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Exploring a new platform to separate Kraft lignin from choline chloride-based eutectic solvents using aqueous mixtures of 1-butanol or ethyl acetate

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ABSTRACT

Eutectic Solvents (ES) have emerged as sustainable media for lignocellulosic biomass delignification. However, the lack of efficient downstream strategies to separate the extracted lignin from the ES-rich mixtures remains a significant industrial bottleneck. This study investigates the potential of aqueous biphasic systems containing organic cosolvents (1-butanol or ethyl acetate) to recover lignin from choline chloride (ChCl)-based ESs. Experimental binodal curves and Kraft lignin partition coefficients (K_L) were determined for 12 systems containing water, a cosolvent, and ES formed by ChCl and six hydrogen-bond donors (three carboxylic acids and three diols). Results indicate that 1-butanol induces larger immiscible regions compared to ethyl acetate, while larger biphasic regions were observed in systems containing acid-based ESs than in those containing diols. To complement the experimental studies, the Merchuk and Hu equations were used to correlate the binodal curves, with the Hu model yielding superior representations ($R^2 \geq 0.999$). Remarkably, Kraft lignin preferentially partitioned into the organic-rich top phase ($K_L > 1$) in all cases. Promising results were achieved for the (water + 1-butanol + ChCl:FA) and (water + ethyl acetate + acid-based ES) systems ($11.3 \leq K_L \leq 29.6$), suggesting these water-cosolvent mixtures are auspicious routes for recovering lignin from acid-based ES media.

KEYWORDS

Kraft lignin; biphasic system; eutectic solvents; liquid-liquid equilibrium; partition coefficient; biorefinery

Introduction

There is worldwide interest in developing new chemical processes that utilize renewable raw materials, aiming to reduce environmental impacts and, whenever possible, achieve biodegradability. The emergence of a bio-based economy is currently one of the main strategies for sustainable growth (Bokhary et al. 2021). In this context, lignocellulosic biomass has been highlighted as a potential candidate for a sustainable and biobased economy, as it is renewable and abundant in nature (Segers et al. 2024). This type of biomass is mainly composed of three types of compounds: cellulose (30–50 wt%), hemicellulose (20–35 wt%), and lignin (10–25 wt%) (Saha 2003). Nowadays, cellulose and hemicellulose have established global markets (Rao et al. 2023; Marinho 2025); however, the valorization of lignin and its conversion into fine chemicals are not fully addressed.

Lignin is a macromolecule of significant interest in the chemical industry, representing a natural, abundant, and renewable source for the production of value-added products. Its structure is based on phenylpropanoid aromatic units and different heteroatoms, making it a unique source of aromatic compounds (Silveira et al. 2015; Horvat 2016). However, due to its high heterogeneity and recalcitrance, its industrial application has been limited mainly to a combustion

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source for energy and steam generation (Zakzeski et al. 2010). In recent years, alternative solvents such as eutectic solvents (ES) and ionic liquids (ILs) have emerged as promising solutions to address common lignin processing challenges (Verdía Barbará et al. 2025; Mqoni et al. 2026).

The specific properties of ES and ILs, such as their ionic nature and strong tendency to form hydrogen bonds with the functional groups available in lignocellulosic materials, result in a high affinity and dissolution capacity for lignin (Smith et al. 2014; Da Costa Lopes et al. 2020). Furthermore, their chemical nature and tunable character also allowed them to be applied for lignin fractionation and depolymerization (del Mar Contreras-Gámez et al. 2023). Although ILs usually exhibit high lignin solubility caapacity (Dias et al. 2020b; Dias et al. 2024), their application in the industry is sometimes limited, as some exhibit high production costs or high toxicity (Malaeke et al. 2018).

In contrast, ES are liquid mixtures, often composed of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), that exhibit, at a specific composition, a melting temperature lower than that of the individual components and of any other mixture composition (Pena-Pereira and de la Calle 2018). Furthermore, these systems—which generally contain at least one component that is solid at room temperature—typically remain liquid across a certain composition range at mild operating temperatures (Abranches and Coutinho 2023). When the eutectic temperature is lower than the ideally predicted value, the term 'deep' may be used to refer to them as deep eutectic solvents (DES) (Teixeira et al. 2022). In recent years, several studies have highlighted ES as a promising class of solvents for industrial-scale biomass pretreatment (Shen et al. 2019) due to their physical-chemical properties, such as relatively low toxicity, lower production costs, and biodegradability, which make them strong candidates for addressing this challenge (Hayyan et al. 2013; Alvarez-Vasco et al. 2016; Hansen et al. 2021).

Regarding ES constituents, organic acids (e.g., formic, acetic, and propionic acids) have been extensively studied as HBDs, showing promising results in lignin extraction processes (Hu et al. 2023), while choline chloride (ChCl) is the most widely used HBA in the ES formulations (Xu et al. 2021). In the context of biomass processing, the combination of organic acids and ChCl to produce an ES is justified by the high delignification capacity of the resulting solvent across various biomass matrices (Li et al. 2019; Kohli et al. 2020). However, some studies have shown that acidic ES can cause lignin degradation due to the severity of its reaction with lignin bonds (Lynam et al. 2017; Tan et al. 2019; Kohli et al. 2020; Tian et al. 2020). In contrast, alcohol-based ES—such as those composed of ChCl paired with ethylene glycol, 1,3-propanediol, and 1,4-butanediol—generally exhibit lower delignification rates but preserve the lignin structure by minimizing degradation (Jablonsky et al. 2019; Hong et al. 2020). Although some ES are considered effective solvent media for lignin extraction, the subsequent recovery of the solvent remains poorly addressed, requiring the development of alternative strategies.

