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Research Paper

Molecular Spectroscopic (FTIR and UV-Vis) Analysis and In Vitro Antibacterial Investigation of a Deep Eutectic Solvent of N,N-Dimethyl Urea-Citric Acid

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Abstract

The intriguing pursuit of environmentally friendly solvents with tailored properties for diverse applications is a focal point of numerous studies, encompassing precursor selection, thorough characterization, and the exploration of potential applications. The study aims to assess the physicochemical properties and antimicrobial activity of deep eutectic solvents (DES) produced from N,N-dimethyl urea (DMU) and citric acid (CA), highlighting differences from their individual precursors. Various mass ratio variations of (DMU, solid) and (CA, solid) (DMU:CA = 1.0:1.0; 1.0:1.5; 1.0:2.0; 2.0: 1.0; 1.5:1.0) have been tested to make DES solvents through the melt process. Both types of blends generally melt at a temperature of 80°C. The overall liquid resulting from the melting of solids was generally clear in color. Molecular analysis using an infrared spectrophotometer showed some insignificant shifts from one product to another, compared with DMU and CA as precursors. Likewise, analysis using a UV–Vis spectrophotometer, when the entire sample was dissolved in demineralized water (2 mg/mL), showed no difference in the spectrum. In addition, functional group analysis using a spectrophotometer showed some minor changes, mainly shifts in peaks due to changes in the DMU:CA ratio. This may be due to the interaction of the hydrogen donor and the hydrogen acceptor in DES. All samples showed absorption peaks in the ultraviolet region of 202-210 nm. The resulting DES application showed growth inhibitory activity for *Staphylococcus aureus* and *Escherichia coli* bacteria in all products produced. The same analysis of the two types of precursors used showed that only CA had activity, but DMU did not have similar activity.

Keywords

N,N-dimethyl Urea, Citric Acid, Deep Eutectic Solvent, *Escherichia coli*, *Staphylococcus aureus*

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1. INTRODUCTION

The development of solvents that are environmentally friendly and have specific characteristics for various needs is very interesting. This can be seen in various studies in this field, starting with the selection of precursors, characterization, and even the development of various possible applications that can be studied (Silva et al., 2019). One type of solvent that has received attention for study is a solvent developed by mixing two compounds, where one compound is a hydrogen bond donor and the other compound is a recipient of a hydrogen bond donation; the obtained compound is known as deep eutectic solvent (DES) (Smith et al., 2014). The development of DES solvent synthesis, characterization, and application has progressed rapidly over the last decade. This is characterized by the discovery of a compound that has a high melting point, and when mixed with other compounds that have high melting points as well, the result is a mixture that has a low melting point (Cruz et al., 2017). The eutectic point (Teut) essentially determines the melting point of the mixture. The mixture reaches a minimal melting point at the eutectic point (Długosz, 2023). Subsequent developments from the discovery of the basic concepts of DES toward applications have also progressed rapidly until now.

The various types of precursor variations that have been developed by researchers in this field, N,N-dimethyl urea (DMU) and citric acid (CA) are interesting candidates because DMU can act as a hydrogen bond donor and CA functions as a hydrogen bond acceptor (Bafti and Khabazzadeh, 2014). The claimed DES compound (DMU and CA at a ratio of 2:1) is useful in the synthesis of bis(indolyl)-methanes, quinolines, and aryl-4,5-diphenyl-1H-imidazole.

On the other hand, the utilization of other DESs has also been widely developed as a solvent, medium, and even a reactant in several studies related to the preparation of metals and metal oxides and their derivatives (Smith et al., 2014). For example, DES in TiO_2 synthesis (Aghazadeh and Aghazadeh, 2017); metal oxide solubility in DES (Richter and Ruck, 2019); and $NiCo_2O_4$ synthesis (Chen et al., 2020). Another report mentioned that cetyltrimethylammonium bromide (CTAB) and acetic acid are used to modify mesoporous silica (Rafiq et al., 2022). Furthermore, choline chloride (ChCl) and urea are used as DES precursors for the chemical modification of silica particles based on DES (Tang et al., 2014; Gu et al., 2015). Some previous studies have also reported the use of DESs as antimicrobial agents (Garcia-Arguelles et al., 2013; Wikene et al., 2017; Al-Akayleh et al., 2022; Azmi et al., 2021), antioxidants (Inayat et al., 2023), and for environmental remediation (Kaur et al., 2021; Kumar et al., 2019).

