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Optimizing lignin cationization: Unveiling the impact of reaction conditions through multi-response analysis

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ABSTRACT

Lignin, a natural and abundant biopolymer, holds great potential for cosmetic applications. However, its limited solubility and inherently negative charge restrict its use in certain formulations. This is particularly relevant for hair conditioning, where a positively charged polymer is preferred to effectively treat negatively charged damaged hair. To overcome this limitation, lignin extracted from acacia wood residues was chemically cationized, through an etherification reaction, using 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) as cationization agent. The cationization process was optimized through a full factorial design of experiments aiming to maximize both the degree of substitution (DS) and ζ-potential of the lignin derivatives, thereby obtaining more positively charged derivatives. The reaction parameters temperature and CHPTAC-to-lignin molar ratio were selected as independent variables to be optimized. Results revealed that the reaction temperature is the most relevant parameter for lignin cationization, showing a significant positive influence on lignin modification, while the CHPTAC-to-lignin ratio has no significant impact. Under optimal conditions, soluble derivatives with a DS of 0.61 ± 0.03 and a ζ-potential of 18.79 ± 0.30 mV were obtained, confirming the success of the reaction. This straightforward and efficient approach enhances acacia's lignin solubility and charge properties, broadening its application potential. In particular, it offers a promising alternative for hair conditioning, thus contributing to the sustainable development of bio-based cosmetic ingredients.

1. Introduction

Hair is an important feature for humans, playing a crucial role in the protection against external elements, thermoregulation, and social interaction. As a result, any disturbance to hair vitality can have a significant psychological impact. Its overall health and appearance are influenced by several genetic and environmental factors [1]. This drives the vast variety of hair care products available and for the projected growth of the hair care market from USD 99.52 billion in 2023 to USD 213.47 billion by 2032 [2]. These products, including shampoos and conditioners, are primarily designed to cleanse, protect, and enhance hair appearance [3]. One of the most widely used cosmetic products is hair conditioners. These products help preventing static electricity, seal the cuticles, reduce friction, enhance softness and shine, improve manageability, and provide protection [4]. One of the main mechanisms of hair conditioning is the deposition of cationic species to neutralize the negative surface charge of damaged hair [3]. These cationic compounds, usually cationic surfactants or cationic polymers, are very efficient at treating damaged hair. However, they usually have a fossil origin, present low biodegradability, and can cause skin and eye irritation. Additionally, they have a negative impact on the environment, leading to long-term adverse effects on aquatic organisms [5]. Hence, there is a significant interest in replacing traditional petrochemical ingredients with alternatives that have a lower impact on health and the

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environment [6]. In this regard, biocompatible and biodegradable polymers obtained from natural sources are a promising solution to meet this demand.

Lignin, with an estimated availability in the biosphere of about 300 billion tons [7], is considered one of the most abundant renewable resources. It is, thus, regarded as a promising feedstock for the development of biocompatible and biodegradable polymers. Its unique structure, characterized by an aromatic and aliphatic backbone rich in different functional groups, makes it an appealing candidate for the design of sustainable and functional biomaterials [8]. Lignin has been recognized as a valuable asset for cosmetic applications given its remarkable antioxidant and antimicrobial activities, stabilizing properties, and ability to absorb ultraviolet (UV) radiation [9]. These features have led to its use as UV-blocking, whitening, and anti-aging ingredient in cosmetic formulations [10].

Despite its valuable properties for cosmetic products, lignin has never been reported as a hair conditioning agent. This is due to the negative surface charge of unmodified lignin which is not beneficial for this application. However, the presence of hydroxyl, carboxyl, methoxy, and carbonyl groups makes lignin versatile for different functionalization procedures [8]. This is particularly attractive for the development of cationically-modified derivatives as alternatives to traditional fossil-based cationic conditioners. Among the various modification routes, cationization by etherification with a cationic epoxide is a very appealing strategy to obtain cationic derivatives that can interact with negatively charged surfaces, such as damaged hair. This approach is an alternative to lignin amination via Mannich reaction, which has important limitations, such as low yield, the need for aldehydes, harsh reaction conditions, and the use of toxic solvents such as dioxane [11]. As a result, interest has shifted toward reaction involving electrophilic reagents containing quaternary ammonium salts [12,13]. 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) is one of the most commonly used reagents for lignin cationization, with several reports documenting its effectiveness [12,14,15]. It features a reactive site linked to a polar quaternary ammonium group through a short and flexible C3 alkyl chain, along with a polar hydroxyl group [12]. The cationization of lignin with CHPTAC is performed in aqueous alkaline medium, which serves several purposes in this reaction: it acts as a solvent to solubilize lignin and as a catalyst, increasing the accessibility to the lignin's reaction sites by generating a nucleophilic intermediate. The alkaline medium is also responsible for the conversion of CHPTAC into the reactive epoxide, 2,3-epoxypropyltrimethylammonium chloride (EPTAC), also known as glycidyltrimethylammonium chloride (GTAC). CHPTAC is converted to EPTAC by the addition of 1:1 M equivalent of NaOH (Fig. 1, reaction A). The epoxide is then attacked by the lignin nucleophilic intermediate via an oxirane ring-opening reaction, resulting in the desired cationized lignin with the covalently attached 2-hydroxy-3-(*N*,*N*,*N*-trimethylammonium)-propyl moiety (Fig. 1, reaction B) [12,16]. A competing side reaction can occur, where EPTAC undergoes hydrolysis to form a non-reactive diol byproduct, which may reduce the amount of the active modifying reagent (Fig. 1, reaction C). In the presence of excess base, a second hydrolysis reaction can also occur, where the newly formed cationic lignin derivative reacts with water (reaction D), reducing the overall cationization degree. Therefore, the neutralization of the medium after the reaction is complete is essential [16].

Lignin from different sources has been previously cationized with CHPTAC or directly with EPTAC/GTAC and some studies report the optimization of reaction conditions, such as the reaction temperature, time and ratio of the reagents [17–19]. However, to the best of our knowledge, an in-depth statistical analysis of the key parameters affecting lignin cationization by etherification has not been described yet. In addition, most of the works report the cationization of commercially available technical lignins obtained from the pulping processes. For example, kraft lignin is obtained from the alkaline fractionation of wood. The chemical structure of kraft lignin is, therefore, significantly different from that obtained, for example, from acidic extractions, which have been widely reported recently based on the use of acidic deep eutectic solvents (DES). Thus, the study of the cationization of DES-lignin is of scientific relevance to understand the chemistry of these increasingly available lignins.

The aim of this study is to prepare new lignin derivatives with physicochemical properties suitable for future application as hair conditioning agents. Specifically, the derivatives should be water-soluble and cationically charged. To achieve this, lignin with high purity (91.45 %) was extracted from acacia wood using a natural DES (NADES)

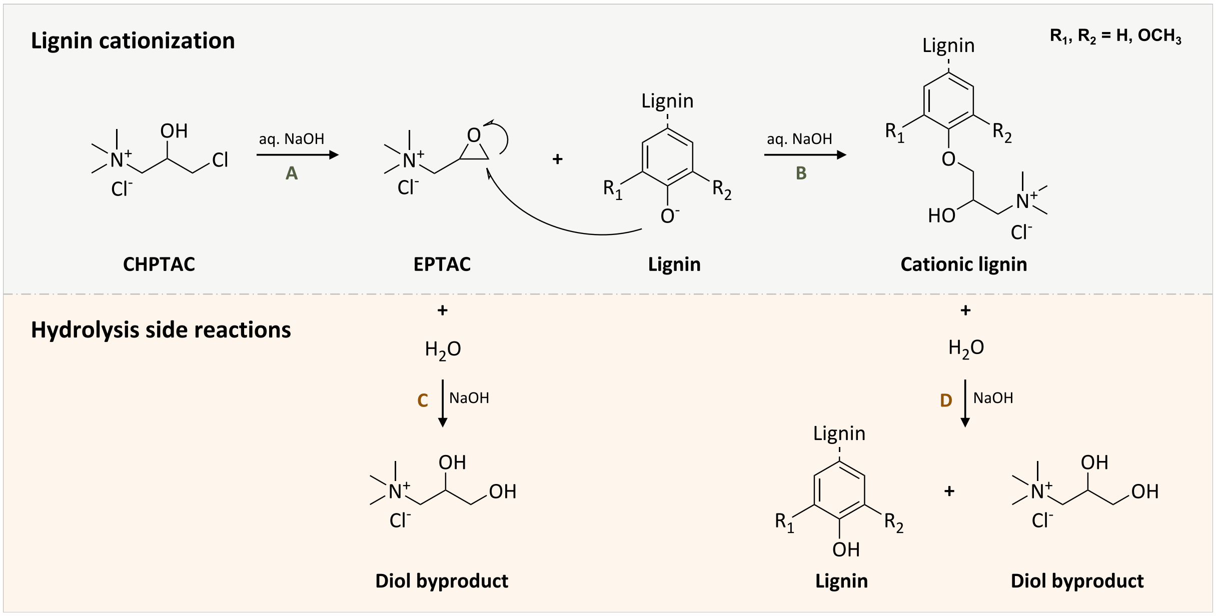

Fig. 1. Cationization of lignin using CHPTAC in NaOH aqueous solution (top). CHPTAC is converted to EPTAC under alkaline conditions (reaction A). EPTAC is attacked by lignin via an oxirane ring-opening reaction, forming the final cationic lignin derivative (reaction B). Competitive hydrolysis side reactions (bottom) can occur simultaneously to lignin's cationization: hydrolysis of EPTAC reduces the availability of active reagent, limiting lignin modification (reaction C); excess of NaOH can further hydrolyze the newly synthesized cationic lignin derivative (reaction D).

employing a previously optimized extraction process, as reported by our group [20], and subsequently cationized using a single-step CHPTAC modification process. The reaction conditions, namely temperature and CHPTAC-to-lignin molar ratio, were optimized using a full factorial design of experiments and response surface methodology to achieve the highest possible cationization by simultaneously maximizing the degree of substitution (DS) and the ζ-potential of the prepared derivatives. Selected derivatives were further characterized by Fourier-transform infrared spectroscopy (FTIR) to verify structural modifications and confirm successful cationization. The obtained water-soluble derivatives are expected to exhibit conditioning properties, thus being possible to be incorporated in future hair conditioning formulations. The proposal of new lignin derivatives as hair conditioning agents represents a novel and advantageous contribution that significantly impacts the cosmetic and biomass sectors by: i) expanding the use of natural-based ingredients in hair care products; ii) offering more environmentally friendly alternatives that will decrease the use of some petroleum-based polyquaterniums with high toxicity to the aquatic organisms; iii) maximizing the utilization of biomass (since cellulose is already commercially used in hair conditioning products), promoting the complete use of the biomass; i*v*) promoting a circular economy and decreasing waste generation by increasing the value of lignin obtained from industrial residues as a low-cost raw material for future valorization and commercialization; v) implementing new multi-active natural ingredients as it can act as UV-filter, emulsifier, and antimicrobial agent.

