Chapter

2

Materials and Methodologies

Contents

This chapter describes the materials (with their source and purity) used in the present work. Furthermore, this chapter provides a detailed overview of many experimental setups employed in this study.

2.1 Introduction

The present work involved conducting various experimental methods to study different DES systems and the solution behaviour of surfactants in Deep eutectic solvents, DESs (with and without water). These experimental methods included measurements of conductivity, surface tension, steady-state fluorescence, rheology, contact angle, pH, density, refractive index, polarizing optical microscopy (POM), etc. Various characterization techniques were also used such as Fourier transform infrared spectroscopy (FT-IR), Nuclear Magnetic Resonance (^{1}H-NMR), and UV-Visible spectroscopy.

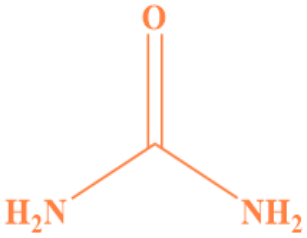
In the beginning of this chapter, the sources of material utilized in the study are listed, followed by a description of the experimental technique employed. Furthermore, several characterization techniques are comprehensively explained and discussed.

2.2 Materials

- The materials utilized throughout the course of this study are listed in **Table 2.1**, which includes details regarding their source and levels of purity.
- All surfactants, additives (salts), components of DES (choline chloride, urea, glycerol, thymol, menthol, camphor), probes, quenchers, and drugs were utilized in their original form without further purification. Desiccators were used to store hygroscopic substances.
- The water utilized for the preparation of solutions was passed through a double distillation process using alkaline $KMnO_4$ in a distillation set-up (made entirely of Pyrex glass). The water exhibited a specific conductivity within the range of 1-2 µS. cm^{-1}
- Glassware was properly cleaned using freshly made chromic acid and distilled water, rinsed with acetone, and oven-dried before use.
- The structures of all the utilized materials including components of DES (quaternary salts, urea, ethylene glycol, glycerol, thymol, menthol, camphor), DES (reline, glyceline, aquolines, ternary DESs, hydrophobic DESs), surfactants (conventional and

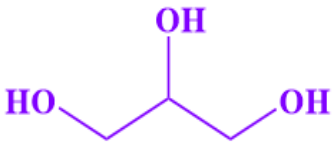
gemini), additives (salt), probe (pyrene), quencher (CPC), and drug (curcumin) are shown in **Schemes 2.1-2.3.**

Choline Chloride

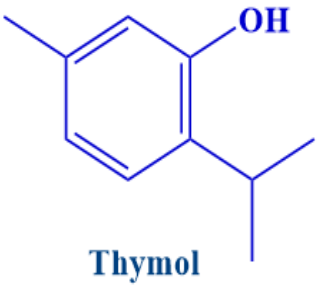


Urea

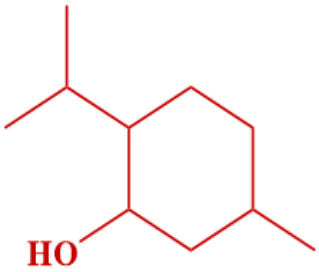
Ethylene glycol



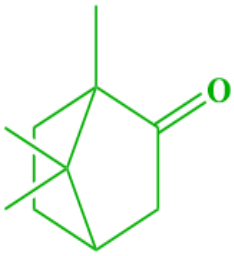
Glycerol



Thymol

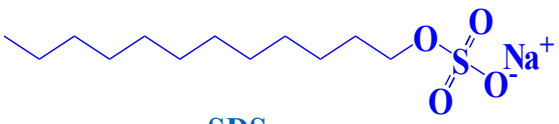


Menthol



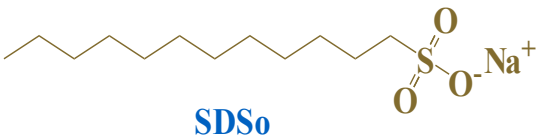
Camphor

Scheme 2.1: Structures of components of DESs.