Based on the literature review that has been conducted, no research has been found related to the modification of the weight ratio of DMU and CA precursors in the synthesis of DES, which is associated with its capacity as an antibacterial agent, especially against *Escherichia coli* and *Staphylococcus aureus*. Therefore, this research is directed at providing fundamental information related to the development of DES based on DMU and CA and its application in the fields of health and environmental.

2. EXPERIMENTAL SECTION

2.1 Materials

DMU (Sigma-Aldrich), CA (Merck), oil palm leaves (OPL), and demineralized water were used in the experiment. Various types of glassware are commonly used in chemistry laboratories to support experiments. Some of the instruments used in this research are UV–Vis and FTIR spectrophotometers. Bacteria *S. aureus* ATCC 6538 and *E. coli* ATCC 8739 have been used in this research.

2.2 Preparation of DMU:CA Deep Eutectic Solvent

Some ratios of the DES prepared using DMU and CA are shown in Table 1. The ratios of DMU and CA that have been used in this research are a modification of the reported procedure (Bafti and Khabazzadeh, 2014).

Table 1. Variation in Precursor Weight for the Preparation of the DMU-CA DES

Compound	Ratio (w/w)						
DMU	0	1.0	1.5	2.0	1.0	1.0	1.0
CA	1.0	1.0	1.0	1.0	0	1.5	2.0

The mixed compound was heated at 80°C until all the solid materials had melted. The obtained clear solution was kept at room temperature for further experiments, such as characterization and application in inhibiting *E. coli* growth. The infrared spectra of the DES samples were measured under ambient conditions on a Fourier transform infrared (FTIR) spectrophotometer (Bruker, USA) in the spectral region of 400 to 4000 cm^{-1}. The UV–Vis spectra of the samples will be measured under ambient conditions on a UV-Vis spectrophotometer (Agilent, Germany) in the spectral range of 200 to 800 nm.

A 24-hour-old microbial culture was prepared, and then 1 oz was taken and inserted into the diluent (sterile TSB) and stirred until homogenized. The solution obtained was measured for %T using a UV–Vis spectrophotometer at a wavelength of 580 nm until 25%T was obtained. On the other hand, TSA solution was made, poured into as much as 20–25 mL, and allowed to solidify. A total of 0.1 mL of microbial suspension was taken, placed on the surface of the solid media, and leveled with a sterile swab. Each sample that had been applied to the disc media (10% w/v concentration, positive control, and negative control) was placed on the solid media that had been given microbes. The positive control for *S. aureus* antibacterial testing is tetracycline, and the positive control for antibacterial testing of *E. coli* is chloramphenicol. The media was incubated for 24 hours aerobically at 30-35°C. The final stage is the observation of the clear zone that has formed.

3. RESULTS AND DISCUSSION

3.1 DES Preparation and Molecular Analysis

DES preparation was carried out with various variations, as shown in Table 1 in the Methods section. The dissolution process starts slowly, with the melting process occurring in each precursor. The process is carried out with a water bath system; the vial containing the mixture of DMU and CA is placed in a glass cup containing a certain amount of water heated to a temperature of 80°C, while DMU itself has a melting point of 104.4°C and CA has a melting point of 153°C. Previous researchers have discovered that the interaction of hydrogen donors and hydrogen acceptors can cause the dissolution process to occur at this low temperature (Długosz, 2023). The possible reaction mechanism is inspired by the structure proposed by other research results (Strauss et al., 2020), as shown in Figure 1, and the resulting material can be seen in Figure 2.

