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Research Article

# Stability of Cholinium Chloride-Based Deep Eutectic Solvents with Carboxylic Acids: A Study on Ester Formation

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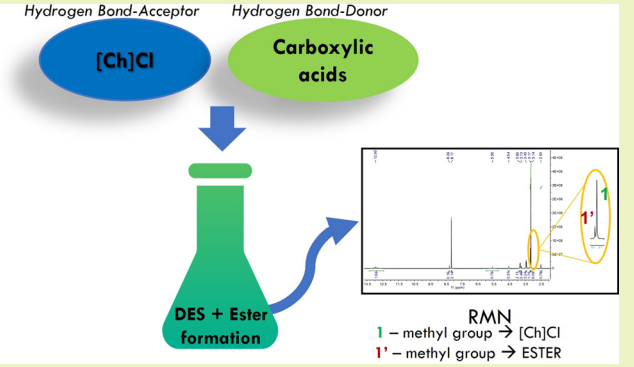
**ABSTRACT:** Deep eutectic solvents (DES), especially those derived from cholinium chloride ([Ch]Cl), have been studied as alternative solvents for various chemical processes. Despite their widespread use, the understanding of the possible formation of impurities in these solvents, such as esters, is still incomplete. In this study, the thermal and long-term stability of [Ch]Cl-based DES were investigated. Specifically, three [Ch]Cl-based DES with carboxylic acids (acetic acid, formic acid, and lactic acid - and their mixtures with water were investigated. The results show that the DES formed with [Ch]Cl: formic acid has lower thermal and long-term stability, but the addition of water to DES significantly reduces the rate of ester formation. The DES [Ch]Cl: lactic acid, and [Ch]Cl: acetic acid were found to exhibit good long-term stability. However, [Ch]Cl: lactic acid has the disadvantage of containing a significant amount of lactide ester originally. It is noteworthy that the esterification in the preparation of the DES [Ch]Cl: acetic acid only took place at temperatures up to 40 °C and no further impurities were formed, making it the most suitable DES for processes at this temperature. In summary, the choice of acid appears to influence ester formation, with temperature accelerating the reaction and the addition of water playing a crucial role in slowing the rate of ester formation. In the long term, however, ester formation appears to be unavoidable, as esterification continues even after its production. Therefore, investigating [Ch]Cl: carboxylic acid combinations, with a focus on the ester content and its influence on the process, is essential.




**KEYWORDS:** *esters, esterification, acetic acid, lactic acid, formic acid, green solvents, RMN*



## 1. INTRODUCTION

Deep eutectic solvents (DES) are a class of alternative solvents formed by mixing a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA).[1,2] According to Martins et al.,[2] DES are defined as mixtures of two or more pure compounds whose eutectic point temperature deviates significantly from the ideal temperature and remains below the operating temperature. Among the HBA, cholinium chloride ([Ch]Cl) stands out, while various other compounds, including carboxylic acids, amides, and sugars have been proposed as HBD.[3]

Although there are five DES types, those of type III based on [Ch]Cl and carboxylic acids have attracted considerable interest due to their versatility, low cost, and low toxicity. However, the assessment of toxicity can be controversial and depends on the DES rather than the class as a whole.[4−6] These solvents have been proposed for various chemical processes, including extraction of bioactive compounds,[7,8] metal recovery,[9,10] purification and solubilization of compounds,[11−13] lignocellulosic biomass processing,[14] removal of microplastics from water,[15] production, and purification of biodiesel,[16−18] among various other applications.

Nevertheless, DES based on [Ch]Cl: carboxylic acids leads to the formation of secondary compounds by esterification reactions, which can occur independently of the preparation method, as reported by Rodriguez-Rodriguez et al.[19] Thermal stability studies using different preparation methods, such as heating with stirring and stirring at room temperature, showed that while heating led to lower viscosity of DES, the use of high temperatures increased ester formation. This behavior was attributed to the esterification reaction between the carboxylic acid and the OH group of the cholinium cation.[19,20] This behavior was first observed by Florindo et al.,[20] who reported impurity levels of 5−30 wt % with the heating method, while the grinding method yielded pure mixtures without ester formation. The evaluation of heating, grinding, and freeze-drying preparation methods was also evaluated in the

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preparation of DES based on L-proline, glycolic acid, and DL-malic acid.[21] The authors also observed that in these DES, the esterification reaction occurred regardless of the preparation method, with the grinding method leading to the lowest amount of esters. However, it should be kept in mind that, as shown by van den Bruinhorst et al.[21] and Rodriguez-Rodriguez et al.[19] water can be used to reduce the esterification reaction rate of DES. Since the esterification reaction is an equilibrium process, the gradual production of water during the reaction is expected to reduce the reaction rate until an equilibrium limit is reached over time.

