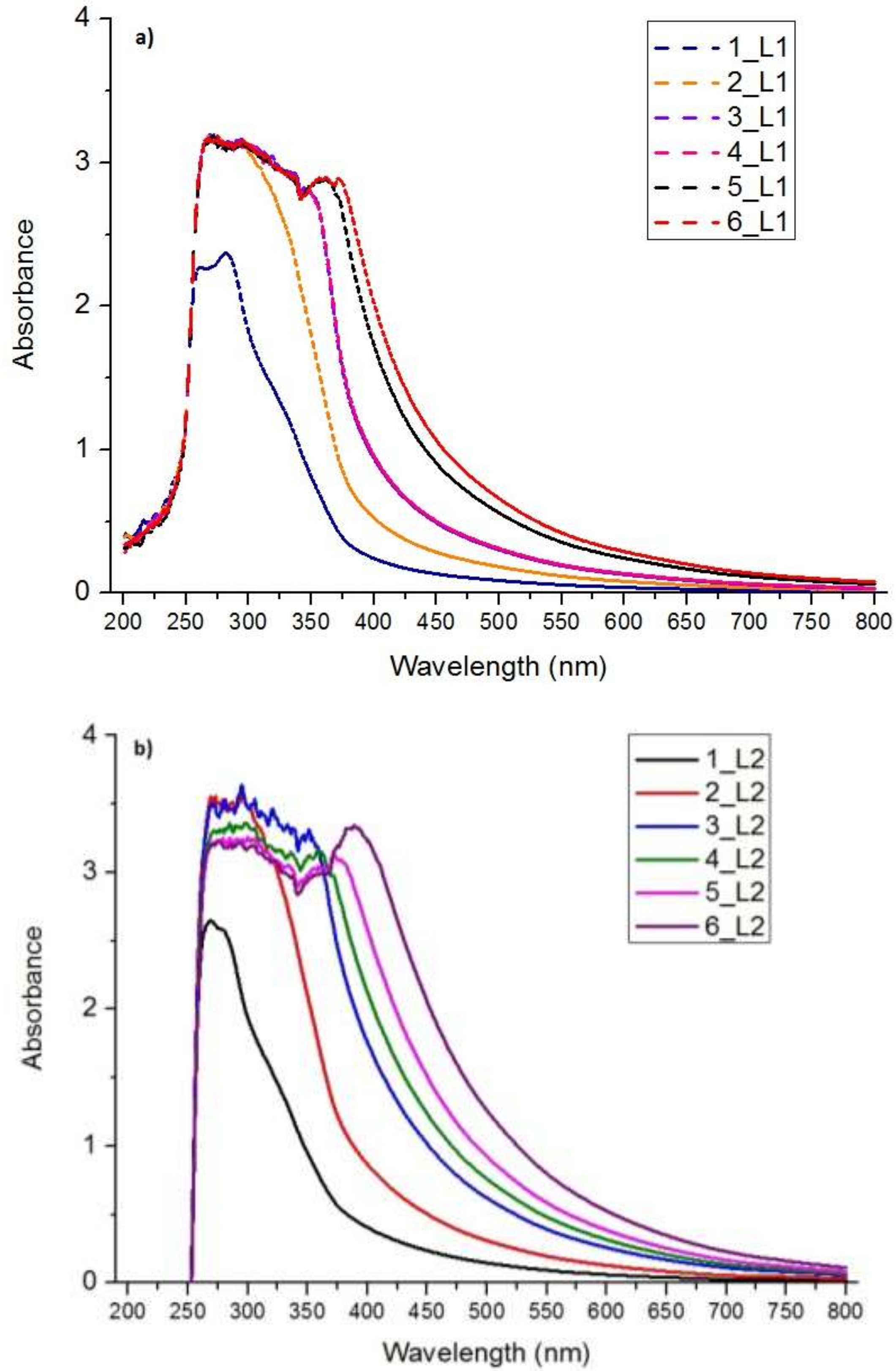

Fig. 2. UV-visible absorbance spectra of two lignin standards (L1 – lignin lignoboost (**Fig. 2a**); L2 – straw lignin (**Fig. 2b**)) at different concentrations of lignin in a deep eutectic solvent (Table 3).