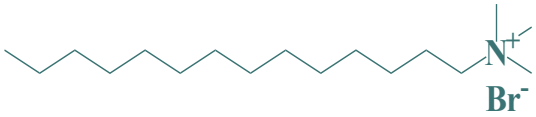


SDS

CTAB



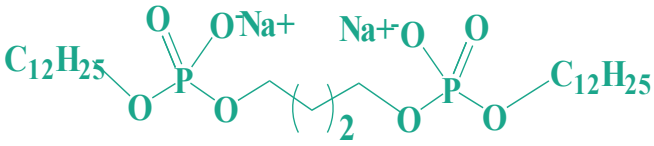
SDSo



TTAB

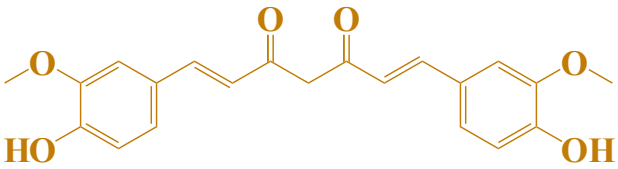
SDBS

DTAB

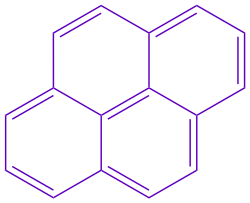


12-4-12 A

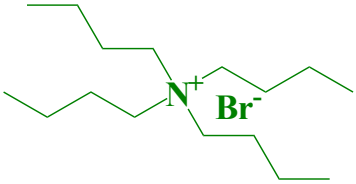
Scheme 2.2: Structures of surfactants.



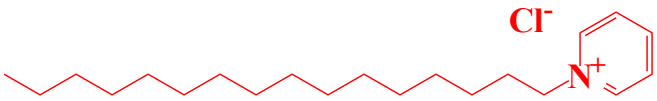
Curcumin



Pyrene



TBAB



CPC

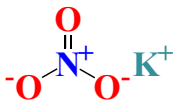
Sodium chloride

Sodium bromide

Sodium nitrate

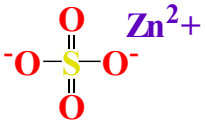
Potassium chloride

Potassium bromide



Potassium nitrate

Cadmium chloride



Zinc sulphate

Scheme 2.3: Structures of additives, probe, quencher, and drug.

Table 2.1: List of materials utilized in the present work.

Material used	Purity	Source
DES components		
Choline chloride	99%	TCI, India
Urea	99%	Sigma Aldrich, USA
Ethylene glycol	≥ 99%	Sigma Aldrich, USA
Glycerol	≥ 99%	Merck
Thymol Crystal	99%	Loba Chemie, India
Menthol Crystal	99%	Loba Chemie, India
Camphor	99%	Loba Chemie, India
Surfactants		
SDS	> 99%	Sigma Aldrich, USA
SDSo	> 99%	Sigma Aldrich, USA
SDBS	> 99%	Sigma Aldrich, USA
12-4-12A	-	Synthesized
DTAB	99%	Loba Chemie, India
TTAB	99%	Loba Chemie, India
CTAB	99%	Loba Chemie, India
Probe and quencher		
Pyrene	98%	Sigma Aldrich, USA
CPC	99%	Loba Chemie, India
Salts/additives		
TBAB	≥ 99%	Sigma Aldrich, USA
$ZnSO_4$	99%	ACS Chemicals, India
$CdCl_2$	99%	ACS Chemicals, India
KBr	≥ 99%	Sigma Aldrich, USA
KCl	≥ 99%	Sigma Aldrich, USA
KNO_3	≥ 99%	Sigma Aldrich, USA
NaBr	≥ 99%	Sigma Aldrich, USA
NaCl	≥ 99%	Sigma Aldrich, USA
$NaNO_3$	≥ 99%	Sigma Aldrich, USA
Drug		
Curcumin	>97%	TCI, India

2.3 Methods

2.3.1 Preparation of samples

Different surfactant stock solutions were prepared by measuring the necessary quantity of each surfactant in suitable standard volumetric flasks that had been cleaned and dried. These stock solutions were then utilized to prepare samples, either with or without a second component such as quaternary salts, additives, or drugs. The sample concentrations were modified by adding the necessary amount of stock solution based on the specific physical measurements carried out, such as surface tension, fluorescence measurements, cloud point (CP) measurements, spectrophotometry, etc. Most of the stock solutions were prepared in DES and DES-water mixture.