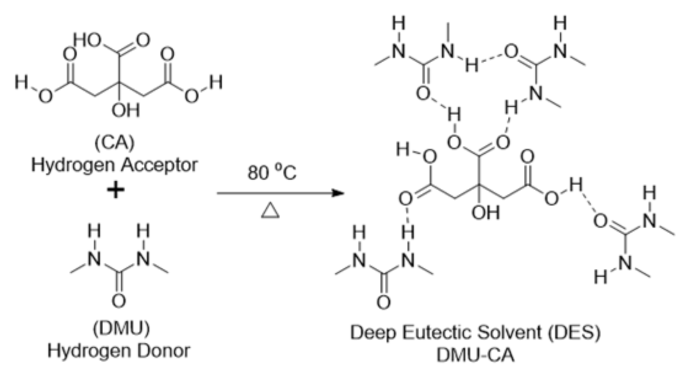

Figure 1. Plausible DMU-CA DES Formation Mechanism at Low Temperature

Based on Figure 2, it can be seen that the color of the solution is generally transparent to yellowish. No experimental evidence was obtained to determine the viscosity of the liquid, but in this case, the DMU:CA ratio of 1.0:1.5 and 1.0:2.0 gives a rather high viscosity because, as seen from the movement of the liquid, it is very slow to adjust the conditions when the sample bottle is tilted.

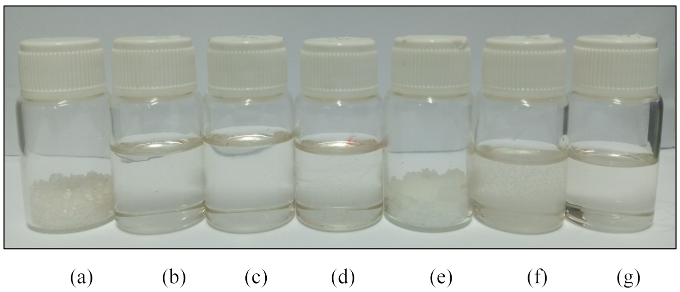

Figure 2. The Mixture of (a) CA; (b) DMU:CA (1.0:1.0); (c) DMU:CA (1.5:1.0); (d) DMU:CA (2.0:1.0); (e) DMU; (f) DMU:CA (1.0:1.5); and (g) DMU:CA (1.0:2.0)

Initial characterization based on FTIR spectrophotometry analysis showed some peak changes, which means that there is a shift in some interactions between functional groups caused by differences in the concentration of each precursor. The appearance of these peaks is shown in Figure 3. Figure 3 shows the FTIR spectra for DMU, CA, and the corresponding synthesized DES. The FTIR spectra show that the DMU has peaks at various positions, such as the peak at 3341 cm^{-1}, which is dedicated to the N−H group of amides, supported by the deformation peak at 1636 cm^{-1}. The peak located at 2981 cm^{-1} is because of the –CH_3 group. A special peak at 1675 cm^{-1} is related to C=O stretching. On the other hand, CA gives a broad peak at approximately 3000–3500 cm^{-1}, indicating the O-H groups of CA, and a special peak at 1721 cm^{-1} corresponds to the C=O group of carboxylic acid. In general, the peaks indicating the N−H groups of DMU and CA overlapped and gave rise to broadened peaks at approximately 3500–2900 cm^{-1}. This may indicate the presence of hydrogen bonding interactions. In addition, the typical N−H peak in the 1570–1485 cm^{-1} region still existed in association with DMU and coexisted with the peak in the surrounding region, indicating the C=O peak of CA.

Using UV–Vis spectrophotometry to investigate solutions of each sample (DMU, CA, and related DES) in water at the same concentration (2 mg/mL) showed that there were no large differences between the samples (Figure 4). All of the compounds had peaks in the UV range of 200–210 nm. This means that there was no change in the chemical structure of the different parts and no creation of new chromophores and did not show any peak in the visible range.