Absorption bands in the lignin spectrum are assignable to a variety of groups or compounds. Lignins contain several functional chemical groups, such as hydroxyl (phenolic or alcoholic), methoxyl, carbonyl, and carboxyl, in various amounts, depending on its origin and the applied isolation process (Sun *et al.* 2001; Gosselink *et al.* 2004). However, it was found that the region of 200 to 400 nm is not suitable for determination of lignin solubilized in DESs. Similar spectral characteristics were identified also by Kline *et al.* (2010), who investigated lignin spectra in the ionic liquid 1-*n*-butyl-3-methyl-imidazolium chloride [Bmim]Cl. In the present work, a partial least squares analysis was applied to the visible spectrum region, 380 to 1100 nm. The absorbance maximum at about 440 nm represented a better solution for lignin quantification.

Table 3. Concentration of Lignin in a Deep Eutectic Solvent

Sample	Concentration of lignin lignoboost in DES1* (mg/g)	Sample	Concentration of straw lignin in DES2** (mg/g)
1_L1	0.087	1_L2	0.08
2_L1	0.133	2_L2	0.196
3_L1	0.236	3_L2	0.407
4_L1	0.351	4_L2	0.486
5_L1	0.762	5_L2	0.602
6_L1	0.784	6_L2	0.858

* choline chloride : lactic acid (1:10)
** choline chloride : lactic acid (1:9)

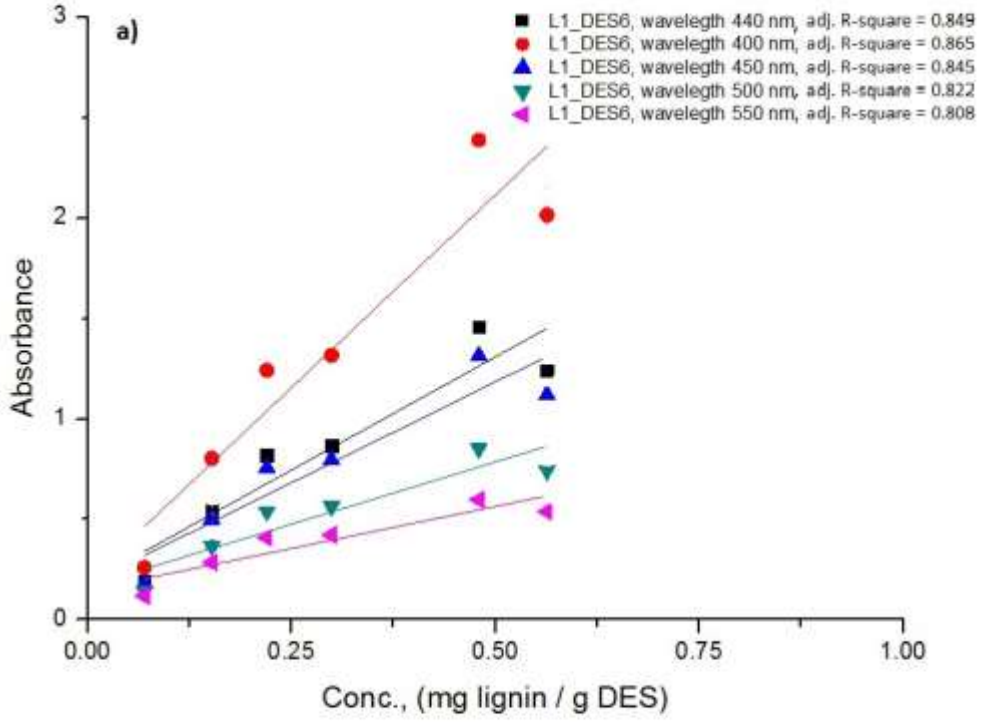

Fig. 3a. Calibration curves for the chosen wavelengths (400, 440, 450, 500, and 550 nm) are shown for different pure lignoboost lignin L1; and deep eutectic solvent: DES6: choline chloride : glycerol (1:2).