2.3.2 Preparation of reline-water mixtures

To prepare a variety of reline-water mixtures, proportionate amounts of reline and water were mixed. These combinations were then utilized to measure a variety of physical properties. The micellization and clouding behaviour were also investigated using the mixtures mentioned above, which included water in reline or reline in water.

2.3.3 Characterization techniques

2.3.3.1 FT-IR

The Fourier transform infrared spectroscopy (FT-IR) [1], which is among the most widely used methods for structural studies, operates on the principle that molecules exhibit different absorption peaks that are related to the frequency of vibration of bonds between atoms inside the component. Several energy levels correspond to the frequencies at which molecules undergo rotation or vibration. Molecules themselves exhibit unique frequencies. In an IR spectrometer, the sample is subjected to infrared radiation of progressively longer wavelengths, and the percentage of absorption is measured as a result of this exposure. The spectrum obtained serves as the molecular fingerprint of the sample due to the absorption of distinct functional groups at their unique frequencies.

FT-IR spectroscopy is a useful method for acquiring hydrogen bonding data and examining functional groups to analyze and determine the structure of a substance [2]. During the synthesis of DESs, their components undergo various transformations. Comparing the FT-IR spectrum of DESs, with their separate components, yields valuable insights on potential

interactions, structural modifications, and the formation of hydrogen bonds within DESs. FT-IR spectra were conducted using a Shimadzu FTIR-8400S instrument from Japan **(Figure 2.1)**. This instrument was designed to cover the spectral range of 4000-400 cm^{-1} in the KBr transmission mode. Pellets were formed by blending the samples with KBr. Certain incidence frequencies matched molecular vibrations when a sample was subjected to radiation. The recorded spectrum represents the frequencies that were absorbed due to resonance. This resonance facilitated the transfer of energy between the incident electromagnetic wave and the molecular vibrations. The formation of DESs was confirmed by FT-IR. DESs were further characterized by ^{1}H NMR in a deuterated solvent.

Figure 2.1: FT-IR spectrometer used for the present work.

2.3.3.2 ^{1}H NMR

^{1}H NMR works on the Nuclear Magnetic Resonance principle exhibited by hydrogen nuclei in the presence of a magnetic field. This methodology involves the application of a strong external magnetic field to a sample, which induces the hydrogen nuclei (protons) in the sample to align in a direction either parallel to or antiparallel to the magnetic field [3]. Radiofrequency pulses are then applied, temporarily disturbing this alignment. As the protons return to their equilibrium state, they emit radiofrequency signals, which are detected and analyzed. The spectrum obtained from the analysis gives significant insights into the chemical environment and interatomic connectivity of hydrogen atoms within the molecules of the sample. This enables the identification and confirmation of the structures of compounds. The ^{1}H NMR spectrum shows chemical shifts and splitting patterns that provide insights into the electronic and magnetic regions surrounding distinct proton groups within the studied molecules.

Figure 2.2: NMR spectrometer used for the present work.

The ^{1}H NMR spectra of all synthesized DESs and their components were acquired using a Bruker Avance 400 NMR spectrometer **(Figure 2.2)** operating at a proton resonance frequency of 400.15 MHz at a temperature of 303 K. The DESs and their components were dissolved in D_2O as the solvent. A volume of 0.6 ml of the solution was put into a 5 mm NMR tube, and the chemical shifts were measured using the ppm scale. The calibration of all spectra is performed using the HOD signal at 4.69 ppm (δ).

2.3.3.3 UV-Visible spectroscopy

Ultraviolet–visible spectroscopy is a technique used to measure the decrease in the strength of a light beam in the visible, near UV, or IR spectrum as it goes through or reflects off a sample surface [4]. Electronic excitation takes place as light passes through the sample. The position of the absorption band corresponds to the wavelength of radiation that possesses the necessary energy for the electronic transition from the ground state to the excited state. Absorption measurements can cover a single wavelength or a broad spectral range. The concentration-absorbance relationship is linear, allowing the determination of analyte concentration in a solution by measuring absorbance at a specific wavelength and applying the Beer-Lambert Law [5] expressed as

$$A = \varepsilon c l \qquad \text{(Equation 1)}$$

where A is absorbance, ε is molar absorptivity, c is concentration, and l is the path length.