The aim of this work is to investigate ester formation in DES based on [Ch]Cl:carboxylic acid, in particular [Ch]Cl: acetic acid, [Ch]Cl: formic acid, and [Ch]Cl: lactic acid, all at a molar ratio of 1:2. In this study, DES esterification, focusing on the percentage of [Ch]Cl esterification, was followed by nuclear magnetic resonance (NMR) under different DES preparation conditions, including temperature (20 to 80 °C), time (up to 14 days), and water content (10 to 70 wt %).

## 2. MATERIALS AND METHODS

**2.1. Materials.** The reagents used to prepare the DES were cholinium chloride ([Ch]Cl) from Sigma-Aldrich (USA, purity >98%), formic acid from Carlo Erba Reagents (France, purity >99%); acetic acid from Honeywell (Germany, purity >99.99%); L-lactic acid from Sigma-Aldrich (France, purity >88−92%). In this work, ultrapure water, which was double distilled, passed through a reverse osmosis system and treated in the Milli-Q plus 185 water purification system, and dimethyl sulfoxide D6 (DMSO) from Eurisotop (USA, purity >99.9%) were used.

The purity of the reagents used in this study reflects typical industrial conditions, where analytical-grade purity is not always feasible. $^1$H NMR spectroscopy was employed to quantify ester formation, excluding any measurable impurities. The natural presence of esters in commercial lactic acid, such as lactide, was accounted for in the calculations. While higher-purity reagents are ideal, the purity levels used here are sufficient to validate the results and are representative of practical industrial scenarios.

**2.2. Preparation of the Deep Eutectic Solvents.** The DES were prepared on magnetic stirrers (Heidolph, MR HEI-Tec, Germany) at 600 rpm with a conduction heating jacket at a fixed temperature until a homogeneous transparent liquid was obtained. The HBA-HBD mixtures evaluated in this study were [Ch]Cl: acetic acid (molar ratio 1:2), [Ch]Cl: formic acid (molar ratio 1:2), and [Ch]Cl: lactic acid (molar ratio 1:2). The individual compounds were accurately weighed within $\pm 10^{-4}$ g on an analytical balance (Sartorius, BCE623I-1S, Germany).

Three key parameters were investigated in the preparation of DES: temperature, time, and water addition. Specifically, first DES were prepared at temperatures of 20, 40, 60, and 80 °C for 2 h, both without and with 10 wt % water addition, in order to evaluate the temperature effect. Then, to evaluate the time-dependent aspects, a kinetic study was carried out over 14 days at intervals of 2, 48, 168, and 336 h at 20 °C for DES without added water. Finally, the effects of water addition were investigated, focusing on water contents of 10, 30, and 70 wt % over a period of 2 h at 20 °C.

**2.3. NMR Spectroscopy.** All the samples (DES and compounds used in the formation of DES) were dissolved in DMSO-$d_6$ and analyzed using a Bruker Avance 300 spectrometer at 300 MHz for $^1$H NMR according to Rodriguez-Rodriguez et al.[19] The pulse program used was zg30, a standard sequence for proton NMR. The spectral width was set to 6893.38 Hz, covering a chemical shift range of 22.97 ppm, which is sufficient for typical $^1$H chemical shifts. A delay time of 1.0 s allowed for nuclear relaxation between each scan and ensured accurate signal intensity. This delay time was based on our evaluation of T1 relaxation times as the signals used for the esterification calculations had T1 values of less than 1.0 s. The acquisition time was 4.75 s, which determines the duration of the free induction decay scan and affects the digital resolution. Each sample was subjected to 56 scans to reduce random noise and improve the clarity of the spectrum through signal averaging. Data processing was performed by using MestreNova software. Signal integration evaluated the area under the peaks and provided information on the relative amounts of the different $^1$H in the samples.

**2.4. Esterification Calculation.** The percentage of [Ch]Cl esterification was determined by analyzing the $^1$H NMR spectrum of DES according to the method described by Rodriguez-Rodriguez et al.[19] Before the NMR analysis is performed, it is important to consider the possible esterification reactions for the DES under study, shown in Figure 1, to enable a comprehensive analysis.

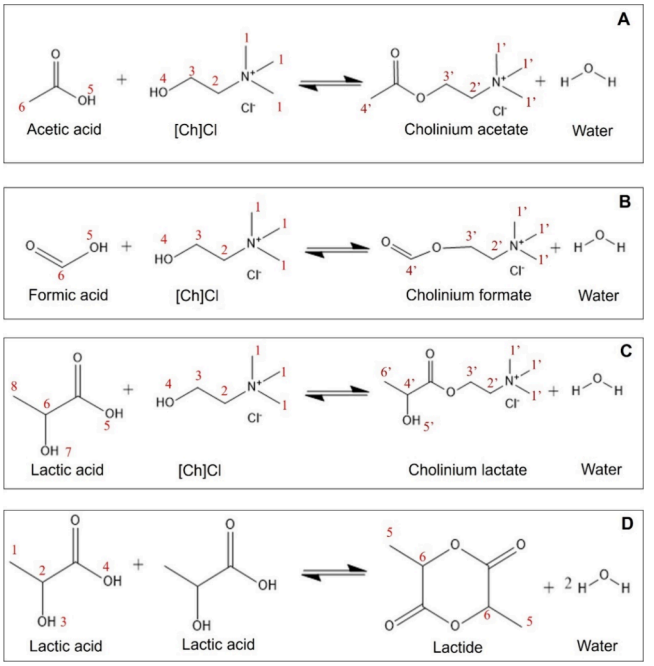


**Figure 1.** Esterification reactions for the systems formed by (A) [Ch]Cl: acetic acid, (B) [Ch]Cl: formic acid and (C) [Ch]Cl: lactic acid, and (D) lactide.