Various lignin standards were tested at different wavelengths and concentrations to calibrate the UV/Vis spectra (Fig. 3a and Fig. 3b). There was a remarkable correlation between the adsorption and lignin concentrations between 0.07 and 0.86 mg of lignin in 1 g of DES. This linear correlation was apparent for the used lignin types and further for the various wavelengths used (from 400 to 550 nm).

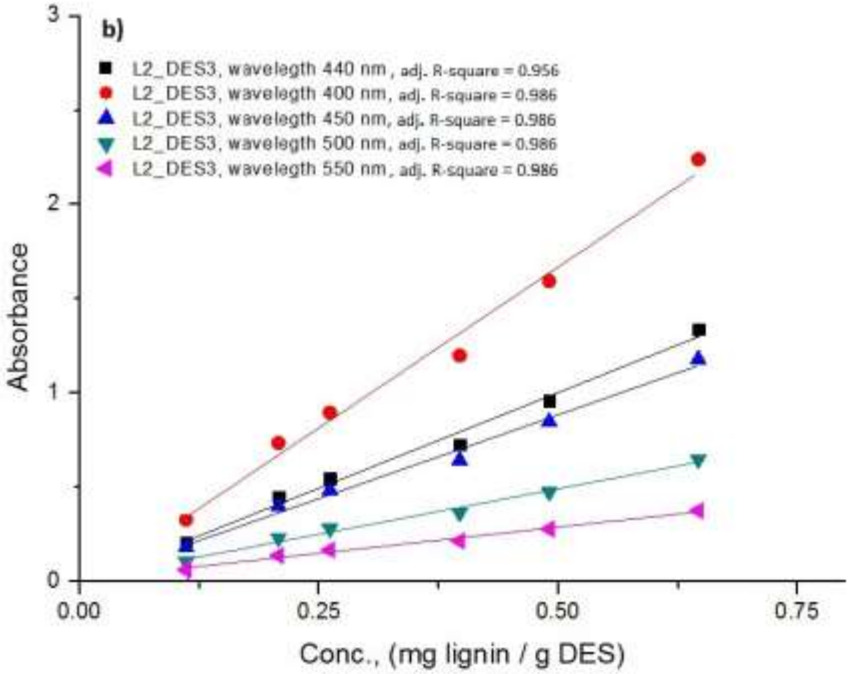

Fig. 3b. Calibration curves for the chosen wavelengths (400, 440, 450, 500, and 550 nm) are shown for different pure straw lignin L2; and deep eutectic solvent: DES3: choline chloride and ethylene glycol (1:2).

Based on promising results obtained by Kline *et al.* (2010), the lignin content in DESs was quantified using spectrophotometry at a wavelength of 440 nm. In the experiment, lignin was dissolved in DES and prepared at 80 °C, and the absorbance spectra were measured. Plots of absorbance at this wavelength *vs*. lignin concentration in the DES for different lignin standards are depicted in Fig. 4. These experimental results are depicted in Fig 4. Different types of deep eutectic solvents and of lignins were compared. The proposed method allows quantification of lignins irrespective of the type of DES used. The concentration of the solubilized lignin was determined with little or no pretreatment of analysed samples using a short list of easily available chemicals.

At the absorbance at 440 nm, the $A_{440\ \mathrm{nm}}$/lignin concentration plot exhibited a fair linear relationship in most measurements. The correlation coefficient R^2 ranged from 0.849 to 0.998; individual equations are shown in Table 4. Figure 5 illustrates the relationship between absorbance at 440 nm and the concentration of the solubilized lignin for lignoboost lignins (L1) and a straw lignin (L2), as described by Eq. 1,

$$\mathrm{A} = -\,0.01221 + 2.46982 \times Conc.,\ \mathrm{R}^2 = 0.8912 \qquad (1)$$

where *Conc* is mg lignin / g DES.