While the focus of this work is the future incorporation of these cationic lignin derivatives into hair care products, the enhanced water solubility and charge density that results from etherification with CHPTAC may also promote their use in a broader range of applications beyond the cosmetic industry. Cationic lignin derivatives have shown potential in various sectors, including as flocculants for wastewater treatment [21,22], emulsifiers for emulsion stabilization [23,24], carriers in drug delivery systems [25,26], or as antimicrobial materials [27,28]. This broad range of applications highlights the potential of cationically modified lignin as a sustainable and multifunctional platform for the development of advanced materials in both industrial and biomedical fields.

2. Materials and methods

2.1. Materials

Acacia lignin (91.45 wt% purity) was obtained as described in section 2.1.1. 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC, 60 wt% aqueous solution) was purchased from Sigma Aldrich. Sodium hydroxide (NaOH, 99 %) and hydrochloric acid (HCl, 37 wt%) were obtained from José Manuel Gomes dos Santos, Lda., and Fischer Scientific, respectively. Dialysis membranes Spectra/Por™ pre-wetted standard RC with a cut-off of 2 kDa from Spectrum™ were used for lignin dialysis. All chemicals were used as received unless stated otherwise. All references to water in this study refer to deionized water. Chloroform-*d* (99.8 atom % D), pyridine (anhydrous, 99.8 %), 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (95 %), chromium(III) acetylacetonate (99.99 %) and cholesterol (≥99 %) were purchased from Merck, Sweden, and used for ^{31}P NMR analysis.

2.1.1. Acacia lignin

Lignin was extracted from acacia wood (*Acacia dealbata* Link) using the optimized method described in our previous work [20]. Briefly, lignin was extracted using a natural deep eutectic solvent (NADES) composed of lactic acid, citric acid, and choline chloride at a molar ratio of 0.6:0.3:0.1. The extraction was carried out using a solid-to-liquid ratio of 1:10 (g of wood to g of NADES), at a temperature of 140 °C for 2 h, in a Teflon-lined stainless-steel reactor. At these conditions, lignin extraction yield is 99.63 ± 1.24 % [20]. The extracted lignin presented a purity of 91.45 ± 1.03 wt%. The molecular mass of the repeating unit (C9) of the lignin was determined to be 207.86 $g\bullet mol^{-1}$, as described in the supplementary material (section 1), and was used to calculate the molar ratios for the cationization experiments.

2.2. Lignin cationization with CHPTAC

For the chemical modification of lignin with CHPTAC, approximately 500 mg of lignin were weighted into a round-bottom flask. Then, 1 M aqueous NaOH solution was added and stirred until complete dissolution of lignin. The volume of NaOH solution was adjusted for each experiment based on the total quantity of lignin and CHPTAC used, as described by eq. (1) to achieve a fixed NaOH ratio of 1, unless mentioned otherwise. This adjustment was necessary to ensure the presence of one molar equivalent of NaOH per CHPTAC to facilitate the formation of the reactive EPTAC species. Once dissolved, the calculated volume of CHPTAC, corresponding to a CHPTAC-to-lignin molar ratio of 1.3–3.8, was slowly added to the alkaline solution of lignin, in small portions, over 15 min, under continuous stirring. Then, the mixture was allowed to react under reflux with continuous stirring at the desired temperature (30–70 °C) for 3 h. After cooling to room temperature, the aqueous alkaline solution was neutralized using 1 M HCl and transferred into a dialysis membrane (cut-off of 2 kDa). Dialysis was performed using deionized water to remove unreacted CHPTAC/EPTAC, salts, and other low molecular weight byproducts until the conductivity of the dialysis medium matched that of deionized water. Once completed, the purified cationic lignin was oven-dried at 70 °C and stored in a desiccator until use.

$$\text{NaOH ratio} = \frac{n_{NaOH}}{n_{lignin} + n_{CHPTAC}} \tag{1}$$

2.3. Optimization of cationization conditions

To determine the optimal conditions for the lignin cationization, a full factorial design of experiments was conducted. Two continuous variables at three levels were evaluated: temperature, ranging from 30 to 70 °C, and CHPTAC-to-lignin molar ratio, ranging from 1.3 to 3.8. The variable limits were defined based on previous works described in the literature [12,29]. The effect of these selected variables was evaluated through 10 independent experiments, comprising 9 different conditions plus a replicate at the central point. A summary of the conducted experiments is presented in Table 1.

The degree of substitution (DS) and the ζ-potential of the synthesized derivatives were selected as the independent response variables to be optimized in the cationization process, both of which were targeted for maximization. Each of these estimated responses, represented as Y, can be fitted to the quadratic expression described in eq. (2):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{1\bullet 2} X_1 X_2 + \beta_{1\bullet 1} X_1^2 + \beta_{2\bullet 2} X_2^2 \tag{2}$$

where Y is the estimated response (DS or ζ-potential), β_i, $\beta_{i\bullet j}$, and $\beta_{i\bullet i}$ represent the linear, crossed, and quadratic coefficients, respectively, and X_i are the independent variables (X_1 is the reaction temperature and X_2 is the CHPTAC-to-lignin molar ratio).

The obtained results were treated using the software JMP® Pro 17, employing ANOVA and Standard Least Squares regression with a 95 % confidence level.

2.4. Characterization of lignin derivatives

2.4.1. Elemental analysis (EA)

The carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) contents of the cationic lignin samples were determined by elemental analysis using an ECS 8040 CHNS-O Organic Elemental Analyzer from NC Technologies. The nitrogen content (wt%) was used to estimate the DS, which represents the amount of quaternary ammonium moieties introduced per repeating unit of lignin. For this calculation, the structure and mo-

Table 1
List of experiments performed, along with experimental and predicted data obtained for DS and ζ-potential. The DS was determined by the nitrogen content of the modified lignin, assuming that the unmodified lignin has the following chemical structure: $C_9H_{6.01}O_{2.98}(OCH_3)_{1.46}$.

Temperature (°C)	Molar ratio	Observed DS	Predicted DS	Deviation (%)	Observed ζ-potential (mV)	Predicted ζ-potential (mV)	Deviation (%)
30	1.30	0.34 ± 0.01	0.36	4.01	−21.45 ± 0.12	−21.38	0.34
	2.55	0.32 ± 0.06	0.30	7.66	−19.58 ± 3.18	−18.70	4.49
	3.80	0.31 ± 0.01	0.32	2.05	−17.31 ± 0.84	−18.26	5.51
50	1.30	0.38 ± 0.00	0.37	2.73	10.32 ± 0.46	10.00	3.10
	2.55	0.35 ± 0.00	0.34	1.10	13.09 ± 1.50	12.13	7.34
	2.55	0.33 ± 0.00	0.34	2.99	12.58 ± 0.26	12.13	3.61
	3.80	0.38 ± 0.01	0.38	1.14	10.29 ± 1.30	12.02	16.87
70	1.30	0.50 ± 0.01	0.50	0.65	19.34 ± 3.61	19.58	1.27
	2.55	0.48 ± 0.00	0.50	2.88	20.64 ± 3.40	21.17	2.59
	3.80	0.57 ± 0.01	0.56	1.88	21.30 ± 1.54	20.52	3.67

lecular weight of the repeating unit of the unmodified acacia lignin was determined as detailed in the supplementary material (section 1). The repeating unit of lignin was defined as $C_9H_{6.01}O_{2.98}(OCH_3)_{1.46}$ and with an estimated molecular weight of $M_{lignin} = 207.86\ g \bullet mol^{-1}$. The DS was calculated using eq. (3), where M_{lignin} is the molecular weight of the C9 repeating unit of the acacia lignin used (i.e., 207.86 g•mol^{-1}), N is the nitrogen content determined by elemental analysis, 14 is the atomic mass of nitrogen (g•mol^{-1}), and 151.66 is the molecular weight (g•mol^{-1}) of EPTAC.

$$DS = \frac{M_{lignin}\left(g \bullet mol^{-1}\right) \times N\,(\%)}{\left[14\left(g \bullet mol^{-1}\right) \times 100\,(\%)\right] - \left[151.66\left(g \bullet mol^{-1}\right) \times N\,(\%)\right]} \tag{3}$$

2.4.2. ζ-potential

ζ-potential of unmodified and cationized lignins was determined by electrophoretic light scattering in a Malvern Zetasizer NanoZS equipment (ZN 3500). In a typical test, 2–3 mg of lignin were added to 10 mL of deionized water and magnetically stirred until complete dissolution or until a homogeneous dispersion was obtained. Samples were then placed in a folded capillary cell and analyzed in duplicate, with a total of 6 measurements per replicate. Results are reported as the average and standard deviation of the 12 values measured per sample.

For the assessment of the ζ-potential curves across different pH values, lignin solutions/dispersions were prepared using the same procedure mentioned above and then the pH adjusted with either HCl or NaOH solutions. The pH range studied was 4–10, being recorded with a previously calibrated pH-meter, and the ζ-potential in those conditions measured.

2.4.3. Fourier-transform infrared spectroscopy (FTIR)

Structural modification of lignin after the cationization was assessed by Fourier-transform infrared spectroscopy (FTIR). The FTIR spectra of the initial unmodified lignin and of the cationic lignins were recorded on a Jasco FT/IR-4200 spectrometer operating in ATR mode. A total of 64 scans were recorded per sample in the wavenumber range of 500–4000 cm^{-1}, with a resolution of 4 cm^{-1}. For a more easily comparison of the relative intensities of the peaks, all spectra were normalized by area, dividing the absorbance by the total area of the spectrum.

2.4.4. ^{31}P NMR

The unmodified lignin and one selected cationic lignin (DS = 0.63) were characterized by ^{31}P NMR to quantify the content of carboxylic, aliphatic, and phenolic hydroxyl groups, before and after the cationization. The lignin samples were phosphitylated according to the procedure described by Meng, et al. [30], and analyzed in a Bruker Avance II+ 600 MHz spectrometer. A total of 64 scans were collected with an acquisition time of 1.0 s and relaxation delay of 5.0 s. All the other settings were defined as described elsewhere [30]. Cholesterol was used as internal standard due to its higher stability in long-term experiments and sample storage.

2.4.5. Average hydrodynamic diameter (Z-average diameter)

The average hydrodynamic diameter (Z-average) as well the polydispersity index (PDI) of the prepared cationic lignin derivatives were determined by dynamic light scattering (DLS) in a Zetasizer NanoZS equipment from Malvern Instruments. A 532 nm laser and a backscatter detection angle of 173° were used for the measurements. The samples were prepared in the same way as for the ζ-potential measurements and analyzed in duplicate with, at least, two measurements per replicate.