Figure S1 in the Supporting Information illustrates an example of a $^1$H NMR spectrum integration to determine the percentage of [Ch]Cl esterification. First, the characteristic signal of the [Ch]Cl methyl groups (labeled 1) was identified, and the integration was normalized to 9 based on the sum of protons associated with the three methyl groups. Subsequently, the esterification signal became clear when the hydrogen atoms of the [Ch]Cl methyl groups (signal 1) shifted upon ester formation (signal 1'). Similarly, the hydrogen atoms of the ethylene group of [Ch]Cl (signal 3) partially split during esterification (signal 3'). The hydrogen atoms of the carboxyl group of formic acid (signal 6) were also shifted during ester formation (signal 4'). After integration of the $^1$H NMR spectrum, the molar percentage of [Ch]Cl esterification ($E_{[Ch]Cl}$) was calculated by relating the signal area of the ester methyl group (signal 1') to the sum of the areas of the ester methyl groups (signal 1') and [Ch]Cl (signal 1) according to eq 1. A similar concept was applied for the ethylene group of [Ch]Cl and carboxyl group of formic acid during the ester formation, as represented in eq 1.


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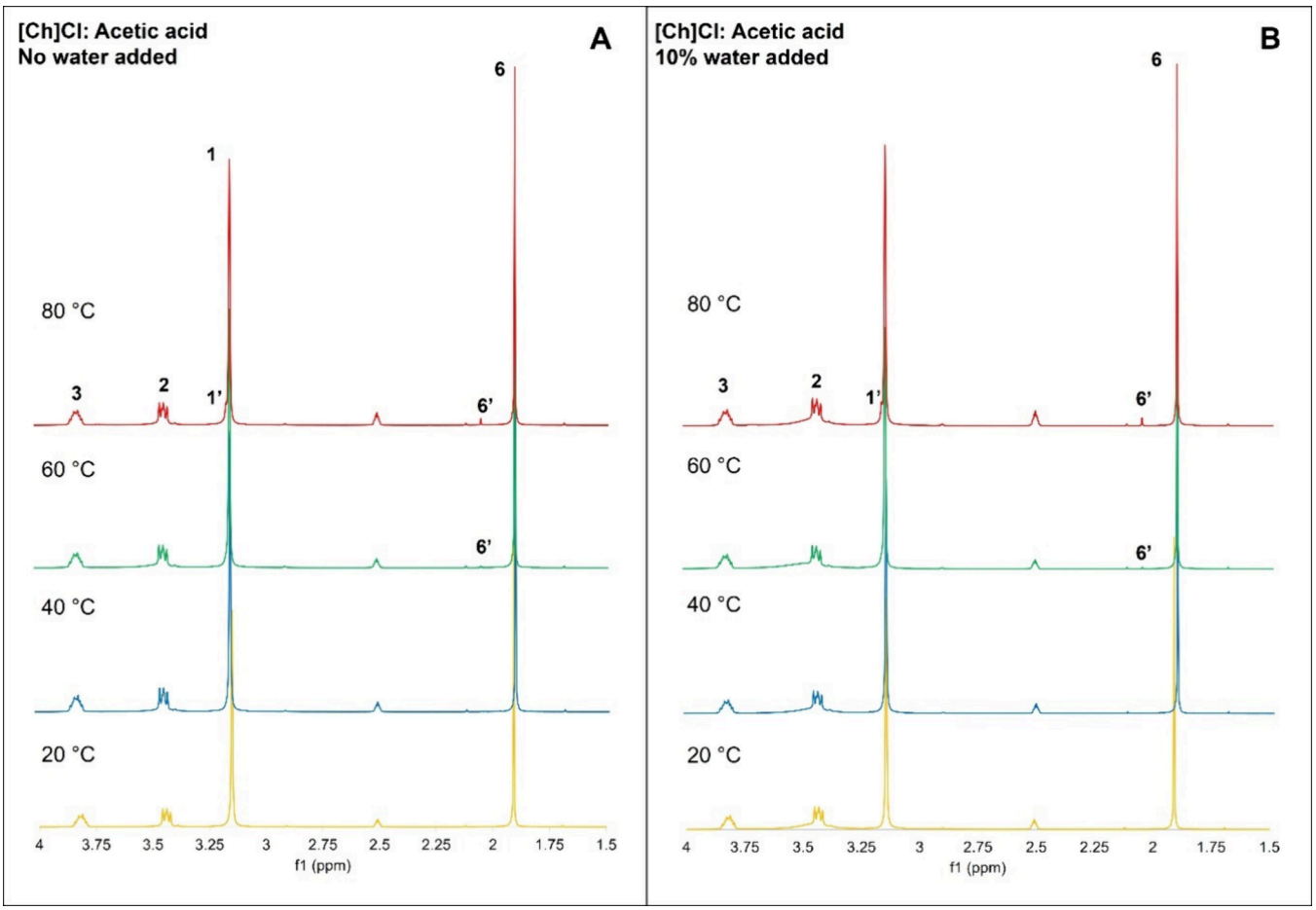