At the industrial scale, several separation strategies have been employed for the recovery of substances from liquid mixtures, including selective precipitation(Kiss et al. 2016), membrane separation, adsorption, and also liquid-liquid extraction (LLX) (Bo and Juanguo 2013). In the case of lignin, precipitation is the conventional method, typically induced by adding cold water as an anti-solvent, followed by vacuum distillation to recover the solvent (Smink et al. 2020a; Soares et al. 2021). However, this procedure requires high water consumption and a significant energy demand (Smink et al. 2020a). Therefore, alternative strategies, such as LLX, must be investigated to provide more efficient routes for lignin recovery and solvent reuse.

LLX is a mass transfer operation wherein solute(s) partition between two immiscible liquid phases (Bokhary et al. 2021). Within the scope of lignocellulosic biomass processing, the potentialities of various organic solvents in selectively fractionating lignin-solvent mixtures through LLX-based processes have been studied (Jääskeläinen et al. 2017; Smink et al. 2020a, 2020b; Gholami et al. 2024). Moreover, some authors have investigated the potential of biphasic systems in promoting the separation of lignin from media rich in ILs (Gogoi and

Hazarika 2019; Dias et al. 2020a) or ESs (Smink et al. 2020a; Altoé et al. 2025).

For instance, Dias et al. (2020a) described the Kraft lignin partition behavior in aqueous two-phase systems (ATPS), studying the influence of different alkyl chains and the number of hydroxyl groups of protic ILs on lignin partitioning. Similarly, Gogoi and Hazarika (2019) evaluated the effects of temperature and the alkyl chain length of the cation in imidazolium-based ILs on ATPS formation and lignin partitioning. Regarding studies involving ES, Smink et al. (2020a) found that specific thermodynamic equilibrium conditions allow for the selective recovery of lignin *via* LLX from ESs composed of choline chloride (ChCl) and lactic acid. Using 2-methyltetrahydrofuran (2-MeMTH) or its aqueous mixtures as cosolvents, they observed that the presence of water greatly increased lignin partition coefficients. More recently, Altoé et al. (2025) investigated the potential of applying (water + 1-butanol or ethyl acetate) systems to separate lignin from betaine-based ES-rich mixtures. The authors found that lignin preferentially concentrates in the cosolvent-rich phase for most studied systems, particularly those containing the ES formed by betaine and 1,4-butanediol. These results collectively suggest that biphasic systems are a promising approach for removing lignin from IL- or ES-rich mixtures.

Following our previous work with betaine-based ESs (Altoé et al. 2025), this study investigates the potential of applying biphasic systems (water-+organic cosolvent) to separate mixtures containing lignin and ChCl-based ESs, which could result from lignocellulosic biomass pretreatment with these solvents. To that end, experimental binodal curves and Kraft lignin partition coefficients were determined for systems composed of water, an organic cosolvent, and a ChCl-based ES at 298.2 K. Additionally, the empirical equations proposed by Merchuk et al. (1998) and Hu et al. (2003) were applied to correlate the obtained binodal curves. The studied ESs were prepared using ChCl as the HBA and six different HBDs, comprising organic acids and diols: formic acid (FA), acetic acid (AA), propanoic acid (PA), ethylene glycol (EG), 1,3-propanediol (PD), and 1,4-butanediol (BD). The cosolvents selected for this study were 1-butanol and ethyl acetate. The choice of 1-butanol was driven by renewed interest in its use as a sustainable solvent in biphasic systems, complemented by recent improvements in its bioproduction by yeast and bacteria (Nann et al. 2013; Dzida 2020). Similarly, ethyl acetate was selected for its biodegradability and low toxicity. Commonly produced by esterification with ethanol and acetic acid (generating water as a by-product), it has been widely employed in industrial processes over the years (Piotrowski and Kubica 2021; Janković et al. 2025). To the best of our knowledge, this is the first work to report liquid-liquid equilibrium (LLE) and lignin partition coefficient data for biphasic systems containing water, a ChCl-based ES, and 1-butanol or ethyl acetate.

Material and methods

Chemicals

Table 1 presents the acronyms, the chemical structures of the ES precursors, their CAS numbers, and the mole HBA:HBD proportions of the ESs used to form the biphasic systems. Three of the precursors used as HBD are diols with alkyl chain lengths ranging from C_2 to C_4, while the others are organic acids with alkyl chain lengths ranging from C_1 to C_3. ChCl was used as the HBA in all ES formulations. In this work, a total of 12 biphasic systems were studied, composed of water, an organic solvent (1-butanol or ethyl acetate), and an ES (ChCl:FA, ChCl:AA, ChCl:PA, ChCl:EG, ChCl:PD, or ChCl:BD).

The biphasic systems were prepared using ES composed of ChCl (>98 wt%, Interlab, CAS number 67-48-1), propionic acid (≥99 wt%, Acros Organics), formic acid (>99% wt%, Dinamica), acetic acid (glacial, Merk), ethylene glycol (>99.5 wt%, Dinamica), 1,3-propanodiol (98 wt%, ACROS), 1,4-butanodiol (>99 wt%, Dinamica), 1-butanol (>99.8 wt%, Sigma-Aldrich), ethyl acetate (>99.8 wt%, Sigma-Aldrich), and bidistilled water. The Kraft lignin, derived from *Eucalyptus urograndis*, was kindly donated by Suzano S/A (Suzano, Brazil). All reagents were used as received from the supplier, without further purification.