2.4.6. Determination of molecular weight by intrinsic viscosity

The viscosity-average molecular weight (Mv) of the unmodified lignin and of two selected modified lignins were estimated by the intrinsic viscosity method, following the Mark–Houwink–Sakurada equation which defines a correlation between the intrinsic viscosity, [η], and the Mv, as described by eq. (4) [31]:

$$[\eta] = K \times Mv^{\alpha} \tag{4}$$

where K and α are constants for a given polymer–solvent system [31].

For the determination of the intrinsic viscosities of the samples, solutions of the selected lignin with a concentration of 30–40 mg•mL^{-1} were prepared in 0.5 M NaOH and their flow times were determined in an automatic capillary viscometer Viscologic TI1 from Sematech, with a capillary of 0.58 mm at 30 °C. Successive dilutions were made until a final concentration not lower than 10 mg•mL^{-1}, and the corresponding flow times were recorded for each dilution. The flow time of the solvent (0.5 M NaOH) was also measured. Two independent replicate samples were prepared and analyzed, with six measurements of flow time recorded per concentration in each replicate. The specific viscosities (η_{sp}) were then determined for each dilution using eq. (5), where t and t_0 represent the flow times for the lignin solution and the solvent, respectively [32].

$$\eta_{sp} = \frac{t - t_0}{t_0} \tag{5}$$

The experimental η_{sp}/c values were then plotted against the solution concentrations (c) and the intrinsic viscosity determined as the intercept with the y-axis (extrapolation to zero concentration), by following the Huggins relationship described in eq. (6), where k_H is the Huggins constant [32]:

$$\frac{\eta_{sp}}{c} = [\eta] + k_H \times [\eta]^2 \times c \tag{6}$$

The Mv of each lignin was estimated by eq. (4), using the following constant values: K = 0.4165 and α = 0.23, as determined by Dong and Fricke for lignin solutions in 0.5 M NaOH [33].

3. Results

3.1. Optimization of lignin cationization

The optimization of lignin's cationization reaction is crucial to enhance lignin's properties and achieve derivatives with the desired functionality. For the intended application of synthesizing cationic hair conditioning agents, positively charged derivatives with sufficient water solubility are needed to ensure their incorporation into cosmetic formulations. With this in mind, lignin derivatization with the cationically charged CHPTAC was assessed to introduce cationic moieties into lignin's structure. The efficiency of the process was evaluated by the DS and ζ-potential, which are crucial indicators of lignin's reactivity and surface charge. It was hypothesized that temperature and CHPTAC-to-lignin molar ratio would be key variables impacting the reaction extent, as higher temperatures could enhance the reactivity of lignin's hydroxyl groups, while the reagent ratio could influence the availability of reactive groups. On the other hand, the reaction time was set to 3 h and no optimization of this parameter was carried out. Previous studies have reported that long reaction periods can actually diminish the DS due to degradation of the reagents or deetherification reactions [34,35]. The NaOH ratio was also fixed at 1 (calculated based on eq. (1)), meaning that the amount of NaOH added was adjusted for each experiment to compensate for the excess of CHPTAC in certain conditions and to avoid hindering the reaction due to limited alkalinity [34].

Using a two factor and three level full factorial design of experiments and response surface methodology, the effects and interactions of the reaction conditions were systematically evaluated. Reaction temperature (30, 50 and 70 °C) and the molar ratio (1.3, 2.55 and 3.8) of CHPTAC-to-lignin (hereafter simply referred to as molar ratio) were selected as variable factors. The DS and ζ-potential of the resulting cationic derivatives were used as independent responses to be maximized. Experimentally, this design consisted of 10 independent experiments, derived from the 9 possible combinations of the three levels across the two factors, plus an additional experiment at the central point (50 °C, 2.55 ratio) to ensure model reliability and assess experimental reproducibility. The replication of the central point is a common practice to improve the precision of the experiments, allowing to estimate the experimental error, while maintaining a minimum number of runs [36]. This type of design enables the investigation of both linear and crossed effects of these variables on lignin modification. Table 1 presents an overview of the conducted cationization experiments, along with the obtained results for DS and ζ-potential. The predicted values for each response, calculated from eqs. (7) and (8) (shown in sections 3.1.1 and 3.1.2, respectively), are also provided. The deviations for each response were calculated as the absolute differences between the experimental and predicted values, expressed as a percentage of the experimental value.

3.1.1. *Optimization of DS*

The DS of the synthesized lignin derivatives is a direct measure of the efficiency of the cationization reaction, as it represents the number of cationic moieties grafted per lignin monomer. Depending on the intended application, lignins with different degrees of cationization may be required, so it is essential to understand the impact of the reaction conditions on DS to either maximize or fine-tune it. In this work, the DOE approach summarized in Table 1 was applied to determine which variables have the greatest impact on DS and determine the optimal conditions, i.e., where the maximum DS is observed.

As observed in Table 1, the experimental DS values ranged from 0.31 ± 0.01 (30 °C, 3.8 molar ratio) to 0.57 ± 0.01 (70 °C, 3.8 molar ratio), which fall within the range of previously reported DS values for different lignins cationized with either CHPTAC or EPTAC, such as *Eucalyptus* Kraft LignoBoost lignin [12], oil palm empty fruit lignin [16], hardwood organosolv lignin [17], or industrial lignin [19]. The experimental results were compared with the predicted DS values calculated from eq. (7), yielding an average deviation of 2.56 %, ranging from 0.65 to 6.18 %, demonstrating the accuracy of the model in predicting the experimental values. In addition, a determination coefficient of $R^2 = 0.983$ was obtained, highlighting the good model fit and its ability to predict a high percentage of the variation in the responses.

Results from the ANOVA allowed the identification of the most relevant parameters affecting the DS of the synthesized derivatives (Table 2). The standardized effects of each term are depicted in a Pareto chart in Fig. 2, highlighting the most significant terms. All terms show a positive influence on the DS response, indicating that the DS of modified lignins increases when the variables are at the highest values within the studied range. The DS was significantly affected by the reaction temperature (X_1), which had the lowest *p*-value (0.0002), significantly below the threshold of 0.05. The quadratic effects of temperature (X_1^2) and molar ratio (X_2^2) also presented significant impact on DS with *p*-values of 0.0073 and 0.0488, respectively. The significance of these quadratic effects indicates that the relationship between DS and both temperature and CHPTAC-to-lignin ratio is not linear, as visually represented in the response surface plot in Fig. 3a). In addition, their positive effect suggests that the influence of these variables becomes more pronounced as their values increase: raising the temperature from 50 to 70 °C leads to a greater increase in DS (0.33–0.38 to 0.48–0.57) compared to increasing it from 30 to 50 °C (from 0.31–0.34 to 0.33–0.38). The molar ratio (X_2) and the interaction term between temperature and molar ratio ($X_1 \bullet X_2$) do not show a statistically significant influence on the response (*p*-value >0.05). This suggests that, within the tested range, CHPTAC-to-lignin molar ratio does not strongly impact lignin cationization, and temperature is the predominant factor driving a higher degree of modification. Although a synergistic effect between temperature and molar ratio was observed, it is not statistically significant (*p*-value = 0.0535).

The coefficients for the quadratic model equation were determined and resulted in eq. (7):

$$DS_{pred} = 0.34 + 0.096\, X_1 + 0.0036\, X_2 + 0.057\, X_1^2 + 0.023\, X_1 \bullet X_2 + 0.032\, X_2^2 \tag{7}$$

Where DS_{pred} corresponds to the predicted DS, X_1 represents the reaction temperature and X_2 the CHPTAC-to-lignin molar ratio. This equation was used to represent the surface response and contour plots for DS, shown in Fig. 3a) and b).

The conditions that lead to the maximum lignin cationization, i.e., those resulting in the highest DS, were also determined (Fig. 3c) and correspond to a reaction temperature of 70 °C and a CHPTAC-to-lignin molar ratio of 3.8. The optimal conditions fall at the extreme values of the tested range for both variables. This result was anticipated since all the factors and their interactions showed a positive effect, meaning that the cationization reaction is enhanced at higher temperatures and ratios. Under these conditions, a DS of 0.56 was predicted, ranging from 0.51 to 0.60.

The results obtained in this work are in agreement with the literature; although the absolute values of DS depend on the reactivity of the starting lignin, the cationization conditions, and on the applied method for the DS quantification, several studies report cationized lignins with DS values ranging from 0.2 to 1.7 [12,16,17,19]. For example, low temperature modification (30 °C) of lignin derived from oil palm empty fruit using CHPTAC resulted in a DS of 0.2–0.3 [16]. In the present work, similar DS values (0.31–0.34) were also obtained when cationization

Table 2
Analysis of variance (ANOVA) of the quadratic model adjusted to the DS of modified lignins. Values with * correspond to variables that have a significant impact on the DS at a significance level of 0.05 (α = 0.05).

Term	Variable	Sum of Squares	F Ratio	*p*-value
X_1	Temperature	5.53×10^{-2}	1.85×10^{2}	0.0002*
X_2	Molar ratio	7.95×10^{-5}	2.67×10^{-1}	0.6325
X_1^2	Temperature • Temperature	7.53×10^{-3}	2.53×10^{1}	0.0073*
$X_1 \bullet X_2$	Temperature • Molar ratio	2.19×10^{-3}	7.35	0.0535
X_2^2	Molar ratio • Molar ratio	2.33×10^{-3}	7.85	0.0488*

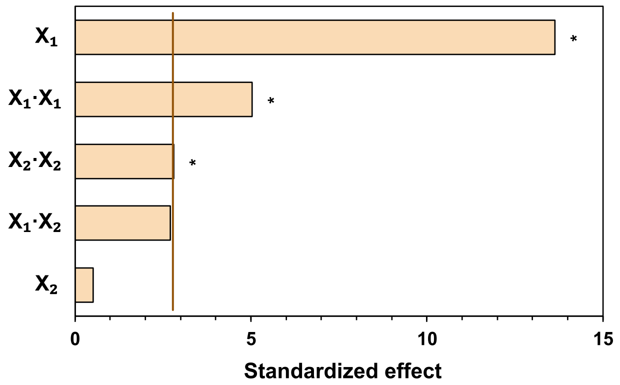

Fig. 2. Pareto chart of the standardized effects obtained from the full factorial design analysis for the DS of modified lignins. X_1: temperature, X_2: molar ratio. The vertical line represents 95 % of confidence: values above this threshold are statistically significant and are marked with *.

was performed at the same temperature. Wahlström, et al. [17] reported DS values of 0.22–0.74 for the cationization of hardwood organosolv lignin at 60 °C. However, it is worth noting that there are differences between the DS calculation methods. Additionally, the cationic kraft lignins prepared by Pinto, et al. [12] showed DS values up to 1.7, which exceed the range reported in this study. This suggests that *Eucalyptus* kraft LignoBoost lignin has higher reactivity than acacia lignin, allowing for more extensive modification.