Figure 2.3: UV-Visible spectrometer used for the present work.

UV-Vis absorption spectroscopy serves as a powerful tool in studying solubilization of drugs. The UV analysis in this study utilized a Shimadzu 1800 instrument from Japan **(Figure 2.3)**.

2.3.4 Physico-chemical properties measurements

2.3.4.1 Rheological measurements

Rheometry is a method used to determine viscosity by applying a controlled stress or strain to a sample material, causing it to undergo deformation. The rheometer measures the shear stress applied on the material and the consequent shear rate or deformation to determine viscosity [6]. The correlation between the applied stress and the resulting deformation is subsequently examined to measure the viscosity of the substance. This methodology is especially useful for determining the flow characteristics and viscosity of substances like polymers, fluids, or complex fluids in various conditions. It helps in understanding their rheological properties and practical applications in fields ranging from materials science to industrial processes.

Figure 2.4: Rheometer used for present work.

The rheological measurements were conducted using a rotational rheometer model HAAKE MARS (Modular Advanced Rheometer System), manufactured by Thermo Fisher Scientific, in Karlsruhe, Germany **(Figure 2.4)**. A concentric cylinder geometry was employed for rheological measurements. The measurements were conducted at a temperature of 303 K. Temperature control during the experiments was done by employing a thermostat having a temperature range of 273 to 453 K, ensuring precise temperature maintenance with an accuracy of ± 0.01 K. Shear viscosity (η_s) vs shear stress plots obtained from the data were used to acquire zero shear viscosity (η_0). This experiment was performed for all DESs and different DES-water mixtures (water in reline and reline in water). Relative viscosity (η_r) has also been measured by the Ubbelohde viscometer using water as the standard solvent.

2.3.4.2 Electrical conductivity measurements

The measurement of electrical conductance in solutions depends on the ability of ions to transport an electric current. When ions are present in a solution, they facilitate the flow of electricity. The conductance is directly proportional to the concentration of ions, providing valuable insights into the electrolytic properties of solution and ion mobility [7]. This method aids in understanding the electrochemical properties and ion mobility within solvents, which is essential for their utilization in diverse chemical processes.

Figure 2.5: Conductometer used for present work.

The specific conductance (κ) of DESs and DES-water mixtures was measured using EUTECH cyber scan CON510 conductometer **(Figure 2.5)**, Singapore (cell constant 1 cm^{-1}). An AQUASOL digital calibration solution (κ~2764 $\mu S.cm^{-1}$), Rakiro Biotech Sys Pvt was used

to calibrate the conductometer. All measurements were performed at constant temperature (in a water thermo state having temperature controlling accuracy within ± 0.1 K). The sample was equilibrated for 30 m at a constant temperature before acquiring conductivity data. The measurement process was repeated for each composition.

2.3.4.3 pH measurements

pH is a fundamental physical characteristic that significantly influences chemical processes. The pH factor plays a crucial role in the utilization of DESs in catalysis, biological processes, gas absorption, and metal treatment [8,9]. pH measurements on various DES systems have been performed using pH meter CL 54+, Ajmer, India **(Figure 2.6)**. Calibration of the glass electrode was performed using the buffer solutions (pH 4,7 and 11) that were specifically designed for the equipment. The pH was measured by immersing the electrode (which had been cleaned with deionized water and dried) into the DES system. The electrode was left in the solution until the pH measurement stabilized, and then the readings were recorded. The pH study of DESs was conducted three times, and the average results were computed. Before measurement, each sample was pre thermostated (30 m) at 303 K.

Figure 2.6: pH meter used for the present work.

2.3.4.4 Surface tension measurements

Surface tension is a characteristic of liquids that results from the attractive forces between molecules on the surface of the liquid. The interfacial tension refers to the force exerted per unit length at an interface between a liquid and a gas, often air. This force is to reduce the surface area of the liquid [10]. The Du Nouy ring method is a usual technique applied for measuring the surface tension of liquids. The basic concept of this method is based

on the equilibrium of pressures exerted on a thin liquid film created by a platinum or platinum-iridium ring. The process involves immersing the ring in the liquid, which facilitating the formation of a liquid film across the ring. As the ring is slowly withdrawn from the liquid, the force required to detach the liquid film is measured.