**Figure 2.** H NMR spectra for the DES (A) without addition and (B) with addition of 10 wt % water at different preparation temperatures, during 2 h.

$$E_{[Ch]Cl}(\text{mol}\%) = \frac{(A1')}{(A1' + A1)} \times 100 = \frac{(A2')}{(A2' + A2)} \times 100 = \frac{(A3')}{(A3' + A3)} \times 100 \quad (1)$$

In eq 1, A1' represents the area of the methyl group of esters and A1 the area of the methyl group of [Ch]Cl, A2' and A3' represent the area of the ethylene group of esters and A2 and A3 the area of ethylene group of [Ch]Cl, as shown in Figure S1 in the Supporting Information.

For the [Ch]Cl: lactic acid system, in addition to the formation of cholinium lactate esters, there was the formation of a lactide ester, already present in commercial lactic acid (Figure 1D). To calculate the molar percentage of lactide esterification ($E_{[Ch]Cl}$), the signals of the methyl group of lactic acid (signal 1) and that of lactide (signal 5) were considered. The calculation was carried out according to eq 2:

$$E_{\text{Lactide}}(\text{mol}\%) = \frac{(A5)}{(A1 + A5)} \times 100 \quad (2)$$

where A5 represents the area of the methyl group of lactide and A1 is the area of the methyl group of lactic acid, as shown in Figure S2 in the Supporting Information.

## 3. RESULTS AND DISCUSSION

In this study, the influence of temperature, time, and water concentration on the esterification reaction in three different DES was investigated. Each DES contained [Ch]Cl as HBA and either acetic acid, formic acid, or lactic acid as HBD. The evaluation of the esterification reaction was based on the observed partial $^1$H chemical shift in the NMR spectrum, as described in detail in the experimental section.

**3.1. Effect of the Temperature in Deep Eutectic Solvents Preparation.** To evaluate the effects of temperature, DES preparations were carried out at constant temperatures of 20, 40, 60, and 80 °C, for 2 h for DES without and with 10 wt % of water. The $^1$H NMR spectra for DES [Ch]Cl: acetic acid are shown in Figure 2, further spectra for the remaining DES studied can be found in Figures S3 and S4 in Supporting Information. The quantitative results of the esterification results for all DES studied are shown in Figure 3 (see Tables S1 and S2 in the Supporting Information for more details).

Figure 2 shows that for the DES of [Ch]Cl: acetic acid, without and with 10 wt % water, no clear signal indicating the esterification reaction could be observed up to a temperature of 40 °C at a preparation time of 2 h. When the DES is heated to 60 °C, the methyl group signals of [Ch]Cl (signal 1) show a broadening of the base and the appearance of a protrusion characteristic of a signal that begins to split off (signal 1') and belongs to the methyl group of the cholinium acetate ester. This phenomenon can be observed in DES with and without the addition of water. Looking at the $^1$H NMR of DES heated to 80 °C, a clear increase in the signal intensity of the prominence associated with the signal of the ethyl group can


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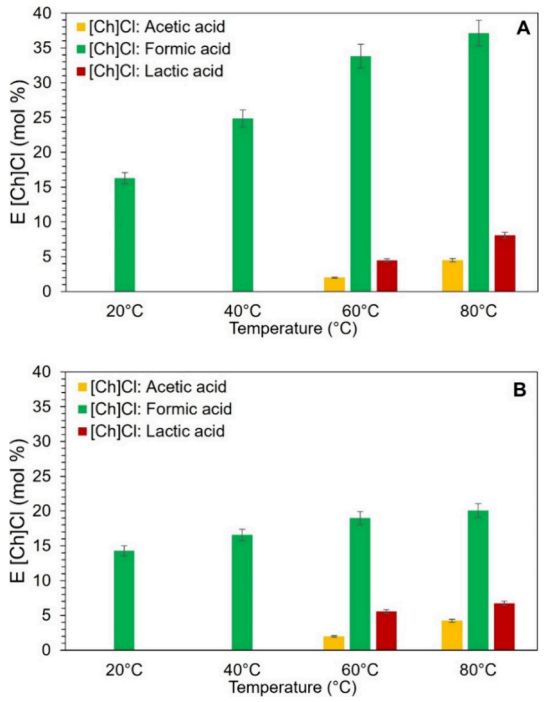