3.1.2. Optimization of ζ-potential

Another important feature to assess the success of lignin's cationization is the determination of the ζ-potential of the derivatives. The ζ-potential is defined as the electric potential at the slipping plane, marking the boundary of the diffuse part of the double layer [37,38]. Although ζ-potential is not a direct measure of the particle's surface charge, this electrostatic potential is related to the surface charge [37] and, therefore, is often considered an indicator of the particle's effective charge [39]. In addition, as it measures the electrostatic state of a solid-liquid interface [37], the magnitude of ζ-potential provides insight into the potential stability of a colloidal system. Generally, particles with highly positive or negative ζ-potential values, greater than +30 mV or lower than −30 mV, are considered strongly cationic or anionic, respectively, and will repel each other, leading to a stable dispersion [37]. On the other hand, when particles have low ζ-potential values (i.e., between −10 and +10 mV), they are approximately neutral and they will have a high tendency to aggregate [37,40].

In this respect, a similar approach to that used for DS optimization was carried out to maximize the ζ-potential of the cationic lignin derivatives (Table 1). Unmodified acacia lignin showed a highly negative ζ-potential of −31.35 ± 4.60 mV, as typically observed for other lignin particles [41–43]. Usually, unmodified lignins present negative values of ζ-potential due to the deprotonation of their different hydroxyl groups [41].

The ζ-potential of the modified lignins range from −21.45 ± 0.12 mV (30 °C, 1.3 molar ratio, Table 1) to 21.30 ± 1.54 mV (70 °C, 3.8 molar ratio, Table 1), indicating that some experimental conditions resulted in positively charged polymers. The developed model showed an excellent fit to the experimental data, with a determination coefficient of $R^2 = 0.997$, indicating a strong predictive capability. An average deviation of 4.88 % was obtained when comparing the experimental results with the predicted ζ-potential values calculated from eq. (8), though the deviation range was broader compared to DS (0.34 to 16.87 %).

Results from the ANOVA allowed to identify the significance of each parameter affecting the ζ-potential of the synthesized derivatives (Table 3). The standardized effects of each term are depicted in a Pareto chart in Fig. 4, highlighting the most significant terms. In contrast to what was observed for the DS, not all terms had a positive influence on the ζ-potential of modified lignins, with only the main effects (X_1 and X_2) positively affecting the response. This means that the ζ-potential of the synthesized lignins increases with temperature and CHPTAC-to-lignin ratio. However, this effect is only significant for temperature (X_1, *p*-value <0.0001), whereas the main effect of molar ratio (X_2) showed a *p*-value of 0.1320, above the 0.05 threshold. The quadratic effect of temperature (X_1^2) also presented significant impact on ζ-potential, with a *p*-value of 0.0002. Similarly to what was observed for the DS, the significance of this quadratic effect suggests that there is a non-linear dependence of the ζ-potential on reaction temperature, as seen in the surface response presented in Fig. 5. However, unlike DS, the quadratic effect of temperature was negative, meaning that the impact of changing the temperature is more pronounced at lower values within

Table 3
Analysis of variance (ANOVA) of the quadratic model adjusted to the ζ-potential of modified lignins. Values with * correspond to variables that have a significant impact on the ζ-potential at a significance level of 0.05 (α = 0.05).

Term	Variable	Sum of Squares	F Ratio	*p-value*
X_1	Temperature	2.38×10^3	1.39×10^3	<0.0001*
X_2	Molar ratio	6.14	3.57	0.1320
X_1^2	Temperature • Temperature	2.77×10^2	1.61×10^2	0.0002*
$X_1 \bullet X_2$	Temperature • Molar ratio	1.18	6.88×10^{-1}	0.4535
X_2^2	Molar ratio • Molar ratio	2.93	1.70	0.2620

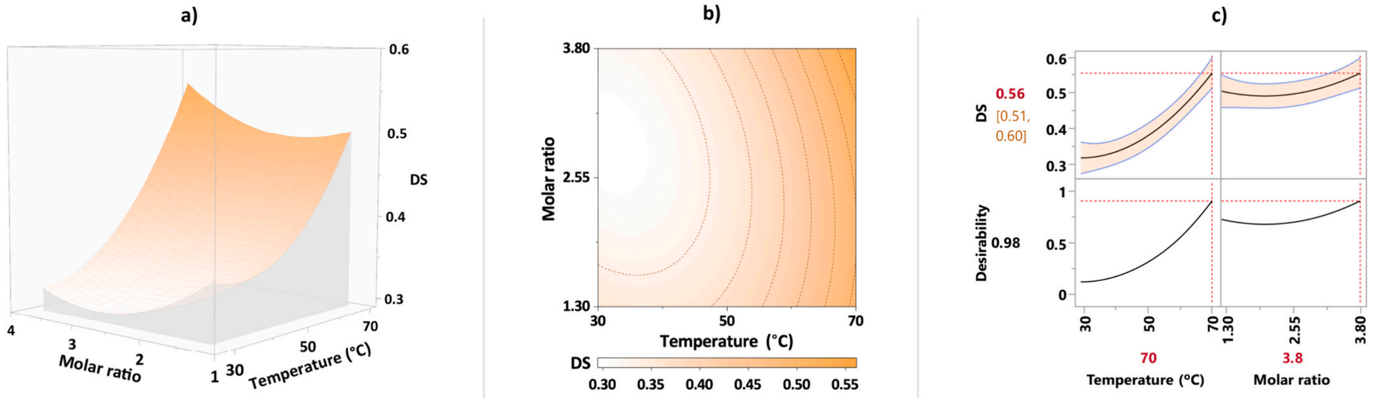

Fig. 3. Surface response (a) and contour (b) plots for the predicted DS as function of reaction temperature and CHPTAC-to-lignin molar ratio; c) desirability prediction profiler illustrating the optimal reaction conditions (70 °C and 3.8 molar ratio) for lignin cationization to maximize the DS value.

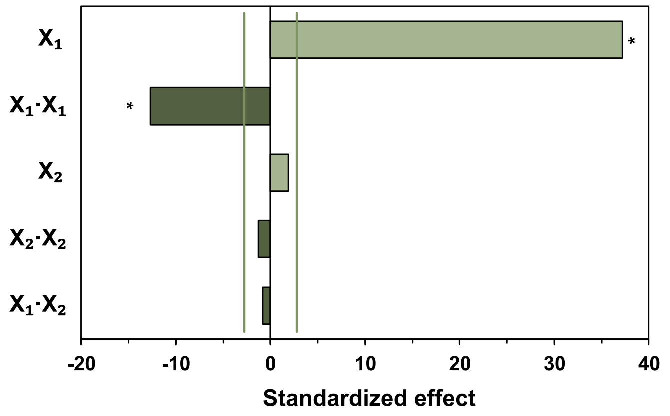

Fig. 4. Pareto chart of the standardized effects obtained from the full factorial design analysis for the ζ-potential of modified lignins. X₁: temperature, X₂: molar ratio. The vertical line represents 95 % of confidence: values above this threshold are statistically significant and are marked with *.

the studied range: increasing the temperature from 30 to 50 °C results in a higher increase of ζ-potential (from −21.45 to 13.09 mV) than changing from 50 to 70 °C (from 10.29 to 21.30 mV). The remaining crossed effects (X_2^2 and $X_1 \bullet X_2$) did not show a statistically significant influence on the response (*p*-value >0.05).

Within the tested range, the CHPTAC-to-lignin molar ratio does not seem to have a significant impact on the ζ-potential of the cationic lignin derivatives. Instead, this response is predominantly controlled by temperature: the higher the temperature, the greater the ζ-potential.

The coefficients for the quadratic model equation were determined and resulted in eq. (8):

$$\zeta = 12.13 + 19.94\,X_1 + 1.01\,X_2 - 10.89\,X_1^2 - 0.54\,X_1 \bullet X_2 - 1.12\,X_2^2 \tag{8}$$

where ζ corresponds to the predicted ζ-potential, X_1 represents the reaction temperature and X_2 the CHPTAC-to-lignin molar ratio. This equation was used to represent the surface response and contour plots for ζ-potential, depicted in Fig. 5a) and b), respectively.

The optimal conditions that result in a maximum value of ζ-potential were determined (Fig. 5c) and were found to be a temperature of 68 °C and a CHPTAC-to-lignin molar ratio of 2.84. At these conditions, a ζ-potential of 21.31 mV was predicted, with a range of 18.93–23.69 mV. The optimal conditions determined correspond to high values for both variables but, in contrast to what was observed for the DS, do not correspond to the highest values for both variables. This effect is caused by the negative crossed and quadratic effects previously mentioned. Although there is a positive effect for the main effects, these become less pronounced at the highest range of the variables. This is particularly visible for the temperature, where both main and quadratic terms were found to be highly significant: an initial increase is observed with temperature, but a plateau is reached for higher temperatures.

A comparison of the ζ-potential values of the synthesized lignin derivatives with literature is not straightforward as these values will depend on the reaction conditions, the raw material, the ζ-potential value of the unmodified lignins and the measurement conditions. However, there is a clear trend where lignin derivatives shift from negative to positive ζ-potential values when cationization is performed under harsher conditions. These values usually also increase with DS: for example, cationized kraft lignin showed ζ-potential values ranging from 12.9 ± 0.5 mV to 30.3 ± 2.6 mV for derivatives with DS values from 0.7 to 1.7 [12]. The authors observed that the surface charge of the derivatives follow the same trend (ranging from 202 ± 2 to 697 ± 4 $\mu_{eq} \bullet L^{-1}$), demonstrating that ζ-potential correlates with particle surface charge, providing in this way a quick method to evaluate the success of the cationization reaction. An increase in the ζ-potential with the CHPTAC ratio was also observed for cationized organosolv lignin, with a maximum of 23.6 ± 0.4 mV at the highest DS of 0.74 [17]. Other studies report the increase of lignins' ζ-potential after cationization while still showing negative values at neutral pH, as seen for lignosulfonate from pinewood [15]. In this case, cationic lignin only exhibits a positive ζ-potential at pH below 2.5. Above that threshold, the presence of sulfonate groups, in addition to the hydroxyl groups, contributed to a negative surface charge.

3.1.3. *Multi-objective optimization*

The global optimization of the lignin cationization procedure involves maximizing both studied responses, i.e., identifying the experimental conditions that simultaneously maximize the DS and the ζ-potential of the resulting cationic lignins. From the separate optimizations, the conditions that lead to the derivatives with the maximum DS and the highest ζ-potential were found to be not the same. However, in general, higher temperatures favor both responses. In order to meet a compromise between optimizing both responses and achieving an efficient lignin cationization, a multi-objective optimization involving both response variables was performed using a Desirability Function Approach (DFA). ζ-potential is an important feature of the resulting lignin derivatives, as polymers with a high positive ζ-potential are desirable for the intend application; thus, this response should be maximized. However, since the DS represents the quantity of cationic moieties introduced per lignin monomer, it serves as a direct measure of cationization efficiency and, therefore, a more reliable indicator of the success of the reaction. Given this, the DS response was weighted higher than the ζ-potential in the DFA, with a ratio of 5 to 1.

