Optimization and kinetic study of ultrasound assisted deep eutectic solvent based extraction: A greener route for extraction of curcuminoids from Curcuma longa

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ABSTRACT

The use of deep eutectic solvents (DESs) as a new extraction medium is a step towards the development of green and sustainable technology. In the present study, nine DESs based on choline chloride acids, alcohols, and sugar were screened to study the extraction of curcuminoids from Curcuma longa L. Choline chloride and lactic acid DES at 1:1 M ratio gave the maximum extent of extraction. Further, DES based extraction was intensified using ultrasound. The impact of various process parameters such as % (v/v) water in DES, % (w/v) solid loading, particle size, ultrasound power intensity, and pulse mode operation of ultrasound was studied. The maximum curcuminoids yield of 77.13 mg/g was achieved using ultrasound assisted DES (UA-DES) based extraction in 20% water content DES at 5% solid loading and 0.355 mm particle size with 70.8 W/cm² power intensity and 60% (6 sec ON and 4 sec OFF) duty cycle at 30 ± 2 °C in 20 min of irradiation time. Kinetics of UA-DES extraction was explained using Peleg’s model and concluded that it is compatible with the experimental data. Additionally, anti-solvent (water) precipitation technique was applied, which resulted in 41.97% recovery of curcuminoids with 82.22% purity from UA-DES extract in 8 h of incubation at 0 °C. The comparison was made between conventional Soxhlet, batch, DES and UA-DES based processes on the basis of yield, time, solvent requirement, temperature, energy consumption, and process cost. The developed UA-DES based extraction can be an efficient, cost effective, and green alternative to conventional solvent extraction for curcuminoids.

1. Introduction

For the development of green and sustainable technology in consideration with their principles and standards; it is essential to evaluate green solvents with low cost and toxicity for the food and pharmaceutical sectors. On the contrary, the usual organic solvents such as chloroform, hexane, methanol, ethanol, and ethyl acetate are remarkably consumed for the extraction of biologically active components from natural sources [1]. Although organic solvents possess important characteristics for the extraction and dissolution of natural compounds, they have inherent limitations such as accretion in the environment due to low boiling point, non-biodegradability, toxicity, and flammability [2]. Furthermore, these solvents give rise to obstacles in green chemistry.

In 2003, Abbott et al. introduced the concept of a green and sustainable range of solvents, i.e., deep eutectic solvents (DESs) that have gained tremendous attention as an alternative to organic solvents [3]. DESs are formed by the combination of halide salt, i.e., hydrogen bond acceptor (HBA), and one or two hydrogen bond donors (HBDs). The most frequently used DESs are choline chloride based as HBA where this non-toxic quaternary ammonium salt is mixed with various HBDs such as carboxylic acids, alcohols, sugars, amines, and vitamins [2]. DESs are a facile versatile medium that can be used in separation, material science, biocatalysis, and organic synthesis with advantages like biodegradability, less toxicity, and low cost [4]. Moreover, presently these compounds are widely studied by the researchers in green extraction of biologically active ingredients from natural sources [5]. However, there is the only drawback of high kinematic viscosity in the range of 200–500 mm²/s, which results in slow diffusivity of DES into cell-matrix during extraction [6]. Water addition and high temperature operation have been reported to decrease the viscosity of DES that enhances the solubility and efficiency of the extraction of desired compounds [7]. However, extraction of water insoluble and heat sensitive compounds like curcuminoids will be limited using these...
techniques. Thus, along with water addition, DESs based extraction has been synergized using novel intensification methods such as ultrasound, microwave, and stirring [8].

In recent years, ultrasound energy has gained huge attention by researchers for greener and cost effective extraction of natural products [9–12]. When ultrasound passes through the medium, it generates various physical phenomena such as shock waves, micro-turbulence, micro-jets, and micro-streaming that augment mass transfer and contact area amongst raw material and solvent. Cavitation separates the desired micro-jets, and micro-streaming that augment mass transfer and contact various physical phenomena such as shock waves, micro-turbulence, micro-jets, and micro-streaming that augment mass transfer and contact area amongst raw material and solvent. Literature review revealed the few reports on ultrasound assisted DESs based extraction of active compounds such as different flavonoids from Radix scutellariae [13], total flavonoid content from Fagopyrum esculentum Möench [14] and Zizia latifolia [15], total phenolics from Morus alba L. [16], and gingerols from the rhizome of Zingiber officinalis Roscoe [17].