Table 1. The HBD and HBA, their chemical structures, CAS numbers, and mole HBA:HBD ratios used to prepare the ESs studied in this work.

HBA chemical structure	HBD chemical structure	HBD CAS number	Molar ratio (HBA:HBD)	Abbreviation
Choline chloride (ChCl)	Formic acid (FA)	64-18-6	(1:2)	ChCl:FA
	Acetic acid (AA)	64-19-7	(1:2)	ChCl:AA
	Propionic acid (PA)	79-09-4	(1:2)	ChCl:PA
	Ethylene glycol (EG)	107-21-1	(1:1)	ChCl:EG
	1,3-propanediol (PD)	504-63-2	(1:2)	ChCl:PD
	1,4-butanediol (BD)	110-63-4	(1:1)	ChCl:BD

Preparation of ES

The HBD and the HBA precursors were placed in sealed glass flasks at the corresponding molar ratio and stirred (at 333.2 K) on a shaking bath (304-DE T5, Ethyk Technology, Brazil) until a clear, homogeneous solution was formed (Ruesgas-Ramón et al. 2017; Škulcová et al. 2017; Hou et al. 2018). Following preparation, the ES flasks were allowed to cool to room temperature, and their water content was determined by Karl Fischer titration (831 KF coulometer, Metrohm, Switzerland). The measured water content was accounted for in the composition calculations for all LLE and partitioning experiments. The prepared solvents were stored in a desiccator under vacuum conditions before use.

Binodal curves

All LLE experiments were carried out at (298.2 ± 0.1) K and ambient pressure (94.3 ± 0.2 kPa) in a jacketed glass cell (21 cm^3) attached to a thermostatic water bath (TE-184, Tecnal, Brazil) to maintain a constant temperature, following the procedure described in detail in our previous work (Altoé et al. 2025). In brief, the mixture was maintained under continuous agitation using a magnetic stirrer to ensure thorough mixing throughout all assays. The binodal curves were determined experimentally by the cloud-point titration method (Jafari and Jouyban 2021). In total, experiments were carried out for twelve systems, resulting from the combination of the six selected ESs (ChCl:FA, ChCl:AA,

ChCl:PA, ChCl:EG, ChCl:PD, and ChCl:BD) with water and either 1-butanol or ethyl acetate.

The experiments were conducted as follows: a known quantity of distilled water was placed in the cell under constant magnetic stirring (200 rpm). Then, the organic cosolvent (1-butanol or ethyl acetate) was added dropwise until turbidity was observed, indicating the formation of a second phase. Subsequently, ES was slowly titrated into the biphasic system until the turbidity disappeared, restoring a single homogenous phase. The amounts of ES, water, and 1-butanol/ethyl acetate were measured using an analytical balance (AX 200, ± 1×10^{-4} g, Shimadzu, Japan), and the first point of the binodal curve was recorded. This procedure was repeated at different concentrations until the immiscibility region was fully delimited.

The experimental binodal curves data were adjusted to the model proposed by Merchuk et al. (1998) (Equation (1)) and Hu et al. (2003) (Equation (2)) as follows:

$$Y = Ae^{(BX^{0.5}-CX^3)} \tag{1}$$

$$Y = e^{(A+BX^{0.5}+CX+DX^2+EX^3)} \tag{2}$$

where Y and X are the cosolvent and water mass fractions, respectively, and A, B, C, D, and E are adjustable parameters obtained by nonlinear regression of the experimental data from the binodal curves. For the modeling, the ES was treated as a single pseudo-component. This pseudo-ternary approach is a necessary simplification for applying these mass-balance-based models (Li et al. 2022; Mashhaditafreshi and Haghtalab 2024; Vessally and Rzayev 2024).

The fitting of the Merchuk and Hu parameters was performed using the *curve_fit* function from the *scipy.optimize* library in Python (version 3.12.11), which utilizes the Levenberg–Marquardt algorithm (Moré 1978). To evaluate the quality of the fit, the Mean Squared Error (MSE) and coefficient of determination (R^2) were calculated based on the comparison of the experimental and the calculated LLE data, according to Equations (3) and (4), respectively:

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}(Y_{\mathrm{exp}} - Y_{\mathrm{calc}})^2 \tag{3}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_{\mathrm{exp}} - Y_{\mathrm{calc}})^2}{\sum_{i=1}^{n}(Y_{\mathrm{exp}} - \overline{Y}_{\mathrm{exp}})^2} \tag{4}$$

where n is the number of experimental data points; Y_{exp} and Y_{calc} are the experimental and calculated mass fractions of the cosolvent along the binodal curve, respectively, and $\overline{Y}_{\mathrm{exp}}$ represents the mean experimental cosolvent mass fraction.

Kraft lignin partitioning

Partition coefficients for Kraft lignin (K_L) were determined by preparing five mixtures at known compositional points. Kraft lignin was initially dissolved in ESs, followed by the addition of water and the cosolvent (1-butanol or ethyl acetate). The mixtures were homogenized by agitation on a vortex mixer (K45-2820, Kasvi, Brazil). Subsequently, the mixtures were kept in a thermostatic water bath (TE-184, Tecnal, Brazil) at 298.2 K for 24 h to achieve equilibrium. Then, the top and bottom phases were carefully separated and weighed for subsequent quantification and density measurements. The latter was conducted at (298.15 ± 0.01) K (DMA 4500 density meter, Anton Paar, Austria), following the ASTM D7042-11a method. The standard deviation of the obtained density values is estimated at 0.01 $kg{\cdot}m^{-3}$.