**Figure 3.** Molar percentage of [Ch]Cl esterification ($E_{[Ch]Cl}$) for DES (A) without addition and (B) with addition of 10 wt % water at different preparation temperatures during 2 h.

be observed (signal 1'). Moreover, the acetic acid methyl group signal at 1.91 ppm (signal 6) gradually disappears when the temperature increases from 60 to 80 °C, accompanied by the appearance of signals at 2.05 ppm (signal 6'). These observations emphasize the significant influence of the preparation temperature on the stability of the DES. These results are also confirmed by the esterification values of [Ch]Cl, which reached 4 mol % at 80 °C, while they were 0 mol % at 20 °C for both the DES without and for the DES with added water (see Figure 3). Additionally, it is worth mentioning that [Ch]Cl: acetic acid esterification results observed exclusively result from the cholinium acetate without the formation of any other esters.

Looking at the results related to the preparation of [Ch]Cl: formic acid DES without the addition of water and with 10 wt % addition of water at different temperatures over a period of 2 h, the $^1$H NMR spectra (Figure S3 in the Supporting Information) show a separation of the signals characterizing the methyl group (3.14 ppm, signal 1) of [Ch]Cl and the methyl group of the cholinium formate ester (3.17 ppm, signal 1'). These results were observed for all temperatures studied in the preparation [Ch]Cl: formic acid DES. Similarly, it was also observed that the signals of the ethylene groups of [Ch]Cl at 3.43 ppm (signal 2) and 3.80 ppm (signal 3) split into 3.73 ppm (signal 2') and 4.54 ppm (signal 3') upon ester formation. Moreover, the intensity of the signals corresponding to ester formation gradually increases with temperature, as shown in Figure S3 in the Supporting Information for the signals labeled 1', 2', 3', and 4'. This observation is confirmed by the determination of the percentage of [Ch]Cl esterification (please see Figure 3). For [Ch]Cl: formic acid without the addition of water, an increase in the cholinium formate ester content of about 131% was observed when DES was incubated at 80 °C compared to 20 °C (from 16 mol % at 20 °C to 37 mol % at 80 °C, Figure 3A). For [Ch]Cl: formic acid with 10 wt % water added, the ester content also increases, albeit to a lesser extent, by about 43% when the DES is kept at 80 °C compared to 20 °C (from 14 mol % at 20 °C to 20 mol % at 80 °C, Figure 3B). Consequently, the presence of water reduces the formation of esters by approximately 46% at 80 °C. However, a more detailed discussion on the effect of water will be provided later. In addition, in the case of [Ch]Cl: formic acid, no esters other than cholinium formate were formed.

Looking now at the results of the preparation of [Ch]Cl: lactic acid without and with the addition of water (i.e., 10 wt %) for up to 2 h at different temperatures, the $^1$H NMR results are shown in Figure S4 in the Supporting Information. It should be noted that, as mentioned in the experimental section, in this case, the lactic acid used for DES formation already contained an ester, namely lactide ester formed by the condensation of two molecules of lactic acid (see Figure S2 in the Supporting Information, where the signals number 5 and number 6 correspond to chemical shifts of the hydrogen protons of the lactide molecule). This observation was also made by Morais et al.[22] when using DES from [Ch]Cl: lactic acid (1:10 molar ratio). They suggested that the formation of lactide could confirm the polymerization of lactic acid, as lactide is a common intermediate in this process.[22] The ester content of the lactic acid remained almost constant at all temperatures studied, i.e., at about 19 mol % (see Tables S1 and S2 in the Supporting Information). The only exception was the [Ch]Cl: lactic acid composition with 10 wt % water at 80 °C, where the ester value was slightly lower, namely 17 mol %. No evidence of decomposition mechanisms other than lactide was found when evaluating the degradation of lactic acid.

Finally, regarding the results of the [Ch]Cl: lactic acid, as shown in Figure S4 in the Supporting Information, no characteristic signals of the esterification reaction were observed up to a temperature of 40 °C, beyond the lactide esters already present. However, at temperatures of 60 and 80 °C, chemical shifts corresponding to the hydrogens of the methyl groups of the ester at 3.17 ppm (number 1') and the OH group at 4.48 ppm (number 5') were observed. Both groups were associated with the formation of cholinium lactate when the mixture was heated above 40 °C. Specifically, [Ch]Cl esterification increased from 4 mol % at 60 °C to 8 mol % at 80 °C, an increase of approximately 81%, as shown in Figure 3A for the system without added water. It should be noted that these values do not take lactide into account. When examining the [Ch]Cl esterification values for the [Ch]Cl: lactic acid with the addition of 10 wt % water, only a 21% increase was observed (from 5.56 mol % at 60 °C to 6.74 mol % at 80 °C, see Figure 3B and Table S2 in the Supporting Information). Thus, the addition of 10% water at a temperature of 80 °C led to a reduction in the formation of cholinium lactate, although the specific influence of water will be investigated in more detail later. Moreover, when evaluating the degradation of [Ch]Cl: lactic acid, in contrast to the other DES, two mechanisms must be taken into account, as two esters are presented/formed, namely, lactide and cholinium lactate.