Turmeric rhizomes (Curcuma longa L.) from a family of Zingiberaceae are widely grown in Southeast Asia, India, and Indonesia [18]. It contains curcuminoids (yellow pigment) that is the most active component in three polyphenolic forms, namely curcumin (C_{15}H_{10}O_{5}) (70–74%), demethoxycurcumin (C_{15}H_{10}O_{4}) (10–24%), and bisde- methoxycurcumin (C_{15}H_{10}O_{3}) (2–10%) (Fig. 1) which contribute different percentage as per origin and soil condition. Curcuminoids belong to diferuloylmethane group and coexist in keto and enol form while enol form is more stable energetically. Significant work has been carried out on curcuminoids owing to their pharmacological properties against cancer, oxidation, inflammation, diabetes, mutagenesis, and microbial and parasitic contamination [19,20]. In addition, compared to other phenolic compounds, curcuminoids have more potential to enhance nutritional and sensory values [21]. Besides, naturally extracted curcuminoids get granted as a food additive over chemically synthesized as per the Joint FAO/WHO Expert Committee. Curcuminoids were derived from different Curcuma species using several conventional extraction processes viz. using hydrotrpse [22], hot and cold percolation [23], steam distillation [24], use of alkaline solution [25] and organic solvents [26]. Though, these methods undergo limitations such as higher requirement of temperature, extraction time, and solvents that lead to poor efficiency of extraction and high energy consumption. Also, the presence of solvent traces in curcuminoids hampers the quality of naturally derived products in the food industry. Curcuminoids being temperature sensitive reduce their bioactivity at a higher temperature during extraction. Thus, the novel extraction process like DES based extraction driven by ultrasound will be useful to intensify the extraction of curcuminoids from C. longa.

The recent work on the extraction of curcuminoids from C. longa was performed using carboxylic acid and sugar DESs [27]. They have investigated three sugar based DESs with citric acid, lactic acid, and malic acid. The present study evaluated the effect of nine choline chloride based DESs with carboxylic acid, alcohols, and sugars for the extraction of curcuminoids from C. longa. The application of direct mode of ultrasound was examined on DESs based extraction where we expect higher curcuminoids yield in a shorter period over conventional solvent and DES based extraction due to cavitation effect. Optimization of various processes and ultrasonic parameters establish the higher curcuminoids yield. A comparison study has also been done with conventional extraction approaches to determine the extent of process intensification. Additionally, Peleg’s model has been applied to evaluate the extraction kinetic parameters for UA-DES based extraction of curcuminoids from C. longa.

2. Materials and methods

2.1. Materials

Dried turmeric powder of C. longa species and 95% pure curcuminoids standard were given by Konark Herbals and Health Care, Mumbai, India. Glycerol (> 99%), ethylene glycol (> 99%), 1,4-butanediol (> 98%), lactic acid (99%), malic acid (99%), citric acid (> 99%), and choline chloride (98%) were purchased from S. D. Fine Chemical, India. Xylose, glucose, and fructose were obtained from Hi Media Laboratories Pvt. Ltd., India. Analytical grade ethanol (99%) and HPLC grade acetonitrile, methanol, and tetrahydrofuran (THF) were procured from Thomas Baker Chemicals Pvt. Ltd. India. Deionised water, purified through Millipore Milli Q 50 HPLC grade, was used.

2.2. Preparation of DESs

In the present study, the stirring and heating method was used for the preparation of DESs. Choline chloride (HBA) was mixed with different HBDS as per molar ratios reported earlier by Dai et al. [6]. The mixture was stirred at 70 °C temperature until the clear homogeneous fluid formation. Nine different choline chloride based DESs were prepared to study the extraction of curcuminoids (Table 1).

2.3. Solubility test

The solubility of standard curcuminoids was checked in ethanol and prepared DESs. An excess amount of standard curcuminoids was mixed in ethanol and different DESs and stirred in a conical flask at 40 °C for 3 h. The solutions were centrifuged at 8000 rpm to remove undissolved curcuminoids. Further, supernatants were collected, diluted with the mobile phase, and vortexed to form a homogeneous solution. The concentration of samples, i.e., solubility of curcuminoids (mg/mL) was obtained using HPLC and shown in Table 1.

2.4. Extraction methods

2.4.1. Soxhlet extraction

Soxhlet extraction was considered as a standard method to compare the ultrasound assisted DESs based extraction. Soxhlet was carried out as per our previously reported study [28]. In brief, 5 g of turmeric powder was kept in a thimble and fixed with a 250 mL ethanol containing a round bottom flask. Ethanol was refluxed for 12 h. The obtained curcuminoids yield was taken as reference, i.e. 100%.

2.4.2. Batch extraction

The DES based batch extraction was performed by taking a measured quantity of turmeric (C. longa) powder in a glass reactor of 50 mL capacity, and the required amount of deep eutectic solvent was added to it. For homogeneous mixing, the glass reactor was provided with four
punched bladed glass turbines. Samples were taken out at regular time intervals, diluted, filtered, and then evaluated for curcuminoids content using HPLC. For comparison, batch extraction was also performed with ethanol as optimized in the earlier report [28]. The various parameters affecting the curcuminoids extraction were optimized, and obtained results were compared with ultrasound assisted DES based extraction.

2.4.3. Ultrasound assisted DES (UA-DES) based extraction

Ultrasound assisted extraction of curcuminoids was performed in deep eutectic solvents with a direct mode of sonication. Ultrasonic probe of 12 mm tip diameter, 22 kHz frequency, and 200 W output power was dipped (0.5 cm deep from the surface of the solvent as reported by Dey et al. [10]) in two neck round bottom flask (50 mL capacity) containing 20 mL of DESs and measured amount of turmeric powder. The mixture was irradiated with pulse mode of ultrasound for 30 min, and samples were withdrawn at a regular time interval. Samples were further diluted, centrifuged, and quantified for their curcuminoids content using HPLC.