To quantify Kraft lignin partition coefficients, sample aliquots (approximately 0.1–0.3 cm^3) collected from the top and bottom phases were transferred to empty sealed flasks, precisely weighed on an analytical balance (AUY220, Shimadzu, Japan), and dissolved in dimethyl sulfoxide (DMSO). The solutions were then analyzed *via* UV-Vis spectrophotometry (Mini 1240, Shimadzu, Japan) at 280 nm. Lignin concentrations in each phase were determined using a previously established calibration curve ($R^2 > 0.999$) (Altoé et al. 2025). Prior to analysis, all samples were diluted with DMSO to ensure that the measured absorbances fell within the calibration curve's linear range. To eliminate potential background interference from solvents or cuvettes, baseline corrections were systematically applied before each measurement. Furthermore, preliminary UV-Vis scans of each

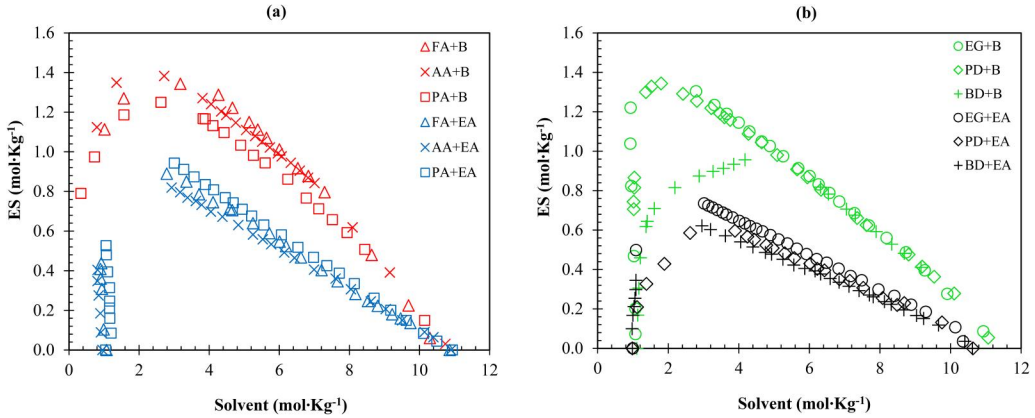

Figure 1. Binodal curves for the studied biphasic systems at 298.2 ± 0.1 K and 94.3 ± 0.2 kPa. (a) exhibits the curves for systems with acid-based ES; (b) shows the curves for systems containing alcohol-based ES.

individual phase-forming component (water, 1-butanol, ethyl acetate, and the neat ESs) confirmed the absence of overlapping of absorption bands at 280 nm. This methodology has also been validated in previous studies from our group (Dias et al. 2020c; Dias et al. 2020a; Altoé et al. 2025), as lignin exhibits a characteristic absorbance at 280 nm due to the presence of aromatic rings in its structure (Kline et al. 2010). The partition coefficients (K_L) were calculated according to Equation (6):

$$K_L = \frac{C_L^T}{C_L^B} \tag{5}$$

where C_L^T and C_L^B are the Kraft lignin molar concentrations on the top and bottom phases, respectively.

Results and discussion

Binodal curves

Knowledge of binodal curves is crucial for developing new LLE-based processes, such as extraction, purification, or separation, as the determination of the immiscibility region provides essential information for designing new strategies and technologies. The ChCl-based ESs used in this study are hydrophilic due to the ability of both precursors to form hydrogen-bond networks (Gabriele et al. 2019; Gajardo-Parra et al. 2020). Previous studies demonstrate that choline chloride has a strong affinity for water (Vilas-Boas et al. 2020; Ferreira et al. 2022), predominantly driven by hydrogen bonding and electrostatic interactions. Similarly, the organic acids and diols used as HBDs in this work are fully miscible with water (Moosavi and Rostami 2017; Clavilier et al. 2025). This behavior is attributed to the presence of a carboxyl group (–COOH) in the acids and two hydroxyl groups (–OH) in the diols, which facilitate interactions with water molecules.

Conversely, as previously reported, the less polar 1-butanol and ethyl acetate are partially soluble in water (Góral et al. 2006, 2009), a behavior confirmed in this study. These solvents possess longer alkyl chains than fully water-miscible short-chain alcohols (e.g., ethanol, 1-propanol, 2-propanol), resulting in stronger hydrophobic interactions that limit their aqueous solubility. Furthermore, these solvents have fewer available hydrogen-bonding sites compared to the diols used to formulate the ESs, which explains their lower affinity for water.

The binodal curves determined at 298.2 K and ambient pressure (94.3 kPa) are presented in Figure 1. These curves are plotted in molality units to minimize biases arising from differences in molecular weight among the studied ESs and to facilitate a fair comparison of their ability to form biphasic systems. The experimental LLE data measured for the binodal curves are shown in Tables S1 and S12 of the Supplemental Material (SM) in mass percentages (w_t%).

For some of the studied systems, particularly those with ethyl acetate, the binodal curves lack data points in regions with high ES content, where the visual identification of the immiscibility point using the titration method is more challenging. A similar behavior was reported in our

previous work for aqueous systems containing betaine-based ESs and 1-butanol or ethyl acetate (Altoé et al. 2025).