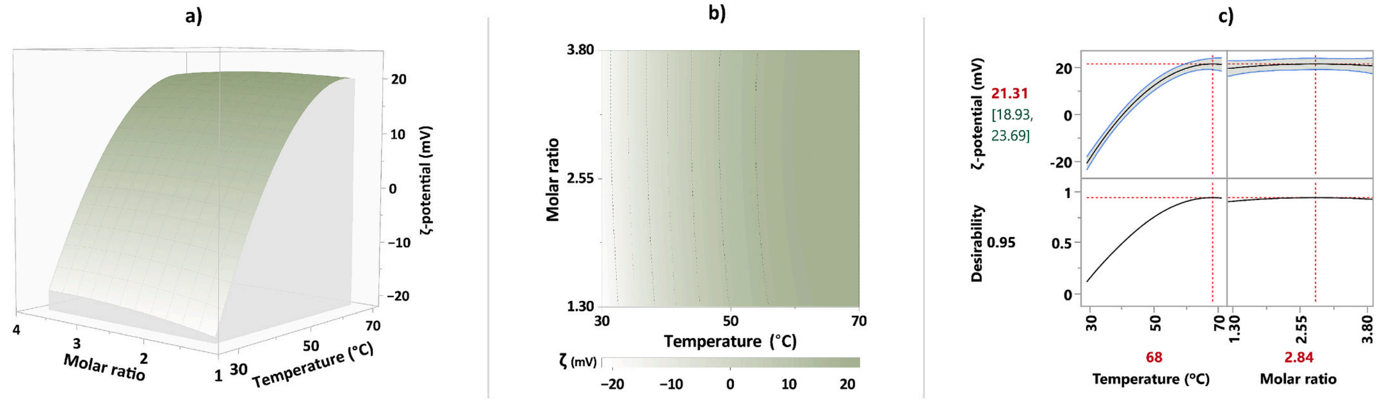

Fig. 5. Surface response (a) and contour (b) plots for the predicted ζ-potential as function of reaction temperature and CHPTAC-to-lignin molar ratio; c) desirability prediction profiler showing the optimal reaction conditions (68 °C and 2.84 molar ratio) for lignin cationization to achieve the maximum ζ-potential value.

The effects of the multi-objective optimization are represented in the Pareto chart in Fig. 6. As observed for the individual responses, temperature (X_1) was the most influential variable, whereas the molar ratio (X_2) does not have a significant impact on the global cationization. The quadratic terms of both variables (X_1^2 and X_2^2) are also found statistically relevant for the overall maximization of lignin modification. However, no significant synergistic effect was observed between the two variables ($X_1 \bullet X_2$).

A global desirability function was used to generate the surface response depicted in Fig. 7a and to identify the optimal conditions to maximize the modification of acacia lignin (Fig. 7b). Due to the higher weighting assigned to DS maximization, the desirability function has a similar profile to that of the DS response. As a result, the optimal conditions identified are the same as those determined in the individual DS optimization: a reaction temperature of 70 °C and a CHPTAC-to-lignin molar ratio of 3.8. Under these conditions, the predicted DS value was 0.56, with a predicted range of 0.51 to 0.60, while the predicted ζ-potential was 20.52 mV, ranging from 17.27 to 23.77 mV. The experimental values obtained for these conditions were a DS of 0.57 ± 0.01 and a ζ-potential of 21.30 ± 1.54 mV, both of which fall within the predicted range. The variation between the predicted and experimental values was −1.75 % for DS and −3.66 % for ζ-potential.

Although the predicted optimal conditions were found at the highest limits of the studied range, it is worth noting that the main effect of the reagent's molar ratio proved to be statistically insignificant in all analyses performed. This suggests that using a higher amount of cationic reagent does not significantly improve any of the evaluated responses, either independently or in the multi-objective approach. The objective of this work is to develop new cationic lignin derivatives that align with green chemistry principles, including waste prevention and atom economy [44] which are of utmost importance in promoting eco-friendly processes. In this sense, the CHPTAC-to-lignin molar ratio was set at its minimum (1.3) in the subsequent studies, as the overall optimal condition. This approach not only minimizes environmental impact but also represents a more economical strategy to obtain lignins with adequate properties. Under these conditions (70 °C and a molar ratio of 1.3), lignin derivatives with a DS of 0.50 ± 0.01 and a ζ-potential of 19.34 ± 3.61 mV were obtained, yielding results comparable to those predicted at harsher conditions, while using nearly one-third of the CHPTAC amount, thereby avoiding wasting reagent. A previous study of the cationization of lignin with EPTAC at three different EPTAC-to-lignin molar ratios (1, 2 and 3) also showed that maximizing the ratio between the reagents does not lead to additional benefits, suggesting that the reaction reaches a stable level at a molar ratio of 2 [19].

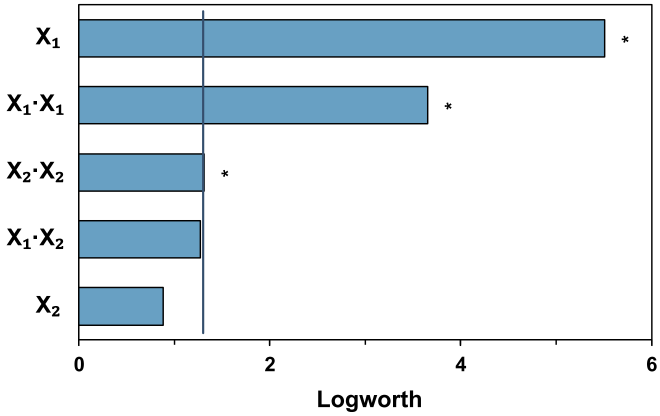

Fig. 6. Pareto chart of the effects for the multi-objective model using a full factorial design for the optimization of lignin cationization. X_1: temperature, X_2: molar ratio. The vertical line represents 95 % confidence: values above this threshold are statistically significant and are marked with *.

Although the optimized temperature corresponds to the highest value of the studied range, higher temperatures were not considered, as they are reported to compromise lignin cationization by etherification with CHPTAC or EPTAC. Maximum cationization of softwood kraft lignin with EPTAC was observed at a temperature of 70 °C, with a decrease in cationization at temperatures above this threshold (80–90 °C), due to EPTAC decomposition and partial hydrolysis of the cationic lignin [18]. A similar trend was observed for cationic hemicelluloses, where increasing the temperature up to 60–65 °C results in derivatives with higher DS, but further temperature increase lead to a decline in DS [35,45]. An initial increase in the reaction temperature favors the reactivity by increasing the kinetic energy of the reagent species and the diffusion of the cationic agent, boosting the cationization reaction [34,35]. However, at higher temperatures, cationization is hindered by reagent degradation, as previously discussed.

Based on the aforementioned findings, the optimal conditions for lignin cationization with CHPTAC in alkaline solution were determined to be a reaction temperature of 70 °C, using a CHPTAC-to-lignin molar ratio of 1.3, for 3 h. These conditions are believed to achieve the best balance between maximizing cationizations efficiency and minimizing environmental impact.

3.2. *Effect of the amount of NaOH on the cationization performance*

In the etherification reaction of lignin with CHPTAC, NaOH acts not only as solvent for lignin dissolution but also as catalyst of the reaction, generating the nucleophilic site within lignin that subsequently attacks the highly reactive epoxy group of EPTAC. In addition, reactions performed with CHPTAC require higher amounts of NaOH than those performed directly with EPTAC, as one equivalent of NaOH is necessary to generate the epoxide EPTAC from the chlorohydrin CHPTAC, which is consumed in the reaction [34]. Consequently, additional NaOH is then necessary to activate the hydroxyl groups of lignin and, therefore, the alkalinity of the reaction medium is a crucial factor in this reaction. This explains why increasing the amount of the cationizing agent does not always lead to an enhanced DS: if the NaOH concentration is not proportionally increased, the alkali may become the limiting reagent [34]. To account for this, during the optimization performed in this work, the content of NaOH used in each experiment was adjusted based on the amount of lignin and CHPTAC added, as pointed in eq. (1). On the other hand, it is well known that under alkaline conditions, EPTAC can undergo hydrolysis, forming diol byproduct (Fig. 1), reducing the yield of the cationization reaction. Besides this undesired side reaction, another competing reaction can further compromise cationization efficiency, that is the hydrolysis of the newly formed cationic derivative (Fig. 1) [18,45]. Hence, the effect of the amount of NaOH used is very important in this reaction: it must be sufficient to solubilize and activate lignin and to generate the reactive EPTAC species, but not so excessive that it promotes hydrolysis of both the reagent and the final product. In an attempt to further improve the DS of the cationized lignins, a small excess of NaOH was added to the reaction medium to evaluate whether it would improve the reaction or lead to the hydrolysis of the reagents and of the newly produced cationic lignin. During the optimization experiments, the NaOH ratio was set at 1, meaning that there was an equimolar ratio of NaOH to the total CHPTAC and lignin monomers. An additional condition was tested using the defined optimal conditions (70 °C, 1.3 molar ratio and 3 h) but with a NaOH ratio of 1.25, which corresponds to a 25 % increase in the base content. The results for the DS and ζ-potential from these two cationizations are presented in Fig. 8.

For the same experimental conditions, i.e., fixed temperature, time, and CHPTAC-to-lignin molar ratio, increasing the amount of NaOH added to the reaction medium by 25 % (from a ratio of 1 to 1.25) resulted in an increase in the DS from 0.39 ± 0.09 to 0.61 ± 0.03. This effect proved to be statistically significant (one-way ANOVA with Tukey test, p-value <0.05), showing that the increase in alkalinity is beneficial, leading to an improvement in the DS and, more notably, to a reduction in the heterogeneity of the resulting material. The same effect was

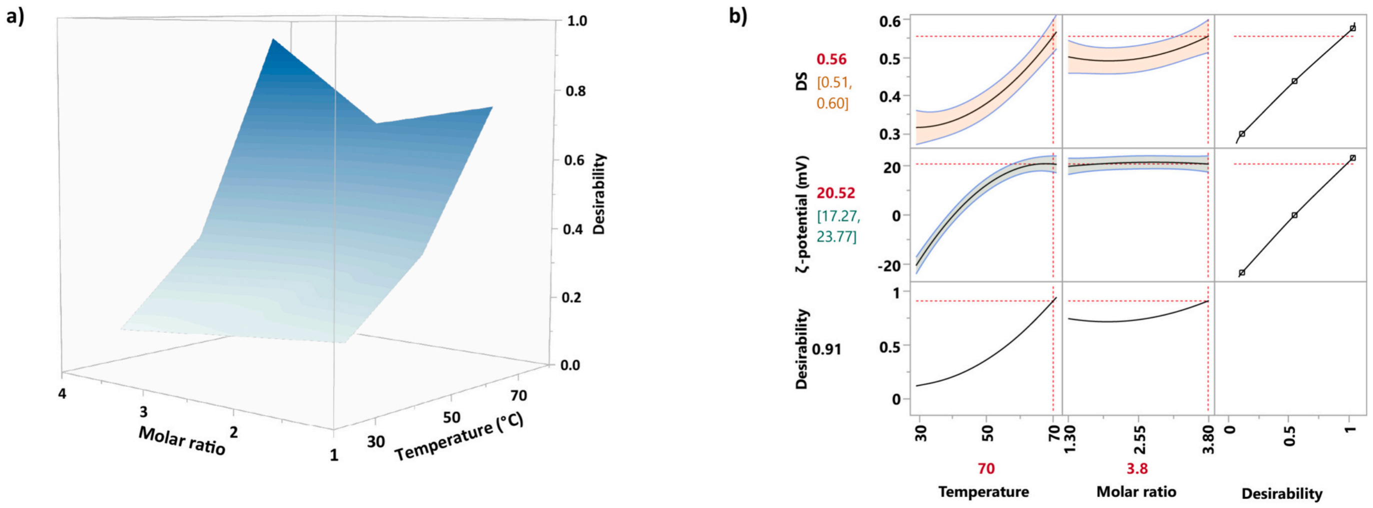

Fig. 7. a) Global desirability surface plot as function of reaction temperature and CHPTAC-to-lignin molar ratio; b) desirability prediction profiler showing the optimal reaction conditions (70 °C and 3.8 molar ratio) for the multi-objective optimization of the lignin cationization process to achieve the maximum DS and ζ-potential. DS response was weighted five times higher than the ζ-potential in the global desirability maximization.