Effect of different choline chloride based acid, alcohol, and sugar DESs (Table 1), HBA:HBD molar ratio, and water % (v/v) in optimized DES was investigated. The process parameters such as % solid loading in DES, raw material particle size, ultrasonic power intensity, and pulse mode operation of ultrasound were optimized to achieve maximum extraction of curcuminoids. To evaluate the actual power dissipated in extraction system and efficiency of ultrasonic probe, the digital thermometer was inserted to measure the temperature rise of extraction mixture with respect to time. To establish reproducibility, all experiments were done in triplicate, and the observed standard deviation was within 5%.

2.5. Determination of curcuminoids content

High performance liquid chromatography (HPLC) of Agilent 1260 infinity model was used to analyze the curcuminoids concentration of samples. Elution of samples was done with THF:acified water (40:60) mobile phase on Kromasil C18 column (Akzo Nobel) of dimensions of 5 mm × 250 mm × 4.6 mm and area recorded at 420 nm. The concentration of curcuminoids (mg/L) was obtained from a standard curve of 95% pure curcuminoids and consequently calculated to mg/g as reported by Patil et al. [9]. The HPLC chromatograph of standard curcuminoids (95%) and extract obtained by UA-DES based extraction method is shown in Fig. 2(A, B).

2.6. Kinetics of ultrasound assisted curcuminoids extraction

In the present study, semi-empirical kinetic model developed by Peleg was applied to determine the initial extraction rate, equilibrium concentration, and extraction rate constant [29]. Due to similarity in the shape of sorption curves, reported Peleg’s model is widely used to explain the extraction curves of biological active molecules from natural sources with modifications [30]. Vetal et al. [12] and Shirsath et al. [31] applied Peleg’s model for explaining ultrasound assisted extraction of natural components. The kinetics of extraction of molecules using a model equation can be given as follows:

\[ M_t = M_0 + \frac{t}{K_1 + K_2 \cdot t} \]  

(1)

where, \( M_t \) is the curcuminoids yield at time t (mg curcuminoids/g turmeric powder), \( K_1 \) is Peleg’s rate constant (min g/mg) and \( K_2 \) is Peleg’s capacity constant (g/mg). As initial concentration of curcuminoids is zero in the fresh solvent, \( M_0 \) term was not considered from Peleg’s equation. Typically, the extraction takes place in two stages; one is first order at the initial stage, and the second is zero order at the later stage of the extraction process. Eq. (2) is modified Peleg’s model, which represents curcuminoids yield against time, as shown below:

\[ M_t = \frac{t}{K_1 + K_2 \cdot t} \]  

(2)

\( K_1 \) (Peleg’s rate constant) and \( K_2 \) (Peleg’s capacity constant) can be obtained from the plot of 1/\( M_t \) vs. 1/t. \( M_t \) was consequently calculated using Eq. (2) at a different time to check model fitting.

2.7. Recovery of curcuminoids

Low vapour pressure of DESs is a drawback in the case of extracted compounds recovery from the DES extract. There are few reports on recovery and recyclability of DES using methods that involve column chromatography, liquid–liquid extraction, recrystallization, adsorption, and supercritical carbon dioxide. Regenerated DES can be reused for the extraction of curcuminoids. In the present work, recovery of curcuminoids was carried out using an anti-solvent precipitation method with water as an anti-solvent as per earlier reported methods [32,33]. In brief, UA-DES based extraction was performed at optimized conditions. The extraction mixture was centrifuged at 10000 rpm for 15 min, and the supernatant was collected in another tube. A known volume of supernatant was taken and kept under stirring, and slowly water was added in different ratios (1:5, 1:10 and 1:20) and further incubated at 30 ± 2 °C and 0 °C for 2, 4 and 8 h. The precipitated mixture subsequently centrifuged at 10000 rpm for 15 min, and precipitates were analysed for their purity against standard curcuminoids (95%) using HPLC.

2.8. Surface morphology of turmeric powder

The surface morphology of turmeric powder (C. longa) was examined to study the effect on structural changes after extraction using

**Table 1**

Screening of different DESs for curcuminoids extraction. All experiments were performed at 3.34% solid loading, 0.355 mm particle size, and 400 rpm agitation speed at 30 ± 2 °C for 2 h.

| DES composition                          | Molar Ratio | Curcuminoids Yield (mg/g) | Density (g/cm²) | Solubility of curcuminoids (mg/mL) | Appearance               |
|-----------------------------------------|-------------|---------------------------|-----------------|-----------------------------------|-------------------------|
| DES reported [27]                       |             |                           |                 |                                   |                         |
| Glucose: citric acid (15% water)        | 1:1         | 43.55                     | 1.44            | 6                                 | Light yellow viscous    |
| DES 1 ChCl: Glycerol                    | 1:2         | 41.12                     | 1.15            | 4                                 | Transparent liquid      |
| DES 2 ChCl: Ethylene glycol             | 1:1         | 39.17                     | 1.11            | 6.3                               | Transparent liquid      |
| DES 3 ChCl: 1,4 butane diol*            | 1:3         | 42.78                     | 1.05            | 6.8                               | Transparent liquid      |
| DES 4 ChCl: Lactic acid                 | 1:1         | 58.87                     | 1.16            | 13.7                              | Transparent liquid      |
| DES 5 ChCl: Malic acid                  | 1:1         | 44.21                     | 1.27            | 7.2                               | Light yellow viscous    |
| DES 6 ChCl: Citric acid                 | 2:1         | 54.43                     | 1.31            | 9.1                               | Light yellow viscous    |
| DES 7 ChCl: Xylose                      | 1:1         | 47.3                      | 1.33            | 8.4                               | Light yellow viscous    |
| DES 8 ChCl: Glucose                     | (2:1)       | 51.66                     | 1.23            | 8                                 | Light yellow viscous    |
| DES 9 ChCl: Fructose*                   | (2:1)       | 50.84                     | 1.28            | 8.3                               | Light yellow viscous    |