In summary, the ester content of all DES increased with increasing temperature. In particular, [Ch]Cl: formic acid showed the highest tendency to esterification, with [Ch]Cl esterification increasing from 16 mol % at 20 °C to 37 mol % at 80 °C without added water and from 14 mol % at 20 °C to 20 mol % at 80 °C with 10 wt % added water. Our results agree with those of Rodriguez-Rodriguez et al.,[19] who observed that


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for all [Ch]Cl-based DES and various acids (lactic acid, glycolic acid, levulinic acid, malic acid, oxalic acid, glutaric acid, and malonic acid) the degrees of esterification were directly proportional to the temperature increase. In this work, we observed no esterification of [Ch]Cl in the [Ch]Cl-based DES prepared with acetic and lactic acid up to a temperature of 40 °C. It is worth noting that acetic acid does not contain an ester, whereas lactic acid itself already contains an ester. In contrast to our results, van den Bruinhorst et al.[21] reported ester formation in proline-based DES with glycolic and malic acid, even at room temperature.

**3.2. Effect of the Time in Deep Eutectic Solvents.** Considering the different time intervals at which researchers prepare and use DES and their reusability, we investigated the temporal effects on DES esterification. The esterification reaction for the three DES studied was observed over different times (time storage), 2, 48, 168, and 336 h, at a constant temperature of 20 °C. Figure 4 shows the results of the percentage of [Ch]Cl esterification over time for DES without added water at 20 °C.

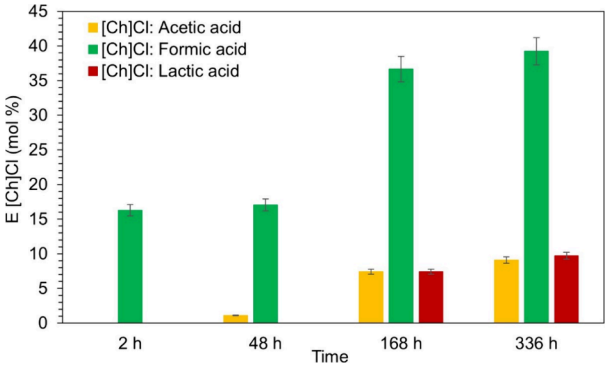


**Figure 4.** Molar percentage of [Ch]Cl esterification ($E_{[Ch]Cl}$) for DES prepared without addition of water at 20 °C, measured at different preparation times.

The [Ch]Cl: acetic acid and [Ch]Cl: lactic acid DES exhibited [Ch]Cl esterification values of 0 mol % after 2 h of preparation, which increased to 9 and 10 mol %, respectively, after 336 h of storage. It is noteworthy that the lactide content in [Ch]Cl: lactic acid DES remained almost constant during the storage period (see Table S3 in the Supporting Information). In contrast, the [Ch]Cl: formic acid mixture showed a significant increase in ester content by about 141%, rising from 16 to 39 mol % during the 336-h storage period.

For all DES tested, the increase in [Ch]Cl esterification within the first 48 h of storage was not significant (e.g., [Ch]Cl: acetic acid content was 0 mol % after 2 h and 1 mol % after 48 h). A notable increase in esterification occurred after 168 h (e.g., [Ch]Cl: acetic acid content was 1 mol % after 48 h and 7 mol % after 168 h). Comparing esterification values between 168 and 336 h showed no significant difference (e.g., [Ch]Cl: acetic acid content was 7 mol % after 168 h and 9 mol % after 336 h). Therefore, it seems that most ester formation occurs in the first 7 days, indicating that the system is approaching equilibrium. Van Den Bruinhorst et al.[21] also investigated the effect of time on the esterification reaction of the DES formed by [Ch]Cl: glycolic acid. They observed that the esterification reaction continues even after preparation, yielding a proline ester content of approximately 5.5 mol % after 85 h. Rodriguez-Rodriguez et al.[19] also observed that the ester content of [Ch]Cl with glycolic acid and [Ch]Cl with lactic acid increased to 14 and 11 mol %, respectively, after 11 months of preparation. Since the esterification reaction produces water and is an equilibrium reaction, both authors concluded that the production of water gradually suppresses the reaction rate and reaches a limit/equilibrium over time. The presence of water shifts the equilibrium toward hydrolysis and decreases the overall rate of ester formation, highlighting the importance of controlling the water content in [Ch]Cl: carboxylic acid DES, as discussed in more detail in the next section.