The binodal curves shown in Figure 1 display distinct profiles, indicating that the addition of ES alters the LLE of the water/organic cosolvent systems. In general, regions from the binodal curves with high concentrations of cosolvent exhibit low ES content, whereas larger amounts of ESs are typically observed in regions with high water contents and moderate cosolvent levels. This observation aligns well with the strong hydrophilicity character of the studied ES constituents and with findings from previous works investigating the LLE behavior of ES + water-+ organic cosolvent systems (Gomes et al. 2024; Souza et al. 2024; Altoé et al. 2025). Due to their low mutual affinity and density differences, the top phase is expected to be rich in 1-butanol (806 $kg{\cdot}m^{-3}$, at 298.2 K) (Dzida 2020) or ethyl acetate (894 $kg{\cdot}m^{-3}$, at 298.2 K) (González et al. 2007), while the bottom phase will likely contain a higher water content (997 kg/m^3, at 298.2 K) (González et al. 2007).

As observed in Figure 1a,b, the binodal curves obtained in systems containing 1-butanol exhibit considerably larger immiscibility regions than those obtained for systems with ethyl acetate. A similar behavior was recently reported by Altoé et al. (2025), who also observed that 1-butanol is more effective as a phase-separating agent when added to aqueous betaine-based ES mixtures than ethyl acetate. From a technical standpoint, the more extensive biphasic regions observed for the systems with 1-butanol are advantageous for LLX-based processes, as they represent a larger potential working area. Such systems provide greater operational flexibility, accommodating a wider range of feed compositions without exiting the biphasic condition.

Although not as pronounced as the cosolvent effect, the binodal curves in Figure 1 show that the ES plays a significant role in the size of the biphasic systems formed. For the water + ES + 1-butanol systems, the following order regarding the extent of the binodal curve was observed: AA ≈ FA > PA ≈ EG ≈ PD > BD. Regarding the acetate-based systems, the ability to form a biphasic system can be ranked as follows: PA > FA > AA > EG > PD > BD. While the acid-based ESs were all prepared at a 1:2 molar ratio (HBA:HBD), the diol-based ESs were formulated at different molar ratios (1:1 and 1:2), making a direct comparison less straightforward. However, it is evident that acid-based ES generally promoted wider immiscible regions for both tested cosolvents when compared to the systems containing the diols. Comparing the studied ESs, those formed by organic acids are generally considered more polar than alcohol-based ones due to the presence of the carboxyl group, which exhibits higher hydrogen bond acidity than the hydroxyl group found in diols (Dai et al. 2013; Mankar et al. 2021). Consequently, this increased polarity promotes stronger intermolecular interactions with water, favoring the segregation of the ES into the aqueous regions and reducing the miscibility of the organic cosolvent. As a result, the biphasic region of these systems spans a larger composition range.

To complement the experimental studies, the binodal curves were correlated with Merchuk's (Equation (1)) and Hu's (Equation (2)) empirical equations. The obtained parameters for the Merchuk and Hu models are listed in Tables 2 and 3, respectively, while the correlated binodal curves are compared with the experimental data in Figures S1 and S12 of the SM.

In general, the Hu model provides a superior representation of the binodal curves compared to the Merchuk model, achieving R^2 values greater than 0.998 and MSE values lower than 0.82×10^{-4} for all the studied systems. Regarding the Merchuk model, good correlations were obtained

Table 2. Regressed parameters and statistical metrics obtained for the correlation of the binodal curves using the Merchuk equation (Equation (1)) for all the studied systems.

ES	*A*	*B*	*C*	R^2	$10^4 MSE$
ES + water + 1-butanol					
ChCl:FA	9.143	−5.266	1.488	0.998	0.60
ChCl:AA	6.677	−4.803	3.134	0.997	0.99
ChCl:PA	6.648	−4.800	3.269	0.998	0.65
ChCl:EG	3.132	−3.213	4.217	0.984	7.85
ChCl:PD	2.927	−3.135	6.713	0.987	7.33
ChCl:BD	4.281	−4.204	0.254	0.998	0.85
ES + water + ethyl acetate					
ChCl:FA	1.324	−1.314	3.468	0.990	7.59
ChCl:AA	1.290	−1.283	3.839	0.991	6.97
ChCl:PA	1.471	−1.774	2.567	0.980	16.0
ChCl:EG	1.289	−1.231	4.059	0.995	2.17
ChCl:PD	1.383	−1.447	2.728	0.994	3.49
ChCl:BD	1.395	−1.460	2.872	0.994	4.40

for the system containing acid-based ESs and 1-butanol ($R^2 \geq 0.997$, MSE $< 1.00\times10^{-4}$), while higher deviations were observed for the systems containing ethyl acetate as the cosolvent ($0.980 \leq R^2 \leq 0.991$, $6.97\times10^{-4} \leq$ MSE $\leq 16.0\times10^{-4}$). For the biphasic systems containing diol-based ESs, $R^2 \geq 0.99$ was obtained for the 1-butanol + ChCl:BD and for all ethyl acetate-based systems described with the Merchuk equation. In contrast, higher deviations ($0.98 < R^2 < 0.99$) were observed for the other systems with 1-butanol as the cosolvent. Notably, the Hu model typically outperforms the Merchuk model when describing the water-rich compositions of the studied systems (Figure S1 and S12 of the SM). A similar behavior was reported by Altoé et al. (2025) for aqueous biphasic systems containing betaine-based ES and 1-butanol or ethyl acetate.

Table 3. Regressed parameters and statistical metrics obtained for the correlation of the binodal curves using the Hu equation (Equation (2)) for all the studied systems.