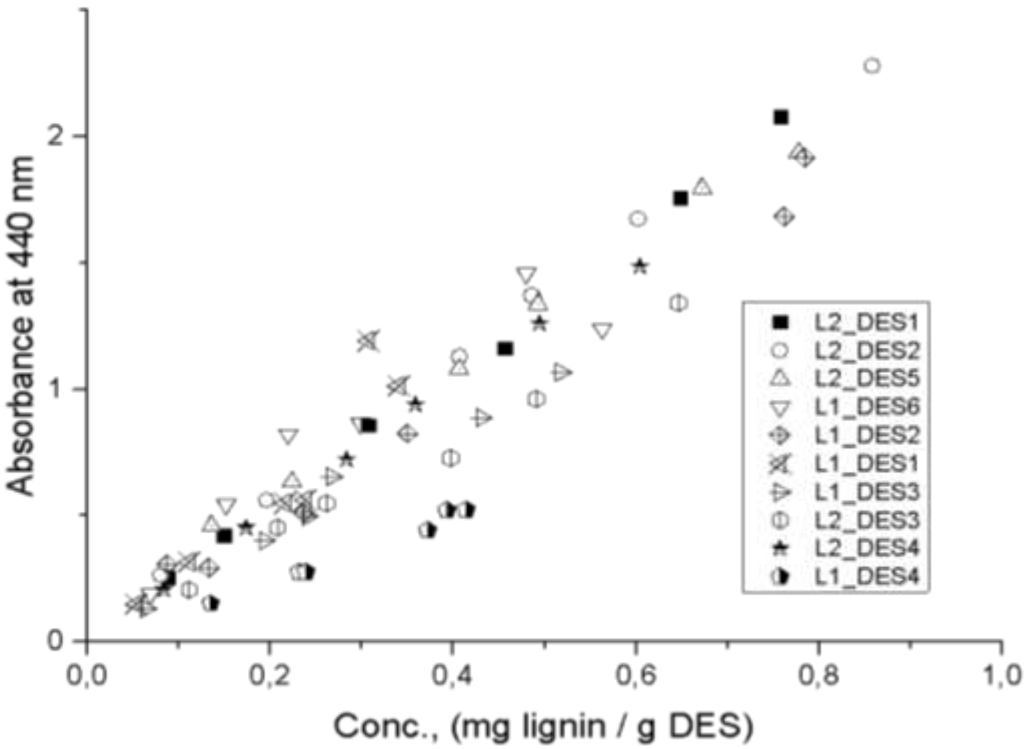

Fig. 4. Absorbance at 440 nm *versus* concentration for solution of lignin dissolved in deep eutectic solvents. L1 – lignin lignoboost; L2 – straw lignin; DES1 – ChCl : lactic acid (1:10); DES2– ChCl : lactic acid (1:9); DES3 – ChCl : ethylene glycol (1:2); DES4 – alanine : lactic acid (1:9); DES5 – betaine : lactic acid (1:2); DES6 – ChCl : glycerol (1:2)

Table 4. Linear Equation for Different Lignin (L1 - Lignoboost Lignin, L2 – Straw Lignin) and Deep Eutectic Solvent, Dependence Absorbance at 440 nm (A) vs. Concentration of Dissolved Lignin in DES (Conc. (mg Lignin / g DES))

Sample	DES	Equation	R-square
L1_DES1	*ChCl : lactic acid	A = −0.08723 + 3.3868 × Conc.	0.8490
L1_DES2	*ChCl : lactic acid	A = −0.02532 + 2.2886 × Conc.	0.9860
L1_DES3	*ChCl : ethylene glycol	A = 0.02081 + 2.04415 × Conc.	0.9814
L1_DES4	Alanine : lactic acid	A = −0.0383 + 1.3514 × Conc.	0.9840
L1_DES6	*ChCl : glycerol	A = 0.18447 + 2.2479 × Conc.	0.8488
L2_DES1	*ChCl : lactic acid	A = 0.00399 + 2.6948 × Conc.	0.9965
L2_DES2	*ChCl : lactic acid	A = 0.0631 + 2.62279 × Conc.	0.9982
L2_DES3	*ChCl : ethylene glycol	A = −0.00907 + 2.02545 × Conc.	0.9858
L2_DES4	Alanine : lactic acid	A = 0.01883+2.4768× Conc.	0.9973
L2_DES5	Betaine : lactic acid	A = 0.12271 + 2.39905 × Conc.	0.9941
*Choline chloride, L1 – lignoboost lignin, L2 – straw lignin			