Note: *Not stable, crystal forms after 6–8 h.
DES and ultrasound combined with DES. It was performed by scanning electron microscopy of JEOL-JSM-6360A, Japan. The samples for SEM analysis were prepared using the standard sputtering protocol. In brief, a small amount of sample was placed on the carbon tape (to prevent the sample loss) and attached to the sample disc. The sample was then coated with thin layer Pt metal (2–20 nm). The process of sputtering was carried out under vacuum for some time. After this, the samples were removed and directly observed under SEM at different magnifications.

3. Results and discussions

3.1. Screening of DESs

The physicochemical properties of DES are mainly dependent on their constituents and molar ratios of HBA to HBD. In the present study, nine different DESs were evaluated to achieve high curcuminoids extraction along with other constant parameters like 3.34% solid loading, 0.355 mm particle size, 400 rpm agitation speed, and 30 ± 2 °C temperature for 2 h. Table 1 depicts the curcuminoids yield obtained with different DESs where the highest extraction (58.87 mg/g) was achieved for choline chloride and lactic acid DES (DES-4) while lowest yield was observed in the case of DES 1. The maximum extraction of curcuminoids was obtained in choline chloride acid based DESs followed by sugar and alcohol based DESs. The extraction using DES is related to many factors such as solubility of targeted compound, hydrogen bonding interaction between HBA and HBD, and viscosity. The maximum solubility of curcuminoids was seen in DES-4, which is comparable with the ethanol solvent (Table 1). Our previous study of batch extraction using different conventional solvents resulted in higher curcuminoids extraction in ethanol solvent [28]. Rao et al. observed enhancement in the solubility of curcumin in 40% lactic acid solution.
(pH 1.2) as curcumin is very stable in acidic pH [34]. Lowest solubility of curcuminoids was observed in DES 1 and thus resulted in less recovery of curcuminoids compared to other DESs. Though, alcohol based DESs have low viscosity in comparison with sugar based DESs; maximum curcuminoids yield was obtained in sugar based DESs. This might be attributed to more hydrogen bonding formation in the case of sugar based DESs. At the same time, high viscosity affects the mobility of the desired compound into the extracting solvent, and this is the reason for the high extent of extraction in DES 1 [35]. Similarly, hydrogen bond interaction is a prominent factor for the solubility of compounds in DESs [36]. Thus, an optimal DES should have a viscosity that is not too high or low, and it is required to select an appropriate solvent to achieve high curcuminoids yield. Based on DESs evaluation, DES-4, i.e., choline chloride: lactic acid (1:1) DES was considered for further study. The synthesized DES of choline chloride and lactic acid (1:1) was confirmed using FT-IR spectroscopy and given in a Supplementary file (Fig. S1). The hydrogen bond formation between HBD and HBA was established by –OH stretching peak at 3300–3400 cm\(^{-1}\) wavelength.

3.2. Molar ratio of DES

The selected DES-4 was further prepared with different molar ratios of HBA and HBD (3:1, 2:1, 1:1, 1:2, 1:3 and 1:4) to investigate the effect of molar ratio of DES on the extent of curcuminoids extraction. All other experimental factors viz. 3.34% solid loading, 0.355 mm particle size, agitation speed 400 rpm, and 30 ± 2 °C temperature for 2 h were kept constant. Fig. 3 illustrates curcuminoids yield obtained at different ratios, where the highest curcuminoids extraction of 65.06 mg/g was observed with 1:4 whereas the lowest yield of 39.54 mg/g was seen at 3:1 M ratio. The mixture of choline chloride and lactic was stable at 2:1 to 1:4 M ratios; however, when ratios changed to 3:1; solid precipitate was formed after 4–6 h. This might be due to insufficient hydrogen bonding between choline chloride and lactic acid at 3:1 M ratio. There are two hydrogen bond formations between one chloride ion from choline chloride and two carboxyl groups from carboxylic acid [6,37]. Thus, excess chloride ion from HBA unable to form bond with lactic acid and resulted in the precipitation of choline chloride [6]. However, DESs containing liquid form of HBG like lactic acid are more fluidic in nature, so at 2:1 M ratio, DES was more viscous than 1:1, 1:2, 1:3 and 1:4 due to half mole addition of lactic acid that of HBA. Though, the molar ratio of 1:2, 1:3 and 1:4 gave higher curcuminoids extraction; there is a slight difference in curcuminoids yield compared to 1:1 M ratio. Also, the use of a large amount of HBD is not considered to be cost effective as operating cost and energy consumption required during separation will be high. Thus, to achieve suitable stability of prepared DES and extraction efficiency, 1:1 M ratio was selected for subsequent study.