ES	*A*	*B*	*C*	*D*	*E*	R^2	10^4*MSE*
ES + water + 1-butanol							
ChCl:FA	17.553	−85.042	119.88	−98.458	44.423	0.999	0.23
ChCl:AA	22.818	−119.84	182.77	−167.64	83.696	0.999	0.19
ChCl:PA	10.983	−54.368	78.151	−70.544	32.519	0.999	0.50
ChCl:EG	5.893	−33.975	56.531	−65.760	35.952	0.998	0.82
ChCl:PD	16.308	−96.381	164.31	−182.66	105.58	0.999	0.59
ChCl:BD	5.489	−26.488	35.236	−30.879	14.298	0.999	0.38
ES + water + ethyl acetate							
ChCl:FA	0.374	−3.056	5.574	−11.67	5.970	0.999	0.58
ChCl:AA	0.336	−2.705	4.704	−10.465	5.206	0.999	0.68
ChCl:PA	0.333	−2.856	5.556	−14.858	9.714	0.999	0.69
ChCl:EG	0.878	−6.080	10.403	−15.415	7.467	0.999	0.38
ChCl:PD	0.587	−4.105	6.703	−11.345	5.529	1.000	0.11
ChCl:BD	0.678	−4.750	8.017	−13.096	6.394	1.000	0.29

Kraft lignin partitioning

The Kraft lignin partition coefficient (K_L) was measured to evaluate the preferential distribution of lignin across all twelve studied biphasic systems. Measurements were conducted in systems with different overall compositions, with the global ES content ranging from 10 to 30 wt% for the 1-butanol systems and from 1.5 to 15.2 wt% for the ethyl acetate-based systems. These values were selected based on the extent of the biphasic regions; as shown in Figures S1–S6, the systems containing 1-butanol exhibit larger biphasic regions than the corresponding systems with ethyl acetate as the organic cosolvent. The obtained K_L profiles as a function of the global ES composition are depicted in Figure 2, while the specific partition coefficients and corresponding overall compositions are listed in Tables S13 and S14 of the SM.

The experimental results revealed that Kraft lignin shows a stronger affinity for the cosolvent-rich top phase, as evidenced by lignin partition coefficients greater than unity in all evaluated systems. This behavior is primarily driven by the hydrophobic character of Kraft lignin (Lisý et al. 2022). Because the bottom phase contains large amounts of water, the hydrophobic effect drives Kraft lignin into the top phase, as it is repelled by the water-rich environment (Sosa et al. 2020; Dias et al. 2024). Although pure ESs typically exhibit a strong affinity for Kraft lignin, previous reports have shown that the presence of large amounts of water sharply decreases interactions between lignin and ES, thereby reducing lignin

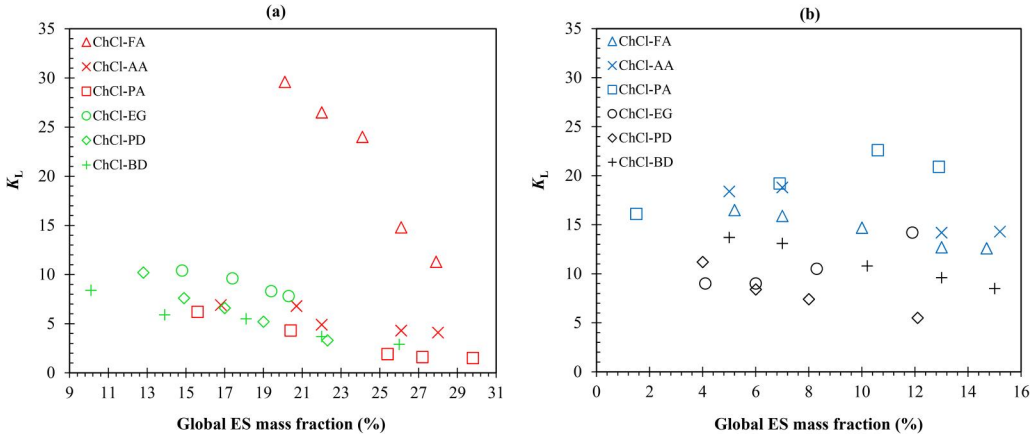

Figure 2. Experimental Kraft lignin partition coefficients at (298.2 ± 0.1) K and (94.3 ± 0.2) kPa. (a) water + ES + 1-butanol; (b) water + ES + ethyl acetate.

concentration in the water-rich bottom phase (Sosa et al. 2020, 2024).

Regarding the evolution of the partition coefficients, the K_L values generally decrease as the global ES content increases for all 1-butanol-containing systems, as well as in three systems containing ethyl acetate (those with ChCl:FA, ChCl:PD, and ChCl:BD). This indicates that lignin is more highly concentrated in the top phase (i.e., the 1-butanol or ethyl acetate-rich phase) when lower concentrations of the eutectic solvent are present. In fact, ChCl-based ESs usually possess a strong affinity for water (Chen et al. 2019; Brusas and Dela Pena 2021), particularly those composed of hydrophilic anions, such as those studied here. Due to their highly hydrophilic character, the ChCl-ESs are expected to be predominantly concentrated in the bottom water-rich phase, as previously reported by Gomes and coauthors for the water + 1-butanol + ChCl:glycerol system (Gomes et al. 2024). Because Kraft lignin exhibits good solubility in ESs formed by ChCl paired with diols or organic acids (Sosa et al. 2020), increasing the overall concentration of ES in the system enhances the capacity of the water-rich bottom phase to partially retain lignin. Consequently, lower K_L values are observed compared to systems with lower ES contents, although lignin partitioning remains favorable to the top organic phase. For the remaining three systems with ethyl acetate (ChCl:AA, ChCl:PA, and ChCl:EG), no clear trend was observed between the partition coefficient profiles and the overall ES composition.