In summary, the stability of [Ch]Cl: acetic acid and [Ch]Cl: lactic acid was higher than that for [Ch]Cl: formic acid. In particular, [Ch]Cl: acetic acid showed a clear advantage as acetic acid did not exhibit ester formation. This property emphasizes its superior stability compared to the other DES, making it suitable for postpreparation storage and possible reuse in multiple cycles. However, additional parameters need to be carefully considered when we consider the reuse of the solvent.

**3.3. Effect of the Water in Deep Eutectic Solvents.** The impact of water addition on DES stability was investigated with varying concentrations: 0, 10, 30, and 70 wt %. The selected amounts of water were based on experiments conducted for the extraction of bioactive compounds,[7,23,24] in which the formation of esters has not yet been taken into account, an aspect that has been often overlooked. The experiments were conducted for a preparation time of 2 h at 20 °C. The [Ch]Cl esterification results are summarized in Table 1.

Looking now at the results for [Ch]Cl: acetic acid, no solvent degradation was observed when different amounts of water were added to this DES, as evidenced by the absence of chemical shifts of the ions in the $^1$H NMR spectra (see Figures S5−S8 in the Supporting Information).

In contrast to the [Ch]Cl: acetic acid DES, the [Ch]Cl: formic acid system showed esterification up to 16 mol %. It is

**Table 1. Molar Percentage of [Ch]Cl Esterification ($E_{[Ch]Cl}$) and of Lactide Esterification ($E_{Lactide}$) for DES Prepared with Different Water Contents, at 20 °C, during 2 h[a]**

| | | % of water added | | | |
|---|---|---|---|---|---|
| | solvents | *NWA | 10 wt % | 30 wt % | 70 wt % |
| $E_{[Ch]Cl}$ (mol %) | [Ch]Cl: Acetic acid | 0.00 | 0.00 | 0.00 | 0.00 |
| | [Ch]Cl: Formic acid | 16.28 ± 0.8 | 14.29 ± 0.7 | 11.85 ± 0.55 | 11.07 ± 0.54 |
| | [Ch]Cl: Lactic acid | 0.00 | 0.00 | 0.00 | 0.00 |
| $E_{Lactide}$ (mol %) | [Ch]Cl: Lactic acid | 18.75 ± 0.9 | 18.87 ± 0.88 | 19.34 ± 0.97 | 19.26 ± 0.96 |
| | Lactic acid pure | 21.26 ± 1.07 | | | |

[a]NWA, no water added.


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noteworthy that the addition of water enhances the stability of the DES, leading to a significant decrease of the esterification reaction up to 32% when 70 wt % water is added (percentage of [Ch]Cl esterification: 16 mol % for DES without water and 11 mol % for DES with 70 wt % water).

For [Ch]Cl: lactic acid, no degradation effect was observed beyond the secondary compounds that were already present in the "pure" lactic acid, the acid used in DES preparation as received from the producer, prior to DES preparation (as shown in Figure S2 in Supporting Information and discussed previously). However, the addition of water led to a significant decrease in lactide content, which dropped from 21 mol % (no water added) to about 19 mol % (70 wt % water added). This decrease can be attributed to the hydrolysis of lactide in the presence of water, producing two molecules of lactic acid.[22,25,26]

In summary, the results indicate a reduction in the degree of esterification of the DES investigated with increasing water addition, as expected taking in account what has been suggested by Van Den Bruinhorst et al.[21] and Rodriguez-Rodriguez et al.[19] In situations in which the presence of esters is undesirable, the addition of water proves to be a crucial factor in controlling their formation. Since esterification is a reversible equilibrium reaction, the equilibrium must be maintained in favor of the reactant. Although water can alter the properties of DES, as reported by Rodriguez-Rodriguez et al.,[19] who found that the addition of more than 51 wt % water to DES-urea:[Ch]Cl (2:1) can turn the mixture into an aqueous solution. However, it is important to consider the specific objectives and carefully weigh the advantages and disadvantages of adding water. Although many researchers prefer to dry the reagents prior to DES preparation, this study shows that such a practice compromises DES stability and shifts the reaction equilibrium toward product formation (esters and water). Moreover, this approach increases the cost and complexity of the work. Another point that should have been taken into account is that many literature studies used DES for the extraction of various compounds such as phenolic compounds,[27] metals,[28] and lignins[29,30] without considering the effects of DES esterification in these processes. In addition, these solvents were used in simulation procedures with models such as COSMO-SAC and COSMO-RS to predict activity coefficients at infinite dilution ($\ln \gamma_i^{\infty}$) without considering the formation of secondary compounds. Not considering these compounds in the modeling may affect the prediction accuracy of the model in solvent screening procedures, especially when bioactive compounds.