3.2 *Staphylococcus aureus* and *Escherichia coli* Growth Inhibition by DMU-CA DES

The application of DES from the five different precursor mass variations to the inhibition of bacterial growth has been carried

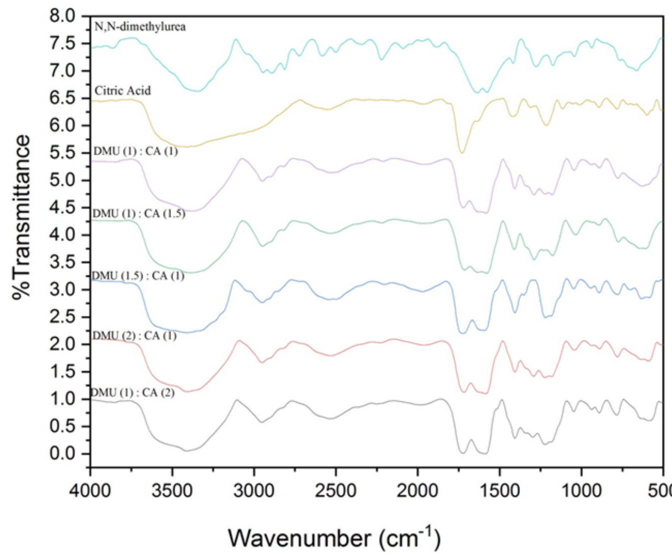

Figure 3. FTIR Pattern of the Various DMU-CA DES

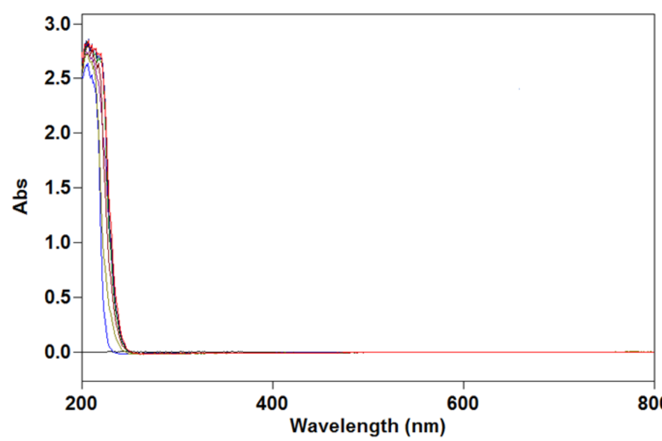

Figure 4. UV–Vis Pattern of the Various DMU-CA DES

out, and the results of the experiments and their explanations can be seen in Figure 5 and Figure 6, respectively. The control samples in this study are the DMU and the CA itself.

In general, all DESs had good inhibition against both *S. aureus* and *E. coli*. It was observed that the activity increased with the increase in the CA to DMU ratio. Meanwhile, DMU itself has no inhibitory activity against either type of microbe. As far as the search for the ability of N,N-dimethylurea goes, no literature has been found that describes its activity to inhibit the growth of *E. coli* and *S. aureus* bacteria. On the other hand, CA had activity against both types of bacteria. This is supported by previous studies that have examined the activity of CA in the process of inhibiting the growth of various types of bacteria, both gram-positive and gram-negative (Ogita et al., 2009; Su et al., 2014; Burel et al., 2021; Li et al., 2023; Akbar et al., 2023). Although there was limited evidence on the antibacterial mechanism of the current DES, it was predicted to operate similarly to other organic compounds. So far, the predictions reveal a general pattern: DES disrupts the bacterial cell membrane and changes the pH of the growing environment to halt bacterial growth.