Figure 2.7: Du Nouy tensiometer used for the present work.

The surface tension of all DES was determined using Khushboo Scientific Pvt. ltd. Tensiometer, India **(Figure 2.7)**. The Du Nouy ring method [11] (using a platinum-iridium ring) was used to measure the surface tensions of all the DESs and DES-water mixtures. Deionized water was used to calibrate the tensiometer. Before each measurement, the platinum-iridium ring was subjected to burning to remove impurities, and the glassware was sequentially rinsed with acetone and distilled water. The surface tension of all synthesized DESs and DES-water mixture was determined at 303 K. Each measurement was replicated three times, and the errors associated with the surface tension measurement were ±0.2 mN m^{-1}.

2.3.4.5 Density measurements

The densities of the synthesized DESs and their mixture with water were measured at the corresponding temperatures (303K, 313K, and 323K) using a 10 mL specific gravity bottle with a core diameter of 1 mm. The mass measurements were performed on a digital electronic balance (CP225D Sartorius AG, Germany) with an uncertainty of 0.01 mg. The uncertainty in the density measurement was $\pm 10^{-4}$ g/cm^3.

2.3.4.6 Contact angle measurements

Contact angle measurement is a method used to analyze the interaction between a liquid and a solid surface [12]. This measurement yields significant data regarding the wetting characteristics of a substance, facilitating the evaluation of its hydrophobic or hydrophilic nature. Contact angle measurement is based on evaluating the angle produced between a liquid droplet, a solid surface, and the surrounding gas or vapor phase. A low contact angle signifies desirable wetting and stronger adhesion between the liquid and solid (hydrophilic), whereas a high contact angle suggests undesirable wetting and weakened interaction (hydrophobic).

Figure 2.8: Goniometer used for measurement of contact angle in the present work.

The contact angle (Θ) of DESs has been measured on Krüss DSA100, Germany, contact angle goniometer **(Figure 2.8)** at 303 ± 0.1 K. 5µL of each aquoline has been delivered using a microliter syringe (durasil) to form a sessile drop on the surface of the glass slide. Contact angle has been taken in triplicate and an average has been reported as the final value.

2.3.4.7 Refractive index measurements

The refractive index (n_D) is expressed as the ratio of the speed of light in a vacuum (c) to the speed of light within the medium (v) [13], as indicated in equation (2).

$$n_D = \frac{c}{v} \qquad \text{(Equation 2)}$$

The refractive indices of the DES-water systems were determined (at different temperatures (303K, 313 k, and 323K)) using an Abbe refractometer **(Figure 2.9)**, which employed a yellow

light beam with a wavelength of 589.3 nm corresponding to the sodium D line. The temperature control system with a water bath, supplied by M/s Frontline Electronics and Machinery Pvt. LTD, India has been used to maintain the constant temperature with an uncertainty of ±0.5 K. Each measurement involved placing a minimum of 1.0 mL sample on the measuring prism to minimize water vaporization at elevated temperatures.

Figure 2.9: Refractometer used for the present work.

A conical yellow laser beam is directed onto the sample from below at various angles of reflection. Subsequently, a microprocessor automatically computes the refractive index of the sample using the collected data. The refractometer was calibrated using deionized water, and the calibration was verified periodically after a few measurements. The maximum measurement error in the values of n was ±0.0002.

2.3.5 Fluorescence measurement

Fluorescence spectroscopy is a scientific method that is based on the principles of molecular fluorescence. It involves the absorption of light by certain molecules, followed by their subsequent emission of light. When a sample is subjected to an excitation light source, molecules absorb photons, promoting electrons to higher energy states. Upon returning to their ground state, these excited electrons emit fluorescent light of longer wavelengths. The fluorescence spectrum, characterized by emission peaks, provides insights into the identity and quantity of specific compounds within the sample [14].