The K_L profiles obtained for the water + 1-butanol + ES systems (Figure 2a) also reveal clear patterns related to the HBD of the ES. For instance, increasing the alkyl chain length of the organic acids (from C1 to C3) or the diols (from C2 to C4) decreases the K_L values. This indicates that as the carbon chain size increases, the hydrophobicity of the ES also increases, thereby enhancing the lignin-ES interactions. Since the ES are expected to concentrate primarily in the bottom phase, solvents formulated with larger HBDs are likely to improve lignin retention in the bottom phase, reducing the partition coefficients compared to systems with shorter-chain HBDs. A similar trend was reported by Dias et al. (2020a) when studying the ATPSs composed of water, acetone, and hydroxyethylammonium-based Protic ILs containing anions equivalent (C1 to C3) to the HBDs studied in this work.

In contrast, lignin partitioning behavior in systems containing ethyl acetate (Figure 2b) is more complex. For mixtures containing acid-based ESs, an opposite trend to that observed for the 1-butanol systems was identified (i.e., the K_L values rank as: PA > AA > FA), while no clear correlation with the carbon chain length was observed for the diol-based ES systems. In the latter, higher K_L values were obtained in the system with 1,4-butanediol than in that with 1,3-propanediol, while the system with ethylene glycol exhibited a distinct upward trend with increasing ES composition, in contrast to the profiles of the other two diol-based systems. A similar absence of a clear correlation between K_L profiles and HBD structure was reported in our previous work (Altoé et al. 2025) for systems composed of water, ethyl acetate, and betaine-based ESs containing the identical diols studied here. These distinctive behaviors observed in systems containing the aprotic polar ethyl acetate suggest that lignin partitioning in such systems is governed by a more complex interplay of hydrophobic and polar forces than in the 1-butanol-based systems.

Among the evaluated aqueous biphasic systems, the most promising results for Kraft lignin recovery were obtained for the ChCl:FA + 1-butanol, as well as the three systems containing organic acid-based ES and ethyl acetate; for such systems, $K_L >$ 10 were achieved across all investigated mixtures. In the context of lignin-ES fractionation, high lignin partition coefficients are highly desirable (i.e., lignin strongly distributed in the upper phase), as the ES is expected to concentrate in the water-rich bottom phase (Smink et al. 2020a; Gomes et al. 2024). The highest partition coefficients were registered for the (water + 1-butanol + ChCl:FA) mixture ($11.3 \leq K_L \leq 29.6$). These high values, combined with the substantially large biphasic region observed for this system (Figure S1), indicate that the water + 1-butanol mixture is a promising platform for recovering lignin from its mixtures with the ChCl:FA solvent. Likewise, the ethyl acetate-water mixture might also be an interesting option for the acid-based ES, particularly for

the ChCl:PA, where $K_L > 16$ was sustained across a wide range of ES compositions (1.5–12.9 wt%).

A comparison with previously reported data highlights the potential of the studied water-organic cosolvent mixtures for separating Kraft lignin from ChCl-based ESs. Dias et al. (2020a) evaluated the Kraft lignin partitioning behavior in ATPSs composed of acetone (cosolvent) and nine different alkanolammonium-based protic ILs, achieving K_L values ranging from 0.6 to 5.0. Although Kraft lignin has a higher reported solubility in acetone (Domínguez-Robles et al. 2018) than in 1-butanol or ethyl acetate (Dastpak et al. 2020), significantly higher lignin migration to the cosolvent-rich top phase was observed in the present study compared to the results reported by Dias et al. (2020a). In a very recent study, Altoé et al. (2025) investigated lignin distribution in aqueous biphasic systems composed of the same cosolvents utilized here and a series of four betaine-based ESs. While their results show a broader range and superior partition coefficients ($3 \leq K_L \leq 456$) compared to our diol-based ES systems, only moderate values ($0.9 \leq K_L \leq 4.6$) were obtained for systems containing an ES formed by betaine and urea (2:3).

Beyond the efficiency of the biphasic system in separating lignin from the eutectic solvent, confirming the structural integrity of the recovered lignin is also important to ensure that no undesired chemical modifications occurred during the process. To evaluate this, a lignin sample recovered from the upper phase of the most promising system (water + ChCl:FA + 1-butanol) was analyzed by 2D Heteronuclear Single-Quantum Correlation Nuclear Magnetic Resonance (HSQC NMR) spectroscopy and compared to the spectrum of the native Kraft lignin. The detailed procedures for the precipitation and recovery of the lignin from the biphasic media, along with the specific HSQC acquisition parameters, are provided in the SM.

The HSQC NMR spectra of the native and recovered Kraft lignin are presented in Figures S13 and S14, respectively. Consistent with standard lignin analysis, we focused our assessment on the oxygenated aliphatic (δC/δH 50–95/2.5–5.0 ppm) and aromatic (δC/δH 100–135/6.0–8.5 ppm) regions, avoiding the pure aliphatic region (δC/δH 5.0–35/0.5–2.5 ppm), which is often congested with signals from contaminants or degradation products (Dias et al. 2024). The main ^{1}H–^{13}C correlation cross peaks, assigned by comparing the experimental chemical shifts with established literature data (Prinsen et al. 2012; Sun et al. 2014; Dias et al. 2020b; Sun 2020; Dias et al. 2024), are summarized in Tables S15 and S16. The corresponding identified lignin substructures are illustrated in Figure S15.