3.3. Water % in DES

As compared to conventional organic solvents, DESs have the major drawback of viscosity, which hinders the diffusion of solvent into cell matrix. Thus, the addition of water in different percentages (10–40 %/v/v) was studied at 1:1 M ratio of HBA:HBD, 3.34% solid loading, 355 particle size, agitation speed 400 rpm and 30 ± 2 °C temperature for 2 h. As shown in Fig. 3, there is a marginal increase in curcuminoids extraction at 10% water content, and this might be due to the decrease in the viscosity of DES, which led to enhancement in the diffusion rate of solvent into cell matrix. Further addition of water, i.e., at 20%, a slight decline in extraction was observed, and from 30 to 40% water, the extraction of curcuminoids drastically reduced. Hydrogen bond interaction between DES and target compound (curcuminoids) gets affected by the addition of excess water [38]. Moreover, an increase in the volume of water decreases the solubility of curcuminoids in DES as curcuminoids are insoluble in water [39].

Effect of % water content was also studied under ultrasonication at 22 kHz frequency, 53.1 W/cm\(^2\) power intensity and 60% duty cycle (6 sec ON and 4 sec OFF), 3.34% solid loading, 0.355 mm particle size at 30 ± 2 °C temperature for 30 min, and obtained results are shown in Fig. 4. Viscosity and surface tension of the extraction medium plays a vital role in the case of ultrasound assisted extraction. Thus, the effect of water addition was also studied under the influence of ultrasound irradiation. The extent of extraction was significantly increased up to 20% water content, unlike conventional batch extraction, and they yield further reduced with an additional increase in water content. This considerable enhancement in curcuminoids yields at 20% water content is attributed to the dissipation of ultrasonic energy in the solvent medium. Ultrasound enhances the mass transfer of desired compound in the extracting medium by promoting greater penetration of DES into the solid cell matrix [9]. Moreover, precipitated curcuminoids due to anti-solvent (water) effect get dispersed in solvent as ultrasonic waves help to break formed particles in uniformed size. Zabihi et al. studied the enhancement in the dissolution of curcumin in deionized water using ultrasound assisted anti-solvent technique, and they found a reduction in particle size of curcumin from 3 µm to 20–120 nm [40].

Fig. 3. Effect of HBA to HBD molar ratio on DES based extraction of curcuminoids. All experiments were performed at 3.34% solid loading, 0.355 mm particle size, agitation speed 400 rpm and 30 ± 2 °C temperature for 2 h.

Fig. 4. Effect of % (v/v) water in DES on extraction of curcuminoids. All experiments were performed at 1:1 M ratio, 3.34% solid loading, 0.355 mm particle size, agitation speed 400 rpm and 30 ± 2 °C temperature for 2 h and for UA-DES 22 kHz frequency, 53.1 Wcm\(^2\) power intensity and 60% duty cycle (6 sec ON and 4 sec OFF) for 30 min.
at 20% water content, there is a considerable decrease in viscosity and surface tension [41], which provides an easy formation of acoustic cavitation [10]. Less viscous fluid exhibits more bubble fragmentation and stable cavitation with larger magnitude compared to viscous fluid [42]. Similar to batch extraction, there was a decrease in the extraction of curcuminoids at 30 and 40% v/v water content in DES. Hence, in the case of ultrasound assisted extraction, 20% v/v water content DES was used for further experiments. FT-IR spectroscopy of 20% aqueous DES-4 was performed and compared with 100% DES-4 to evaluate the effect of water addition on the hydrogen bonding of HBD and HBA. From Fig. S1, it can be confirmed that there is no change in –OH stretching peak at 3300–3400 cm⁻¹ wavelength in 20% aqueous DES-4. Moreover, Francesco et al. studied the effect of water addition in DES and found a gradual weakening of the hydrogen bonding between HBD and HBA up to 50%. Around 75% water addition completely breaks the interactions and hydrogen bonding of DES. Also, they found improved polarity, fluidity, and conductivity of DES with 25% water addition in it [43]. In the present study, experiments were performed with 20% water addition in DES-4, which decreases the viscosity of DES and might be increased the cavitational intensity of ultrasonication in the medium compared to 100% DES-4.