**3.4. Impact of p$K_a$ on Deep Eutectic Solvents Stability and Ester Formation.** The results of this study can be directly attributed to the acid dissociation constants (p$K_a$) of the weak acids used. The p$K_a$ values of acetic acid, lactic acid, and formic acid are 4.76, 3.86, and 3.74, respectively.[31−33] These values show that acetic acid is the weakest of the three acids and is, therefore, the least susceptible to esterification reactions. This higher p$K_a$ means that acetic acid is less likely to donate protons, thereby reducing the propensity for ester formation at lower temperatures. This minimal ester formation is evidenced in the [Ch]Cl: acetic acid DES, particularly at temperatures up to 40 °C, making it the most suitable acid for the preparation of DES among the acids studied. In contrast, formic acid and lactic acid, with lower p$K_a$ values of 3.74 and 3.86, respectively, are stronger acids and have a higher tendency to donate protons, facilitating esterification reactions. The DES with [Ch]Cl: lactic acid also showed remarkable ester formation, which was enhanced by the presence of lactide ester from the beginning. The selection of acetic acid for DES preparation is particularly advantageous due to its higher p$K_a$ value, which minimizes ester formation and maximizes stability. This finding emphasizes the necessity of considering the p$K_a$ values of acids in the design of DES, especially for processes requiring long-term solvent stability and minimal impurity formation

Furthermore, the esterification rates of [Ch]Cl: carboxylic acid systems are influenced by the p$K_a$ values of the acids, with lower p$K_a$ values leading to higher esterification rates. [Ch]Cl: formic acid shows the highest esterification rate, achieving 37 mol % at 80 °C without added water, and 20 mol % with 10 wt % water. This is due to formic acid's low p$K_a$ of 3.75. [Ch]Cl: lactic acid exhibits a moderate esterification rate, reaching up to 8 mol % at 80 °C without water, although the initial presence of lactide ester complicates this system. [Ch]Cl: acetic acid has the lowest esterification rate, with no significant ester formation up to 40 °C, and minimal formation at higher temperatures, attributed to acetic acid's higher p$K_a$ of 4.76. Water addition reduces the esterification rates across all systems. These findings align with previous studies showing that esterification rates increase with temperature and vary according to the acidity of the acids involved.[19,21]

**3.5. Practical Effects of Ester Formation in Industrial Applications of Deep Eutectic Solvents.** The formation of esters in [Ch]Cl-based DES has several practical implications for industrial applications. First, esters as impurities can affect the purity of DES, which impacts the reproducibility of industrial processes. On the other hand, in regulated industries such as pharmaceutical and food industries, the presence of impurities is critical for safety and regulatory compliance. Therefore, monitoring and controlling ester formation is essential to meet safety and regulatory standards and avoid contamination risks.

Long-term stability is critical to the commercial viability of DES. Continuous ester formation can shorten shelf life and require frequent replacement or regeneration, which increases operating costs. Understanding and controlling the conditions for ester formation can improve the stability of DES and extend its shelf life. Ester formation can also change the physicochemical properties of DES such as viscosity, density, and solvation capacity, which affects process efficiency and product quality. In the extraction of phenolic compounds, for example, esters can impair the selectivity and efficiency.

In summary, understanding the factors that influence ester formation in DES is critical to optimizing industrial applications. Controlling ester formation can improve the purity, stability, performance, and sustainability of DES, making them more viable and effective for a variety of applications.

## 4. CONCLUSIONS

In this work, we investigated the effects of various conditions on the stability of DES based on [Ch]Cl: acetic acid, [Ch]Cl: formic acid, and [Ch]Cl: lactic acid. In particular, we investigated the effects of temperature, time, and water concentration on [Ch]Cl esterification. It was observed that in the absence of water, the temperature intensified the esterification reaction. Nevertheless [Ch]Cl: acetic acid proved promising for processes up to 40 °C and showed no or very little esterification or impurity formation at these conditions.


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Over a period of 336 h (14 days) at 20 °C, an increase in esters was observed for all three DES, which was particularly pronounced for [Ch]Cl: formic acid. However, for [Ch]Cl: lactic acid and [Ch]Cl: acetic acid, the increase in esters was small, with the added advantage that [Ch]Cl: acetic acid showed no ester formation, unlike lactic acid, which formed lactide. Although the addition of water could not completely prevent ester formation, it effectively slowed the esterification process. Therefore, ester formation in [Ch]Cl-carboxylic acid-based DES is an inevitable long-term outcome, but the strategic addition of water can effectively slow this process. In addition, the relationship between the $pK_a$ values of acids and the stability of DES highlights the importance of selecting acids with appropriate dissociation constants to minimize impurities and maximize stability, emphasizing the role of acid strength in DES design and application. Nevertheless, a more nuanced assessment of the impact of ester content on DES properties, application performance, and other relevant factors is critical. Further research is needed to expand the practical applications of these solvents. The knowledge gained in this study contributes to both basic research and applications in processes in which DES is used.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.4c02903.

$^1$H NMR spectra for all compounds and tables containing the numerical results of the esterification percentage as a function of temperature with and without the addition of water, as well as a function of time (PDF)

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### Notes

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