Fluorescence spectroscopy is employed for critical micelle concentration (CMC) determination by utilizing the intrinsic fluorescence properties of certain molecules,

particularly fluorescent probes (pyrene) sensitive to changes in the microenvironment upon micelle formation [15]. As surfactant concentrations gradually increase, micelles form, causing a notable alteration in the local environment around the fluorescent probe. At concentrations below the CMC, the probe is usually solubilized in the monomeric state, exhibiting a specific fluorescence spectrum [16]. As the concentration surpasses the CMC, the probe preferentially associates with the micelles, leading to changes in its fluorescence characteristics, such as intensity and wavelength shifts. Monitoring these changes allows the precise determination of the CMC, a critical parameter in surfactant behavior, aggregation, and its applications in various fields, including colloidal and pharmaceutical sciences [17].

Figure 2.10: Spectrofluorophotometer used for the present work.

Fluorescence measurements (micro polarity, apparent dielectric constant, and quenching) have been carried out (using pyrene as a probe) on the RF-6000, Japan Spectro fluorophotometer **(Figure 2.10)** (having a 1 cm quartz cell). Different concentrations of surfactants were used to get fluorescence data in various compositions of aquolines and water in reline/reline in water. Data were acquired with constant excitation at 337 nm, and the slit widths of excitation and emission were also fixed at 3 and 3 nm, respectively. The ratio of Ist and IIIrd vibration maxima (I_1/I_3) in the fluorescence spectra of pyrene was used to obtain CMCs of various surfactants. The uncertainty in the measured CMC was ±0.1 mM L^{-1}.

2.3.6 Acquisition of cloud point (CP) data

The cloud point is the temperature at which a solution containing surfactants undergoes a phase transition, changing from a clear and uniform state to a cloudy or turbid state due to the formation of micelles/other aggregates [18]. Below the CMC, the solution remains clear,

but as the concentration reaches and surpasses the CMC, micelle formation occurs, leading to cloudiness. The cloud point is determined by noting the temperature at which this turbidity becomes visually apparent or by using specialized instruments to quantify the change in optical properties.

The CP values were determined by immersing tubes containing surfactant solutions of fixed composition (surfactant + quaternary salt in DES and DES-water mixture) into a temperature-controlled water bath. The temperature was ramped with an accuracy of ± 0.1 K. The temperature at which turbidity first appeared (or LLPS) and the temperature at which turbidity disappeared upon cooling was recorded visually. The CP was determined by calculating the average of these two temperatures. The technique was repeated for the same sample, and two consecutive readings (within ±0.1 K) were selected as the final CP. Additional experiments have been conducted to assess CP using various types of additives/salts.

2.3.7 Solubilization experiment

The solubility of Curcumin (CCM) has been studied in DESs (with and without surfactants/salt). To determine the solubility of CCM, an excess quantity of CCM was added to the sample bottle that contains the solvent systems (pure DESs, DES + salt, DES + surfactant, and DES + salt + surfactant). The mixture was thereafter agitated for a duration of 24 h and left to attain equilibrium at the ambient conditions. These mixtures were centrifuged and the supernatant was collected, diluted with methanol, and subsequently, absorbance was measured. The absorption spectra were acquired (in the range of 300-600 nm) using the above-mentioned UV–Vis. spectrophotometer having a quartz cell with a path length of 1 cm at 303 K. Pre-weighed CCM is dissolved in methanol, and its absorbance as a function of concentration at a wavelength (λ = 423 nm) is measured to obtain a calibration curve.

2.3.8 Polarizing optical microscopy (POM)

The morphology of the formed micelles in a surfactant-DES mixture (with and without salt) has been observed under a polarizing optical microscope (Nikon Eclipse Ci-Pol, Japan **(Figure 2.11)**) equipped with a double Linkam Mettler stage with a lens (5X, 10X, 20X, and 50X magnification) at 303±0.1 K. A drop of a sample (surfactant in DES with and without K-salt) is placed on the glass slide (covered with the coverslip) with the help of a microliter syringe. The prepared glass slide is placed under the Mettler Stage and then the images were captured by an inbuilt camera (Nikon, Japan) at 20X magnification.

Figure 2.11: Polarizing optical microscope used for the present work.

Since the objective lens power is 20X and the ocular lens magnifies 10 times, the total magnification would be 200 times (20X×10). The size of the magnified object has been determined by ImageJ software. Therefore, the actual size can be obtained by dividing the size acquired from the software by 200.

2.4 References

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