3.4. Optimization of process parameters for UA-DES extraction

3.4.1. Effect of % solid (turmeric powder) loading in DES

Mass transfer at solid–liquid interface varies with the proportion of solids and solvent quantity available at any given operating conditions [44]. Therefore, the influence of solid loading on optimum curcuminoids yield was studied by varying it from 20 to 2.5% in 20 mL of DES with 0.355 mm particle size, 53.1 W/cm² power intensity and 60% duty cycle (6 sec ON and 4 sec OFF) at 30 ± 2 °C temperature for 30 min. Fig. S2 discusses the effect of solid loading on the kinetics of curcuminoids extraction, and it can be seen that with a decrease in solid loading from 20 to 2.5%, there was an increase in curcuminoids yield from 27.83 mg/g to 77.12 mg/g. This significant enhancement in the extent of curcuminoids extraction is attributed to the mass transfer phenomenon. Higher volume of solvent contributes to a greater concentration gradient between plant cell-matrix and extraction medium where generated driving force consequently releasing the curcuminoids from cell-matrix in the DES medium. With a decrease in % solid loading, the cavitation threshold also decreases, and cavitation occurs with ease at lower cavitation threshold [45]. Cavitation threshold is a minimum acoustic pressure required to initiate the growth of a cavity in fluid during rarefaction stage of the cycle. However, higher solid loading gives rise to uneven distribution of cavitation effect and DES will provide less hydrogen bond interaction, which affects the solubility of curcuminoids. Also, the concentration of cavitation nuclei is more with the presence of solid particles in fluid compared to pure fluid [46]. Hence, at high % solid loading, bubble formation increases both in size and number that coalesced into a cluster of vapor, which affects the ultrasonic flow in the extracting medium. The additional decrease in solid, i.e., from 5 to 2.5% loading limits the mass transfer confined in the interior part of plant cell and would not change the driving force between solid and solvent [31]. Unlike batch extraction, maximum curcuminoids yield was achieved at 5% solid loading when extraction was driven by ultrasound. Shirshah et al. reported the extraction of C. amada assisted by ultrasound and observed the highest extraction at 1:25 (w/v) solid to solvent ratio [31]. Ultrasound improves mass transfer rate and breaks raw material particles, which exposes more cells to the solvent medium and thus reduces the required solvent amount. Considering an economic point of view, 5% solid loading was selected for subsequent experiments.

The kinetic constants of variation of % solid loading were calculated from Peleg’s model and plotted in Fig. S2. The Peleg’s model is validated as there is a good agreement for curcuminoids extraction and shown in Table S1.

3.4.2. Effect of particle size of turmeric powder

Cavitational intensity of ultrasound is greatly affected by raw material particle size [31], so the effect of different particle sizes ranging from 0.85 to 0.15 mm was studied at 5% solid loading with 53.1 W/cm² power intensity and 60% duty cycle (6 sec ON and 4 sec OFF) at 30 ± 2 °C for 30 min. The obtained curcuminoids yields have been depicted in Fig. S3. It can be observed that the amount of curcuminoids in DES-4 increased till particle size of turmeric powder was reduced from 0.85 to 0.25 mm and then decreased at 0.15 mm particle size. Smaller particle size offers a larger surface area, which is favorable during extraction. Moreover, it is evident that particle size reduction will increase the exposure of cells to the solvent media as well as to the cavitation induced by ultrasound [47]. The direct mode of ultrasound (probe) application led to the breakage of raw material, which also resulted in particle size reduction during extraction, and thus diffusion path required by solvent to reach cell matrix gets minimized. Shirshah et al. performed surface morphology of Curcuma amada using SEM after ultrasound assisted extraction and found particle size reduction [31]. On the other hand, with a further decrease in particle size (< 0.15 mm), the particle agglomeration was observed at the bottom of flask, and even ultrasonic irradiation could not break it. Along with high surface area, fine particles also possess high surface energy, which tends to form agglomerates. Ohta et al. observed aluminum agglomerates i.e., “particle clump” under sonication when the size of aluminum reduced to 50–150 μm [48]. This resulted in uneven distribution of cavitation effect, and also some of the particles did not get exposed to extracting medium. Also, DES being viscous compared to conventional organic solvents did not provide free flow for very fine particles into extracting medium and created a silent zone during extraction. Thus, decrease in yield of curcuminoids in the solvent (DES-4) was observed at 0.15 mm particle size.

However, typically in most cases, optimum is arrived based on the energy required for size reduction and its ease of separation compared to its extraction efficiency. Hence, considering the parameters of separation and ease of experimentation, 0.355 mm ranged particles were selected as optimum in this study. Peleg’s model fitting parameters (Table S2) were found to be in good concordance with the experiment.

3.4.3. Effect of power intensity

To study the influence of power intensity (17.7, 35.4, 53.1, 70.8 and 88.5 W/cm²) on the extraction of curcuminoids, the ultrasound horn was operated at 20–100 W power, and the respective electrical acoustic intensity was calculated using Eq. (3):

\[
\text{Power intensity, } I = \frac{W}{m^2} = \frac{P}{\pi r^2}
\]

where \(P\) is the actual input power in W, and \(r\) is the radius of ultrasonic probe microtip in cm.