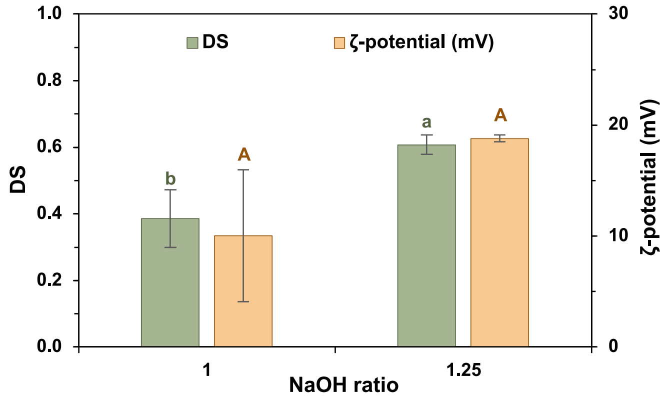

Fig. 8. Effect of NaOH ratio on the DS and ζ-potential of cationized lignins. Reaction conditions: 70 °C, CHPTAC-to-lignin molar ratio of 1.3 and 3 h. NaOH ratio is defined as described in eq. (1). Bars connected with the same letters show no significant difference in DS (lower-case) and ζ-potential (capital) (One-way ANOVA with Tukey test, p-value ≤0.05).

observed for the ζ-potential of the derivatives, although with no significant statistical differences. Nonetheless, a slight increase from 10.02 ± 5.94 to 18.79 ± 0.30 mV was observed when the NaOH content was increased, with a clear positive effect in reducing the variability. The higher reproducibility of the experiments at higher NaOH concentrations is likely due to a better solubilization of lignin in the reaction medium which results in a more homogeneous solution, suggesting that the addition of an excess of NaOH improves lignin cationization. This suggests that the threshold at which the eventual hydrolysis of the newly cationized lignin would limit the reaction efficiency was not reached within this range of alkalinity. The equimolar ratio between NaOH and the reagents does not seem to be the optimal ratio to achieve the highest cationization degree. In this case, it is reasonable to assume that at a NaOH ratio of 1, NaOH becomes the limiting reagent. Possibly, NaOH is partially consumed by the numerous hydroxyl groups of lignin during deprotonation, thereby reducing the amount available to react with CHPTAC and form the epoxide derivative.

The effect of alkalinity on the cationization of lignin has not been reported in the literature to the same extent as other variables such as the reaction time, temperature, and reagents ratio. However, this effect has been explored for the modification of polysaccharides. For instance, in the cationization of hemicelluloses with EPTAC [45], a correlation between the DS of the resulting cationic hemicelluloses and the NaOH dosage was established: increasing the NaOH dosage from 1 to 5 wt%, led to an increase in DS from 0.02 to 0.12 %. However, further increasing the NaOH dosage above this threshold resulted in a decrease in the observed DS to 0.04 %. This suggests that while increasing the NaOH dosage indeed promotes the etherification reaction, there is a competition between the etherification and the decomposition of both the EPTAC and of the newly formed cationic species [45]. Other studies on the production of cationic sugarcane bagasse hemicellulose derivatives have also suggested the existence of an optimal molar ratio of NaOH to the etherifying reagent [46,47]. Specifically, an increase in the NaOH-to-EPTAC molar ratio from 0.10 to 0.25 and 0.50 led to DS values of 0.01, 0.23 and 0.54, respectively. However, when the ratio was further increased from 0.50 to 0.75, the DS decreased to 0.49 [46]. Similar hydrolysis reactions in alkaline aqueous solutions have also been considered in cellulose modification [48,49]. To mitigate the hydrolysis of the epoxy ring in aqueous alkaline media, some authors have suggested a partial or total replacement of water with organic solvents, such as dimethylacetamide [48], dimethyl sulfoxide, isopropanol, or tetrahydrofuran [50].

Regarding lignin etherification, in the study by Pinto, et al. [12], at a fixed reaction temperature of 70 °C, reaction time of 3 h and CHPTAC-to-lignin molar ratio of 2.6, increasing the solid loading of lignin in a NaOH aqueous solution (2 mol•L^{-1}) from 10 to 20 wt% led to a decrease in the DS from 1.3 to 1. Although the increase in solid loading may compromise the accessibility of the lignin molecules during reaction, it must also be considered that doubling lignin concentration effectively halves the amount of available NaOH to dissolve lignin and convert CHPTAC into EPTAC. This reduction may not only compromise lignin's solubility but also limit the degree of CHPTAC conversion, thereby decreasing the quantity of EPTAC available for reaction.

In summary, under the optimized conditions presented above (70 °C, 3 h, CHPTAC-to-lignin molar ratio of 1.3, and NaOH ratio of 1.25) we were able to maximize the DS and ζ-potential of the obtained lignin, yielding a soluble and cationically modified lignin, with low variability. The optimized reaction avoids using harsh conditions or hazardous materials, and enables a successful cationization with a minimal use of cationic reagent, decreasing the environmental impact by reducing the waste generated. It is relevant to note the overall high yield of the entire process. Namely, acacia lignin was obtained from the biomass using a natural-based solvent system that yielded 99.6 % of the lignin present in wood. After the modification, the weight yield of lignin obtained was 87 % ± 4 % of the staring lignin, with minimal losses being mainly attributed to the loss of low molecular weight fractions (< 2 kDa) during the dialysis purification step.

3.3. *Structural analysis of cationic lignin derivatives by FTIR*

To evaluate the structural changes in lignin's structure during the cationization process, particularly the introduction of quaternary ammonium moieties, FTIR spectra of the unmodified and of the various cationized lignins were recorded and compared. The normalized spectra for three selected samples, i.e., unmodified acacia lignin and two cationic lignins with different DS (0.35 and 0.63) are presented in Fig. 9.

Acacia lignin exhibits a FTIR spectrum characteristic of hardwood lignins due to the presence of both guaiacyl (G) and syringyl (S) units. The most intense bands in acacia lignin are at 1029, 1111, and 1209 cm^{-1} (bands 11–13) and are attributed to vibrations in S and G units [51–55]. Vibrations in S units correspond to bands 9, 11, and 12, at 1322, 1209, and 1111 cm^{-1}, while vibration modes in G units result in bands 10 and 13, at 1264 and 1029 cm^{-1}, respectively [20]. A complete analysis of acacia lignin used in this work was recently described by us [20].

After modification, some differences are observed in the spectra of cationic lignins, which depend on the DS of the polymer. Although bands 11–13 remain the most intense in the modified lignins, their intensity relative to other bands decreases, suggesting a relatively lower contribution of vibration modes of the lignin's aromatic moieties compared to the unmodified lignin. This suggests the presence of other functional groups. In addition, changes in the relative intensity of bands 11 and 13, comparing unmodified with the cationically modified lignin, suggest structural changes. The increase in band 13, at ca. 1030 cm^{-1}, indicates the introduction of ether linkages between lignin and EPTAC [18,56]. Additionally, the increase in the relative intensity of band 13 in comparison to band 12 suggests that EPTAC primarily reacts with phenolic OH groups, forming aromatic ethers rather than aliphatic ethers. This is reasoned from the fact that aliphatic ethers typically exhibit the C—O stretching band at 1120 cm^{-1}, whereas the corresponding band for aromatic ethers appears at 1040 cm^{-1} [57]. This outcome was anticipated because, although aliphatic hydroxyl groups can be involved in the reaction with CHPTAC, this reaction is limited and it only occurs in the presence of an excess amount of the reagent [12].

The introduction of quaternary ammonium groups was clearly identified by the appearance of band 14 at 966 cm^{-1}, which is attributed to the C—N stretching in quaternary ammonium groups ($CH_2–N(CH_3)_3$) [58,59]. A slight increase and broadening in the region of 1463 cm^{-1} (band 7) may be caused by the vibrations in methyl groups attached to the nitrogen atom in the quaternary ammonium moieties [60,61]. Since acacia lignin also presents bands in this region, attributed to C—H deformations in methyl and methylene groups [62], a clear assignment to the quaternary moieties is challenging. However, it is possible that the insertion of the cationic groups causes some band overlapping, resulting in the shift and broadening of the bands observed for this range. Band 15, near 915 cm^{-1}, in lignin corresponds to C—H out of the plane vibration in aromatic rings [20]. The increase of this band after modification may be a result of the overlapping with the band associated to the C—O stretching vibration of the newly formed ethers [27,63].

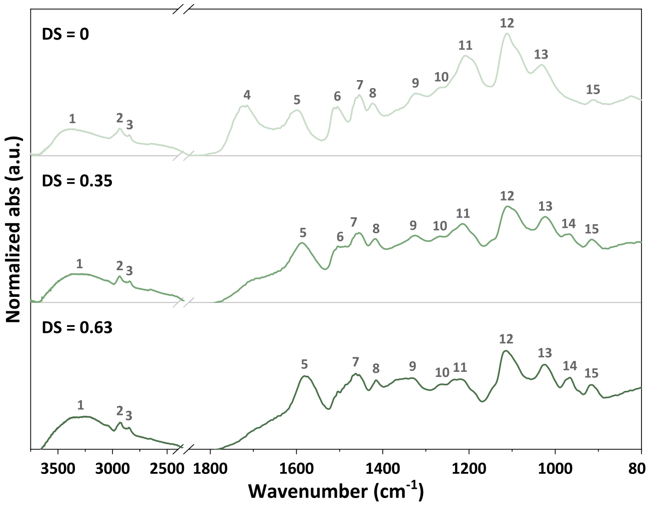

Fig. 9. FTIR spectra of unmodified acacia lignin (DS = 0) and two selected cationic lignins with different DS (DS = 0.35 and DS = 0.63).