As mentioned before, there are papers describing lignin quantification in DESs through absorbance at 280 nm (Kumar *et al.* 2015). Kumar *et al.* (2016) noticed that the absorption maximum varied and was not centered in all cases at 280 nm. They deduced that the most important factor was probably the pH of extracts after applying deep eutectic solvents. When extracting lignins at a pH of 2 to 3, the absorption maximum lies at about 280 nm; with neutral green solvents (pH 6 to 7), an additional peak at 305 to 315 nm was observed. An open question relates to a pH range of 3 to 6. The absorption maximum depends on the DES composition and shifts from 328 nm for ChCl : oxalic acid (1:1) to 265 nm for alanine : lactic acid (1:9) (data not shown). This shift was also observed by Kumar *et al.* (2016), but they did not explain this result.

In this short communication, the lignin content in DES was quantified using the UV/VIS methods through absorbance at 440 nm. One weakness of this mode of lignin concentration determination is that the absorbance is not measured at its maximum but on the slope of spectral curves. However, the R-square values clearly documented that this weak point is dismissable by simplicity and reliability of the absorbance.

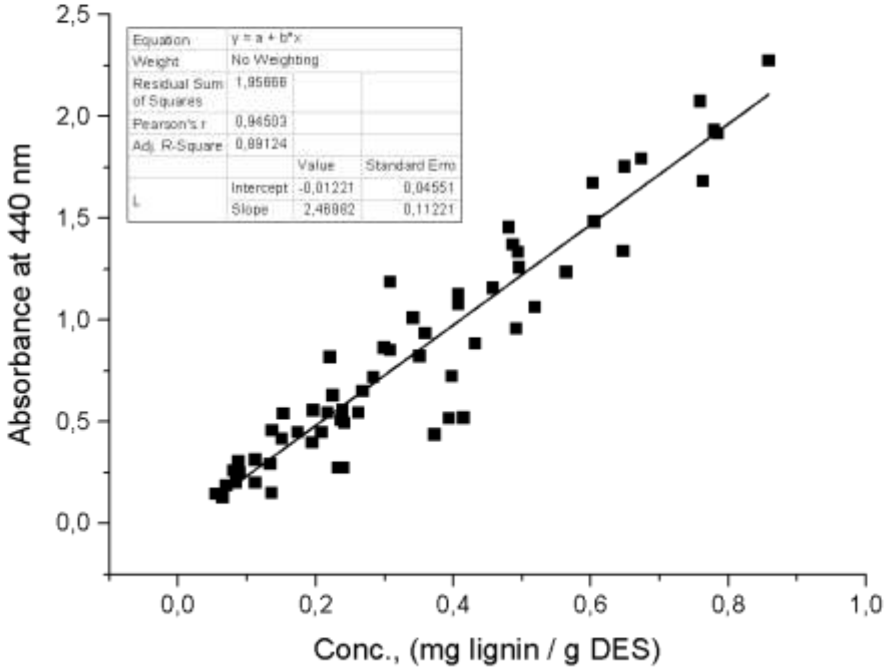

Fig. 5. Absorbance at 440 nm *versus* concentration for solution of lignins (L1 – lignoboost lignin) dissolved in deep eutectic solvents. DES: ChCl : lactic acid (1:10); ChCl : lactic acid (1:9); ChCl : ethylene glycol (1:2); alanine : lactic acid (1:9); betaine : lactic acid (1:2); ChCl : glycerol (1:2)

CONCLUSIONS

1. In this study, UV/Vis spectrometry was investigated as an easy, quick technique for the analysis of lignin dissolved in DES.
2. The concentration of solubilized lignins in different types of DESs was determined without having to significantly modify the analytical process.

3. The UV/Vis spectrometric quantification of solubilized lignins was independent of the type of solubilized lignin.

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