Fig. S4 elucidates the curcuminoids yield achieved at different
power intensities and Peleg’s model predictions. Applied power intensity has a direct correlation with its magnitude, where an increase in power intensity amplifies ultrasonic waves that result in the intensification of cavitation effect [49]. Thus, with an increase in power intensity from 17.7 to 70.8 W/cm$^2$, the extraction of curcuminoids increased from 38.99 to 77.62 mg/g, whereas decreased to 67.12 mg/g at 88.5 W/cm$^2$. Actual power output i.e. power dissipation in the solvent medium is more with increase in power intensity that enhances the curcuminoids extraction in short period of time. Increased power intensity leads to the potent cavities collapse due to travelling of large amplitude of ultrasound through the extraction medium and enhances different physical effects like cell wall disruption, interfacial turbulence, local energy dissipation, and improved solute diffusion [11]. Higher vibration creates shock at the interface between solid matrix and solvent, and hence the curcuminoids extraction efficiency is enhanced. In addition, an increase in power intensity causes a temperature rise of extraction medium (DES), which helps to decrease the viscosity of DES and allowing them to reach to the cell matrix. While, at higher power intensity after prolonged time, there is increase in heat of extraction medium, which might have degraded extracted curcuminoids as it is thermally sensitive [9]. Also, high power intensity results in the agitation of liquid instead of cavitation and limits the circulation of ultrasonic waves through a liquid medium that provides uncontrolled mechanical shear to the extraction system [45]. Moreover, number of bubble formation is more at high power intensity, which creates voids and affects the transmission of ultrasonic waves in the solvent medium [50]. Dey et al. also observed the reduction in β-carotene extraction from *Spirulina platensis* with an increase in electrical acoustic intensity [10]. Thus, 70.8 W/cm$^2$ considered as optimal to achieve higher curcuminoids yield.

Kinetics of variation of power intensity is shown in Table S3, which depicts compatibility between the experimental and predicted concentration of curcuminoids from *C. longa* rhizome powder.

### 3.4.4. Effect of pulsed mode application

Effect of pulsed mode ultrasound was investigated on the extraction of curcuminoids from *C. longa* with other constant parameters viz. power intensity of 70.8 W/cm$^2$, solid loading 5% and particle size of 0.355 mm at 30 ± 2 °C for 30 min irradiation time. Four combinations of pulse time and interval time were 2 and 8, 4 and 6, 6 and 4, and 8 and 2. These combinations correlate to cycle time of 10 s and duty cycles of 20%, 40%, 60%, 80% and 100% (continuous mode). Fig. S5 illustrates the obtained yield of curcuminoids at different duty cycles (pulsed and continuous mode) and highest extraction (79.71 mg/g) was
seen at 80% duty cycle while lowest (71.12 mg/g) was observed at a continuous mode of ultrasound (100% duty cycle). The pulse mode of ultrasonication is recommended by researchers for natural product extraction to enhance the life span of transducers and to avoid excess energy losses [9]. To make any process economically viable at a large scale, the crucial factor is power consumption, which decides the operational time and cost of the process [51]. With the continuous operational mode of ultrasound, the excess heat is generated because of prolonged exposure of ultrasonic waves to the extracting medium. This causes not only uncontrolled cavitation in the solvent medium but also mechanical shear to the plant matrix, which resulted in the degradation of curcuminoids and might have weakened the bonding between the target compound and DES. Hence, less recovery of curcuminoids was observed at a continuous mode operation of ultrasound. This is accordant with previous findings by Pan et al. [52], who studied the extraction of antioxidants from pomegranate peel using continuous and pulsed mode ultrasound and observed that 50% duty cycle was superior to the continuous mode of ultrasound. Due to less energy consumption and an increase in curcuminoids yield, pulse mode at 60% duty cycle was considered optimum over the continuous mode of ultrasound.

The Peleg’s model calculated parameters constant K1 and K2 and C_{eq} of extraction of curcuminoids at different duty cycle have been elucidated in Table S4.

3.5. Recovery of curcuminoids

Low vapour pressure of DESs is a drawback in the case of extracted compounds recovery from the DES extract. However, several methods such as column chromatography, liquid–liquid extraction, recrystallization, adsorption, and supercritical carbon dioxide, have been applied to recover the desired compound from DES extract [16]. In the current work, anti-solvent precipitation approach was explored to recover the curcuminoids from UA-DES extract, where water was used as anti-solvent as curcuminoids are insoluble in it. Table 2 depicts the recovery of curcuminoids from UA-DES extract, where water was used as anti-solvent as curcuminoids are insoluble in it. Table 2 depicts the recovery of curcuminoids at different temperatures, extract to anti-solvent ratio and time. The maximum curcuminoids recovery (41.97%) was obtained at 1:20 (v/v) extract to anti-solvent ratio in 8 h incubation at 0 °C, while lowest was seen at 1:5 (v/v) extract to anti-solvent ratio in 2 h at 30 ± 2 °C. The purity of recovered curcuminoids was 82.22% and characterized by FT-IR against standard curcuminoids (95%) (Fig. 5). Less recovery of curcuminoids might be due to the solubility of curcuminoids in lactic acid solution, as reported by Rao et al. [34]. Similar observations were reported by Nam et al. [33] and Huang et al. [32] for the recovery of flavonoids such as quercetin, kaempferol, and isorhamnetin from, Flos sophorae extract and rutin from tartary buckwheat hull, respectively.