A comparative analysis reveals that the fundamental structural features of native Kraft lignin are fully preserved in the sample recovered from the selected biphasic system. The oxygenated aliphatic regions confirm the retention of key inter-unit linkages, specifically β-O-4′, resinols, phenylcoumarans, and spirodienones. Traces of the xylan backbones are also present in both spectra, albeit less prominently in the recovered sample. Similarly, the aromatic core remains intact post-extraction, with the characteristic syringyl, guaiacyl, and cynnamyl (alcohols, aldehydes, or acids) units clearly present, though with a slight reduction in the cynnamyl derivative signals in the recovered lignin spectrum. Notably, the vertical band observed around δH ~3.7 ppm in the proton dimension of the recovered lignin spectrum corresponds to residual choline from the eutectic solvent. As recently evidenced, choline moieties can become strongly entrapped or even covalently bonded within the lignin matrix during extraction, making complete removal challenging (Hong, Shen, et al. 2020; van Erven et al. 2024). Overall, these NMR results confirm that the core macromolecular structure of Kraft lignin is preserved following extraction with the water + ChCl:FA + 1-butanol system.

The findings reported in this work, alongside those reported by Dias et al. (2020a) and Altoé et al. (2025) demonstrate that selecting a biphasic water-cosolvent system for lignin recovery from alternative pretreatment solvents is a nontrivial task. Nevertheless, our results suggest that 1-butanol and ethyl acetate are promising options for recovering Kraft lignin from the studied ESs. Specifically, the alcohol yielded superior results for the ChCl:FA system, while the ester offered satisfactory partition coefficients across the ESs composed of the three tested organic acids. Moreover,

the data presented here, combined with those reported by Altoé et al. (2025), strongly encourage the use of water + 1-butanol and water + ethyl acetate mixtures in future studies aimed at recovering lignin from other alternative solvents.

Conclusions

This study investigates the formation of biphasic systems composed of an organic cosolvent (1-butanol or ethyl acetate) and a ChCl-based ES (ChC:FA, ChCl:AA, ChCl:PA, ChCl:EG, ChCl:PD, or ChCl:BD) as a potential strategy to recover Kraft lignin from ES-rich mixtures. Experimental binodal curves for twelve (water + organic cosolvent + ES) systems were determined *via* the titration method, and Kraft lignin partition coefficients were measured for all investigated biphasic systems across different overall compositional ranges. To complement the experimental data, the phase boundary data were correlated with the Merchuk and Hu empirical models, with the latter providing the best description across all systems. Finally, the main structural features of the lignin recovered from the most promising biphasic system were compared against those of native Kraft lignin using 2D HSQC NMR spectroscopy.

In general, the measured binodal curves indicate that the addition of 1-butanol favors biphasic system formation compared to ethyl acetate, resulting in substantially larger biphasic regions. Regarding the effect of the HBD structure, larger immiscible regions were observed for acid-based ESs compared to diol-based ESs. This behavior is attributed to the greater ability of carboxylic groups to interact with water than that of the hydroxyl groups present in the diols, which favors the migration of the organic cosolvent to the top phase.

The partitioning studies revealed that lignin preferentially concentrates in the top, organic-rich phases across all investigated systems ($K_L > 1$). The most promising results were obtained for the water + 1-butanol + ChCl:FA system ($11.3 \leq K_L \leq 29.6$) and for the systems containing water + ethyl acetate + organic acid-based ES ($12.6 \leq K_L \leq 22.6$). Conversely, lower partition coefficients were typically observed for the diol-based systems (ranging between 3 and 14). A distinct influence of the solvent nature was identified: in systems with 1-butanol, increasing the HBD alkyl chain length decreased the partition coefficients for both diol-based and organic acid-based ESs. In contrast, an opposite trend was observed for systems containing acid-based ESs and ethyl acetate as the cosolvent (where K_L increased with the chain length of the acid), while no clear trend was found for the (water + ethyl acetate + diol-based ES) systems. The 2D HSQC NMR analyses revealed that lignin recovered from the water + 1-butanol + ChCl:FA system exhibited the same fundamental structural features as the native Kraft lignin.

Collectively, the findings of larger biphasic regions and higher partition coefficients suggest that the water + ethyl acetate mixtures are potential alternatives for recovering Kraft lignin from the acid-based ESs studied herein, whereas 1-butanol stands out as a promising option for separating lignin from the ChCl:FA solvent. To the best of our knowledge, binodal curves and lignin partitioning behavior for these (water + cosolvent + ChCl-based ES) systems are reported for the first time in this study. Beyond expanding the limited experimental dataset for water + ES + additive systems, this work supports the application of aqueous mixtures of 1-butanol and ethyl acetate as efficient agents for recovering lignin from other promising alternative solvents used in biomass delignification routes.

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Authors contributions

CRediT: **Fernanda Sossai Altoé**: Investigation, Software, Validation, Writing – review & editing; **Larissa Kiyomi Tsukamoto**: Data curation, Investigation; **Lenin Pedroni Ivonica**: Data curation, Investigation; **Fabiano Scolfaro Cheri**: Methodology; **Wellington Moreira Corrêa**: Methodology; **Rafael Macedo Dias**: Data curation, Supervision, Visualization, Writing – original draft; **Sérgio M. Vilas-Boas**: Data curation, Supervision, Visualization, Writing – original draft; **Mariana Conceição da Costa**: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of generative artificial intelligence (AI)

During the preparation of this work, the authors used Gemini 2.5 Pro exclusively to improve language and

readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Disclosure statement

No potential conflict of interest is reported by the author(s).

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