Figure 5. Photograph of the Antibacterial Activity Test of DMU, CA, and its Corresponding DES

The results of our study reveal a notable antibacterial activity of the developed DES material, primarily attributed to the presence of CA (Figure 6). Specifically, our findings demonstrate that the DES material can effectively inhibit the growth of both *S. aureus* and *E. coli* bacteria. In comparison to the control compound at an equivalent concentration, the antibacterial activity of the DES material was approximately 25% lower. While this slight reduction in activity may raise some concerns, it is important to emphasize that the observed inhibitory effect remains substantial and biologically significant. Other researchers have conducted comparable research on the use of DES as an antibacterial. For instance, DES produced from CA-proline, CA-glucose-glycerol, and CA-fructose-glycerol have also shown effectiveness as antibacterials against *S. aureus* and *E. coli*. It is explained that the acidic nature of DES due to the presence of CA is one of the keys to its antibacterial activity (Radošević et al., 2018). Another study conducted a thorough investigation of in vitro antibacterials and found that the DES prepared from tetrabutylammonium bromide (TBABr) and formic acid exhibited a good capacity of inhibition towards tested bacterial strains, including *S. aureus* and *E. coli* (Inayat et al., 2023).

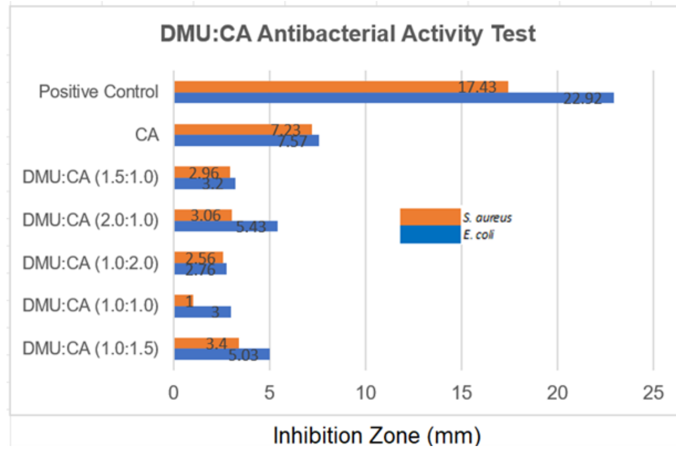

Figure 6. Graphical Representation of Antibacterial Activity Test of DMU, CA, and its Corresponding DES (Note: The inhibition zone value is the average of three repetitions of the experiment)

These results suggest that the DES material holds promise as an effective antibacterial agent, particularly against *S. aureus* and *E. coli* in vitro. Based on the results obtained, it can be seen that, in general, DES has a higher inhibitory activity against *E. coli* (one of the gram-negative bacteria) compared to *S. aureus* (gram-positive bacteria). This prediction is based on the higher cell integrity of *S. aureus* due to its cell wall, which is composed of thicker peptidoglycan than *E. coli* (Mai-Prochnow et al., 2016). The ability to combat these common pathogens is crucial, as they are responsible for a wide range of infections in clinical and environmental settings. The 25% decrease in activity compared to the control compound should not overshadow the potential benefits of utilizing DES materials as antibacterial agents, as the observed inhibitory activity is still robust enough to warrant further investigation and development. Further studies, including in vivo experiments and toxicity assessments, will be necessary to fully evaluate the practicality and safety of these DES materials for potential antibacterial applications. Nonetheless, these findings provide a strong foundation for the continued exploration of DES materials in the field of antibacterial research.

4. CONCLUSION

A DES from the melting process of two compounds, namely, DMU (proton donor) and CA (proton acceptor), was successfully carried out at 80°C with several variations in mass ratio. The results obtained are generally in the form of clear liquids with different viscosities from one another, as seen from the movement of the liquid when the sample bottle is moved. Molecular analysis using FTIR showed that there was an interaction between DMU and CA, as seen from the shifting of several functional group peaks from both compounds. UV–Vis analysis showed no significant difference between DES and various variations with DMU or CA. All DESs formed showed antibacterial activity against *S. aureus* and *E. coli*. The activity was generally derived from CA activity.

5. ACKNOWLEDGMENT

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