Band 5, near 1600 cm^{-1}, is attributed to aromatic skeletal vibrations combined with C═O stretching in lignin [64]. The increase in intensity in this region in the modified lignin spectra, may arise from absorbed water due to the increased hydrophilicity of the lignin derivatives, as previously reported for cationically modified celluloses with CHPTAC [65].

3.4. ^{31}PNMR

The grafting of the nitrogen-containing reagent was clearly supported by the EA and FTIR analyses. The results from the FTIR analysis also suggested that the reaction between EPTAC and lignin do mostly occur at the phenolic OH groups, rather than at the aliphatic OH. The regioselectivity of this modification was further investigated by ^{31}P NMR. This technique allows the quantification of the different types of hydroxyl groups (i.e., aliphatic, phenolic, and carboxylic) that are present in the lignin structure. The ^{31}P NMR spectra of the unmodified acacia lignin (DS = 0) and of the cationically modified lignin obtained using the defined optimal conditions (DS = 0.63) are presented in Fig. 10. Cholesterol, which corresponds to the signal at 144.86 ppm, was used as internal standard for the quantification of the OH content. The content of aliphatic, phenolic, and carboxylic acid OH groups were determined by integration of the regions at 145.4–150, 137.6–144.0, and 133.6–136.0 ppm [30], respectively, which are highlighted in the spectra as shaded regions of different colors. The contents of each functional group for both lignins are listed in Table 4.

In the unmodified acacia lignin, the phenolic OH groups are the most abundant, with a content of 1.81 mmol OH per g of lignin. The amount of aliphatic and carboxylic OH is similar in this sample, being 1.04 and 1.07 mmol•g^{-1}, respectively. This OH profile in the unmodified lignin is common for lignin extracted with acidic DES, known for their high ability to cleave the β–O–4 bonds [66]. This cleavage contributes to increasing the content of phenolic OH groups [67]. The high content of carboxylic acid groups may result from the incorporation of acids from

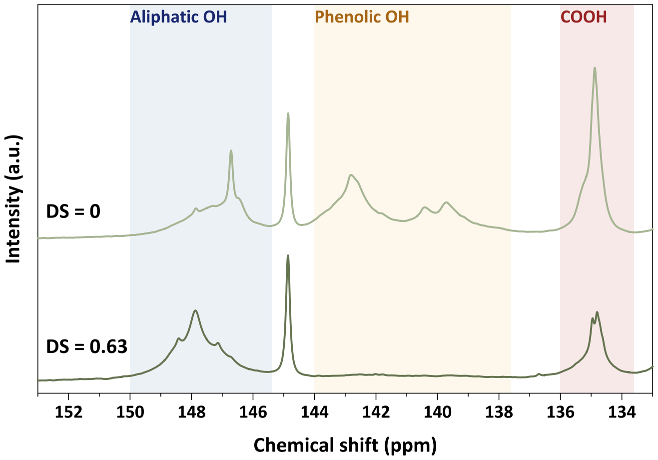

Fig. 10. ^{31}P NMR spectra of unmodified (DS = 0) and cationized (DS = 0.63) lignins.

Table 4

Content of aliphatic, phenolic and carboxylic acid hydroxyl groups determined by ^{31}P NMR for unmodified acacia lignin (DS = 0) and one cationically modified lignin (DS = 0.63). Molar contents (mmol•mol^{-1} lignin) were calculated based on the molar mass of the unmodified lignin (207.86 g•mol^{-1}) and of the modified lignin (303.41 g•mol^{-1})[a].

Hydroxyl groups	Content (mmol•g^{-1} lignin)		Content (mmol•mol^{-1} lignin)	
	DS = 0	DS = 0.63	DS = 0	DS = 0.63
Aliphatic OH	1.04	1.08	217.2	327.6
Phenolic OH	1.81	0.30	375.8	90.6
Carboxylic acid OH	1.07	0.53	222.0	160.2
Total	3.92	1.91	815.0	578.4

[a]) $M_{\text{cationic ignin}} = M_{\text{lignin}} + DS \times M_{\text{EPTAC}}$

the extraction solvent, since acids, such as lactic acid and citric acid (used in this case) are reported to chemically bind to the biomass through esterification during the fractionation process [68–70]. After the chemical modification, clear differences are observed in the spectra of the cationic lignin: the most notable change between both spectra is the almost complete disappearance of signals corresponding to phenolic OH, with a content of only 0.30 mmol•g^{-1}, significantly lower than the initial value of 1.81 mmol•g^{-1}. Thus, in contrast to the initial lignin, where phenolic OH represent the main fraction of hydroxyl groups, in the cationic lignin, phenolic OH are the least abundant hydroxyl groups. This clearly supports the findings from the FTIR analysis, further proving that the reaction occurs at the phenolic OH groups. As for the content of aliphatic OH, it is identical in both samples, being 1.08 and 1.04 mmol•g^{-1} for the cationic and unmodified lignins, respectively. At a first glance, it may seem that no changes occurred at the aliphatic groups, but one must consider that, by grafting EPTAC into the lignin structure, one aliphatic OH group is being introduced per molecule of EPTAC (see Fig. 1). Thus, an increase in the content of aliphatic OH is expected. For a better comparison between the samples, the OH content was converted to molar content, as the changes in the molecular mass of the lignin monomers before and after modification would be considerable at a DS of 0.63 (303.41 vs 207.86 g•mol^{-1}). When comparing the molar contents, a clear decrease in the content of phenolic OH is still observed and this is followed by an increase in the aliphatic OH, supporting the hypothesis that EPTAC is reacting at the phenol group, introducing aliphatic OH groups. This is also supported by the changes observed in the chemical shifts of the aliphatic OH between both spectra, with a widening of the band attributed to these groups in the cationic lignin. This profile also suggests that the aliphatic OH in lignin are also being consumed during the reaction, with a shift of the aliphatic main peak toward higher chemical shifts. Although the reaction occurs primarily at the phenolic groups, the extensive cationization and the presence of an excess amount of CHPTAC may also drive the reaction to occur at the aliphatic groups [12]. As for the observed decrease in the content of carboxylic OH, it is hypothesized to result from the release of carboxylic acid groups from the lignin structure when subjected to severe alkaline conditions. Namely, it must be highlighted the hypothesis that some hydrolysis of the ester bonds formed between lignin and citric acid present in the solvent during the extraction process can occur. The disappearance of some carboxylic acid and/or ester groups were also detected in the FTIR spectra by the decrease in the carbonyl band. Recently, van Erven, et al. extensively studied the incorporation of the DES components into the lignin structure [69]. Namely, the acids present in the DES can form alkali-labile ester linkages that can be then broken by saponification at room temperature in aqueous NaOH solution. Similar results were also reported by Zeng, et al. regarding the esterification of cellulose resulting from fractionation with acidic DES [70]. De-esterification was also reported after stirring the cellulose in an aqueous NaOH solution for 2 h at 50 °C.

3.5. Effect of cationization on the physicochemical properties of lignin

To evaluate the physicochemical changes in lignin after modification, cationic lignins with different degrees of cationization were further characterized regarding their hydrodynamic diameter (*Z*-average diameter). Results for the *Z*-average diameter and PDI of the different cationic lignins are presented in Table 5.

The Z-average diameter of the lignin derivatives ranged from 191 ± 8 to 897 ± 39 nm. This suggests that the degree of cationization of the derivatives affects their hydrodynamic diameter. However, the correlation between DS and diameter is not linear, as represented in Fig. 11a. For samples with lower DS values (DS < 0.34), *Z*-average diameters of 300–450 nm were observed. Then, an increase in size was detected for samples with intermediate DS (0.38), with a maximum of 897 ± 39 nm. Lignins with DS higher than 0.48 showed Z-average diameters below 250 nm. Although this trend in Z-average diameter as a function of DS may initially appear independent of the surface charge of the cationized lignins, a closer analysis reveals a correlation with ζ-potential. From the results in Table 5, it can be observed that the initial increase in the *Z*-average diameter is caused by a decrease in the absolute value of ζ-potential: for low DS values, the derivates are negatively charged, presenting ζ-potential values that can be lower than −20 mV. Increasing the DS to intermediate values causes the ζ-potential to become positive, but more neutral, with a ζ-potential close to 10 mV. Further increasing the DS (DS ≥ 0.48) results in more positively charged derivatives, presenting a ζ-potential of approximately 20 mV. When plotting the Z-average diameter as a function of the absolute value of ζ-potential (Fig. 11b), we can observe a negative correlation ($R^2 = 0.80$) between them, with highly charged particles showing a smaller diameter. The hydrophilicity of these samples likely reduces their tendency to agglomerate while also increasing electrostatic repulsion between particles due to the higher charge.

All cationic lignin derivatives showed relatively high polydispersity as their PDI values ranged from 0.29 ± 0.07 to 0.59 ± 0.18. These values are above the threshold of 0.1 that implies polydisperse particle size distributions [71].

Since the ζ-potential is highly dependent on the measurement conditions, particularly the pH of the solution, further characterization of the derivatives was performed over a wide range of pH values. Solutions/dispersions of the different lignin derivatives were prepared by adjusting the pH to values between 4 and 10 using either HCl or NaOH. The ζ-potential of the resulting samples was then measured and plotted against the respective pH values to evaluate eventual changes in the charge of the polymers according to the pH of the medium. This relationship is illustrated in Fig. 12. As expected, a clear dependence of the ζ-potential with the pH of the medium is observed, with a negative correlation between both. Although all samples show the same general trend, with ζ-potential decreasing with increasing pH, three distinct profile groups could be identified, which correspond to the reaction temperature. This further supports the previous findings where temperature is the main factor influencing both the reaction performance and the physicochemical properties of the lignin derivatives, with low variability within each group. Derivatives synthesized at 30 °C (which show the lowest DS values) exhibit the lowest ζ-potential values and the greatest sensitivity to pH changes. As the reaction temperature, and consequently the DS, increased, the variations in ζ-potential became less pronounced. In particular, samples prepared at 70 °C, remained positively charged across the entire pH range studied. This behavior can be rationalized by the nature of the modification: CHPTAC introduces permanently charged quaternary ammonium groups into the lignin structure, which remain cationic regardless of the pH. On the other hand, the hydroxyl groups in lignin tend to deprotonate as the pH increases. The balance between the number of introduced cationic groups and remaining free hydroxyl groups determines the ζ-potential profile of each derivative. For example, CHPTAC-modified sulfonated lignin has been reported to have a low isoelectric point (2.2) due to the high

Table 5
Z-average diameter and PDI of cationically modified lignin derivatives with different degrees of cationization.