3.6. Surface morphology of turmeric powder

Structural changes in the morphology of C. longa were investigated before and after extraction with DES and ultrasound assisted-DES using scanning electron microscopy. Fig. 6 elucidates the surface morphology of turmeric powder before extraction (Fig. 6A, B) and after extraction by DES (Fig. 6C, D) and UA-DES (Fig. 6E, F) based method at 30X (Fig. 6A, C, E) and 1000X (Fig. 6B, D, F) magnification. Fig. 6 (A, B) specifies an integral and non-porous surface of turmeric powder before extraction while it was damaged in the case of DES based extraction (Fig. 6C, D). Interestingly, in the case of UA-DES based extraction (Fig. 6E, F), the surface has entirely ruptured as well as more porous, and raw material has been slightly milled under direct ultrasound [47]. The synergistic effect of DES and ultrasound is clearly seen on the morphology of raw material. DESs with acidic HBD enable the disintegration of cellulose from the plant cell wall and facilitate the separation of curcuminoids from cell-matrix [16,53]. Along with these advantages of DESs, ultrasound also provides microfracture and porous surface by penetrating cell matrix material resulting in faster extraction of curcuminoids than only DES from solid to solvent medium [31].

3.7. Comparative study

The efficiency of ultrasound assisted DES based extraction was compared with Soxhlet method, conventional batch, and DES based extraction method (Table 3). Batch extraction was performed at optimized parameters according to the previously reported study [28], where the obtained yield was 52.77 mg/g at 40 °C in 180 min. Similar to batch extraction, optimization of DES based batch study was done to compare DES with conventional solvent (data not shown). Optimized DES based extraction gave 66.12 mg/g curcuminoids yield with 1:1 choline chloride:lactic acid DES, 10% (v/v) water content in DES, 1:30 (w/v) solid to solvent ratio, 0.355 mm particle size, 300 rpm agitation
speed at 50 °C in 75 min. When DES was assisted by ultrasound, a significant increase in curcuminoids yield (77.13 mg/g), and a drastic reduction in time from 75 to 20 min was achieved. The Soxhlet extraction (reference method) resulted in 88.96 mg/g extraction in 12 h. Though DESs are more viscous in nature and DES based extraction was carried out at higher temperatures compared to conventional solvent batch method, the significant improvement in curcuminoids content and time reduction has been noticed. This attributes to the higher solubility of curcuminoids in DES than ethanol (Table 1) and dissolution of cellulose cell wall due to acidic HBD of DES [53]. This phenomenon allows the solvent to reach inside the cell, which not only improves the yield of curcuminoids but also reduces the extraction time. Further application of ultrasound intensifies DES based extraction by more disrupting and penetrating cell wall and enhances the rate of mass transfer [9]. Further, the energy consumption and cost required by each process was compared and illustrated in Table 3 (detailed calculations were given in the Supplementary file).

The energy required was estimated as reported in our earlier work [9] for one kilogram curcuminoids extraction by batch, DES based, and UA-DES based approaches (Table 3). The highest energy was consumed by Soxhlet extraction, whereas batch, DES extraction, and UA-DES based extraction showed comparable energy requirement to extract 1 kg of curcuminoids. Soxhlet process requires latent heat of evaporation from the solvent reservoir to thimble and longer extraction time compared to other processes [9]. Owing to cell disruption phenomenon driven by batch, DES, and ultrasound, these methods consume less time compared to Soxhlet and thus resulted in less energy consumption. The process cost was also evaluated to extract 1 kg of curcuminoids for different extraction approaches based on extraction yield achieved by each process. The cost of 1 electric unit (kWh) was taken as 8.78/-.

Though Soxhlet resulted in the highest curcuminoids yield, the cost required by Soxhlet method was very high compared to other processes due to high energy required for processing. On the contrary, the process cost to extract 1 kg curcuminoids is slightly less by UA-DES extraction compared to batch and DES based extraction even though the energy required by these methods was comparable (Table 3). This is attributed to highest curcuminoids yield obtained by UA-DES extraction in less amount of solvent and time. Hence, ultrasound assisted DES based extraction of curcuminoids from C. longa technique is efficient compared to other conventional methods based on product yield, amount of solvent, extraction temperature, time, energy consumption, and process cost.

4. Conclusion

The present work evaluated different choline chloride based DESs as an alternative to conventional solvent for the extraction of curcuminoids from C. longa. The study has also confirmed the ultrasound as a process intensification tool for DES based extraction of curcuminoids. UA-DES based extraction proved as an efficient technique compared to conventional Soxhlet and batch extraction processes. It is clear that UA-DES not only enhanced the curcuminoids yield but also reduced extraction time at 30 ± 2 °C with less amount of DES. In comparison with the reference method, i.e., Soxhlet (100%), UA-DES extraction, and simply DES based extraction resulted in 86.7% and 74.32% curcuminoids yield, respectively. Scanning electron micrographs of turmeric powder after UA-DES extraction affirmed the reduction in particle size along with the ruptured and porous structure of raw material. The curcuminoids recovery of 41.97% was achieved from obtained UA-DES extract using anti-solvent (water) precipitation method where the purity of recovered curcuminoids was found to be 82.22%. In summary, DES has proved to be an efficient, green, and easily accessible solvent for the extraction of natural products. Ultrasound assisted DES extraction (UA-DES) process has shown a significant ability for curcuminoids extraction in all aspects compared to conventional methods.
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.

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Appendix A. Supplementary data

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