Temperature (°C)	Molar ratio	DS	Zeta potential (mV)	Z-average (nm)	PDI
30	1.3	0.34 ± 0.01	−21.5 ± 0.1	324 ± 12	0.47 ± 0.06
	2.55	0.32 ± 0.06	−19.6 ± 3.2	315 ± 21	0.47 ± 0.05
	3.8	0.31 ± 0.01	−17.3 ± 0.8	360 ± 44	0.52 ± 0.05
50	1.3	0.38 ± 0.00	10.3 ± 0.5	897 ± 39	0.59 ± 0.18
	2.55	0.33 ± 0.00	12.6 ± 0.3	442 ± 12	0.29 ± 0.07
	3.8	0.38 ± 0.01	10.3 ± 1.3	653 ± 24	0.33 ± 0.13
70	1.3	0.50 ± 0.01	19.3 ± 3.6	245 ± 28	0.56 ± 0.07
	2.55	0.48 ± 0.00	20.6 ± 3.4	156 ± 14	0.45 ± 0.03
	3.8	0.57 ± 0.01	21.3 ± 1.5	191 ± 8	0.43 ± 0.04

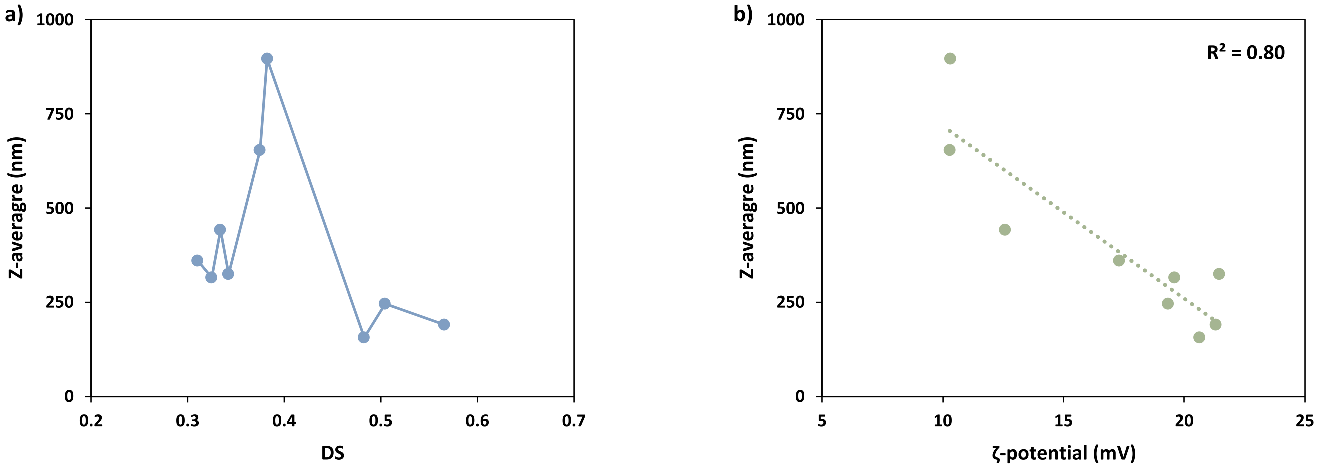

Fig. 11. Influence of a) DS and b) ζ-potential of cationically modified lignins on the Z-average diameter. ζ-potential values are expressed as absolute values.

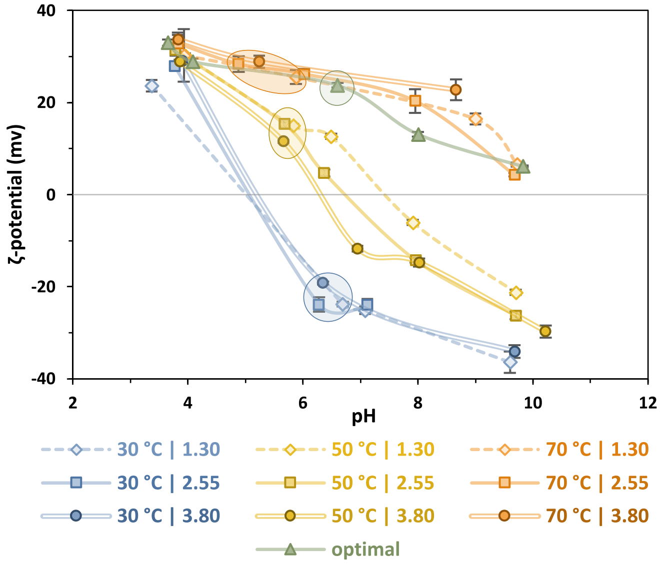

Fig. 12. ζ-potential of the synthesized lignin derivatives as a function of pH. Samples names refer to the conditions used for the synthesis (temperature | CHPTAC-to-lignin molar ratio). The sample obtained under optimal conditions was synthesized at 70 °C, using a CHPTAC-to-lignin ratio of 1.30 with 25 % extra NaOH. Data points within the shaded regions correspond to the aqueous dispersions of each polymer without pH adjustment.

content of anionic sulfonate groups relative to cationic ones [72]. The value of the isoelectric point can be tuned according to the intended application. Lignin derivatives synthesized at high temperatures and with higher DS, may be advantageous for applications requiring stable cationic properties, whereas slightly modified lignins could yield pH-responsive polymers.

For each sample, the pH of the resulting solution/dispersion without pH adjustment is highlighted by the shaded circles. It can be observed that different reaction conditions led to shifts in the natural pH of these solutions. In general, samples cationized at higher temperatures resulted in aqueous solutions with lower pH values. This is attributed to the higher content of cationic moieties introduced during the modification process.

The viscosity-average molecular weight (Mv) of two selected cationic lignin derivatives with different DS was also determined using the intrinsic viscosity method. The Mark–Houwink–Sakurada equation establishes a correlation between the Mv of a polymer and its intrinsic viscosity, $[\eta]$ (eq. (4), section 2.4.6). Therefore, the Mv can be estimated from the $[\eta]$ if the constants for that specific polymer–solvent system are known. Results for the determined $[\eta]$ and the resulting Mv obtained for cationic lignins with DS values of 0.35 and 0.63 are summarized in Table 6. Experimental plots of specific viscosities (η_{sp}) and concentrations used to determine the $[\eta]$ can be found in supplementary material (section 3). No significant effect of the DS on the intrinsic viscosity of the lignins was observed. Consequently, the Mv of the polymers was also not significantly affected (one-way ANOVA with Tukey test, *p*-value >0.05) after modification. Nevertheless, slight changes were observed, suggesting that the relationship between the DS and the Mv is not straightforward. A slight decrease in Mv from 13.53 ± 2.12 kDa to 8.65 ± 1.09 kDa was observed for the lignin with lower DS (DS = 0.35) compared to the unmodified lignin (DS = 0). In contrast, lignin with higher DS (0.63) exhibits a higher Mv, 12.79 ± 2.73 kDa, closer to that of the unmodified lignin. The lack of statistical significance in these differences restricts the possibility of withdrawing clear conclusions for the effect of reaction conditions and DS on the Mv of the polymers. However, it still indicates that there is a slight decrease in the Mv when cationization is performed at lower temperatures and alkalinity, and a

Table 6
Intrinsic viscosity [η] and viscosity-average molecular weight (Mv) of unmodified acacia lignin (DS = 0) and two selected cationic lignins with different cationizations degrees (DS = 0.35 and 0.63). Same letters connecting the results represent no statistical differences in Tuckey test ($\alpha = 0.05$).

Sample	Reaction conditions	[η] ($mL \bullet g^{-1}$)	R^2	Mv (kDa)
DS = 0	n.a.[1]	3.71 ± 0.14^a	0.954 ± 0.025	13.53 ± 2.12^a
DS = 0.35	A[2]	3.35 ± 0.10^a	0.997 ± 0.003	8.65 ± 1.09^a
DS = 0.63	B[3]	3.66 ± 0.18^a	0.980 ± 0.008	12.79 ± 2.73^a

[1] n.a.: not applicable
[2] A: temperature: 50 °C, CHPTAC-to-lignin ratio: 2.55, NaOH ratio: 1
[3] B: temperature: 70 °C, CHPTAC-to-lignin ratio: 1.3, NaOH ratio: 1.25.

possible recondensation happens when harsher conditions of temperature and alkalinity are employed. This phenomenon of lignin recondensation in alkaline medium was also previously hypothesized by Du, et al. for lignin cationized with EPTAC [73].

4. Conclusions

In this study, lignin extracted from acacia wood was chemically modified by grafting quaternary ammonium groups to improve its solubility and surface charge. The cationization of lignin was performed by reacting it with the reactive epoxy group of EPTAC, which was prepared in situ from the conversion of CHPTAC under alkaline conditions. The cationization reaction conditions, namely reaction temperature and CHPTAC-to-lignin ratio, were optimized using a three-level full factorial design of experiments and a response surface methodology. A multi-objective optimization was employed to simultaneously optimize the DS and the ζ-potential of the obtained lignin derivatives. The reaction temperature was found to be the most relevant parameter for achieving a higher lignin cationization, whereas the CHPTAC-to-lignin molar ratio had no statistically significant effect within the studied range. The alkalinity of the reaction medium (NaOH ratio) was also found to affect the degree of cationization, as it governs the conversion of CHPTAC to the reactive EPTAC species and the activation of the lignin hydroxyl groups. After optimization, a DS of 0.61 ± 0.03 and ζ-potential of 18.79 ± 0.30 mV was achieved. The optimal conditions were found to be a reaction time of 3 h, temperature of 70 °C, CHPTAC-to-lignin ratio of 1.3 and NaOH ratio of 1.25. The successful grafting of the quaternary ammonium groups was confirmed by infrared spectroscopy.

Lignin cationization via etherification with CHPTAC proved to be a straightforward method, performed at mild temperature and time conditions, capable of converting the highly negatively charged and water insoluble acacia lignin into water soluble and positively charged derivatives. In the future, these cationic derivatives will be studied as potential hair conditioning agents to replace the currently used fossil-based agents, which are highly toxic to aquatic organisms. It is expected that these lignin-based derivatives will offer a safer, biodegradable, and sustainable alternative, reducing environmental harm while effectively repairing damaged hair. This work aligns with circular economy principles, aiming to reduce the environmental impact of cosmetic formulations by valorizing lignin into commercially valuable products.

CRediT authorship contribution statement

Catarina Fernandes: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Leandro Cid Gomes:** Investigation. **Diana Bernin:** Resources. **Luís Alves:** Writing – review & editing, Supervision, Conceptualization. **Bruno Medronho:** Writing – review & editing, Supervision, Conceptualization. **Maria Graça Rasteiro:** Writing – review & editing, Supervision, Resources, Conceptualization. **Carla Varela:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2025.167345.

Data availability

No data was used for the research described in the article.

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