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Applicability of Deep Eutectic Solvent as a Sustainable Reaction Medium in Biocatalytic Multicomponent Synthesis

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Abstract

Over the last decade, deep eutectic solvents (DESs) have gained recognition as a promising alternative to conventional organic solvents, from both environmental as well as technological perspectives. Due to its inherent biological properties these eutectic solvents serve as ideal reaction media with whole cells and isolated enzymes which have shown improved enzyme stability and activity translating to high conversion of product. In this article, we have reported Baker's yeast (*saccharomyces cerevisiae*) catalyzed one-pot three component condensation of aryl aldehydes, thioglycolic acid, and amines in deep eutectic solvent leading to 4-thiazolidinones successfully. This article presents a successful one-pot three-component condensation of aryl aldehydes, amines and thioglycolic acid, catalyzed by Baker's yeast (*Saccharomyces cerevisiae*) in a deep eutectic solvent, leading to the efficient synthesis of 4-thiazolidinones. The chloride-based deep eutectic solvent (DES) used in this methodology can be effectively reused and recycled up to five consecutive runs without any significant loss in catalytic activity.

Keywords: Deep eutectic solvents, biocatalysis, thiazolidinones, sustainable and greener synthesis

Introduction

Over the past few decades, biocatalysis has gained significant importance in organic transformations due to its eco-friendly nature, enabling reactions under mild and sustainable conditions while minimizing the use of hazardous chemicals [1-4]. Enzymes offer high regioselectivity, chemoselectivity and stereoselectivity, making them ideal for producing pure compounds, particularly in pharmaceuticals [5]. Derived from renewable sources, biocatalysts are cost-effective, biodegradable and energy efficient [2]. Biocatalysts have broad substrate scopes and are well suited for large-scale manufacturing processes, further emphasizing their value in green and efficient chemical transformations. Whole-cell biocatalysis by using yeast has demonstrated significant applicability in the field of pharmaceutical and medicinal chemistry. Notably, the whole-cell or enzymatic transformations have also been reported under non-aqueous conditions, including in ionic liquids or neat substrates among other solvent-free reaction media [4, 6] To improve the solubility of substrates and products, the usage of non-conventional reaction media in biocatalysis provides a practical and enzyme-compatible framework. Various ILs have demonstrated efficiency in biocatalytic processes; though, their application is limited by concerns related to high-cost and toxicity [4, 7]. To overcome these challenges, deep eutectic solvents (DESs) have recently emerged as a new generation of ionic liquids, offering customizable properties making them a promising alternative [8-10].

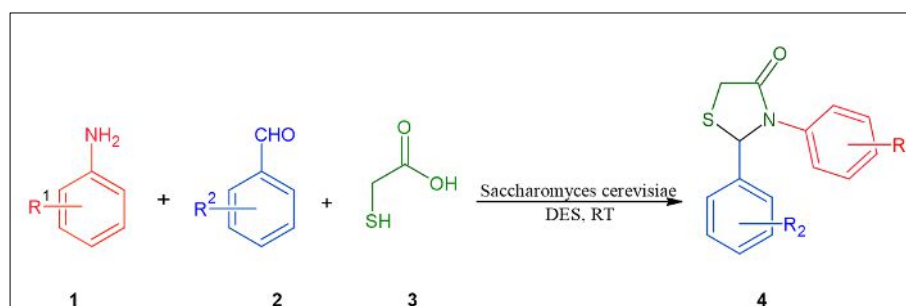
Baker's yeast or *Saccharomyces cerevisiae* can carry out several organic transformations under mild reaction conditions [11]. It is available easily, economical and non-hazardous. Baker's yeast and lipase can catalyze several condensation reactions to synthesize biologically active structurally diverse heterocyclic compounds like polyhydroquinolines [12], dihydropyridines [13], dihydropyrimidines [14], benzotriazoles [15],

benzimidazoles ^[16] and quinoxaline ^[17]. The biotransformation reactions using deep eutectic solvent studied so far involves aminolysis, lipase-catalyzed transesterification, epoxide hydrolysis, Knoevenagel condensation and N-alkylation of aromatic amines ^[9]. Baker's yeast was also explored for different enantioselective reductions of carbonyl compounds ^[18].

Thiazolidinones are thiazolidine derivatives which have a sulfur atom at the position 1, nitrogen atom at the position 3 and a carbonyl group at the position 2, 4, or 5. Thiazolidinone derivatives are among the well-studied heterocyclic moieties. Its presence in a well-known penicillin drug marked the earliest remarkable discovery of its natural existence ^[19]. This scaffold is considered biologically important due to its broad spectrum of pharmacological activities, including antioxidant ^[20], antifungal ^[21], antitubercular ^[22], antiviral ^[23], anti-inflammatory and anticancer agents ^[24] properties.

Considering the importance of thiazolidinone derivatives, in this article, the applicability DES was explored as a better solvent system for biocatalytic transformation. The cyclocondensation of benzaldehyde, aniline and thioglycolic acid was selected as a model reaction for the synthesis of 4-thiazolidinones (Scheme 1).

Results and Discussion



Scheme 1: Baker's yeast catalyzed the synthesis of 4-thiazolidinones

1 g of Baker's yeast with 8 cm³ of eutectic solvent was found to be sufficient to carry out the reaction at normal room temperature. Under these optimized reaction conditions, a set of substituted thiazolidinone derivatives were synthesized.

The choline chloride based eutectic solvents are well known to offer enzyme stability and activity. These results validate that the eutectic solvents may truly become promising non-conventional reaction media for whole-cell biocatalysis, in which enzymes are used for organic transformations may be

Initially, a multicomponent reaction of benzaldehyde (5 mmol), thioglycolic acid (5 mmol) and aniline (5 mmol) was carried out at room temperature in presence of 1 g of Baker's yeast by using 8 cm³ of different eutectic solvents which are made up of thiourea, urea, oxalic acid, succinic acid, glycerol, malonic acid with choline chloride quaternary ammonium salt in a different ratio. Choline chloride: Glycerol ChCl: Gly (1:2) was found to be excellent eutectic solvent producing excellent results at room temperature 1 g of Baker's yeast.

When the model reaction was carried out by using DES ChCl: Gly without Baker's yeast as a blank reaction at normal room temperature low yield of desired product was obtained. However, in the presence of Baker's yeast under the same conditions, the reaction offered an 80% yield of the desired product within 26 hours.

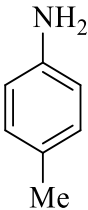
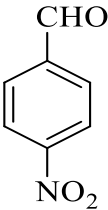
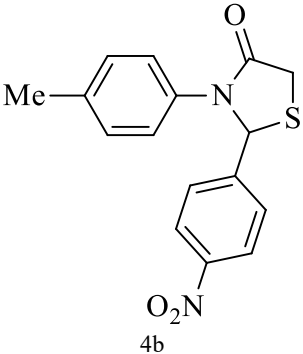
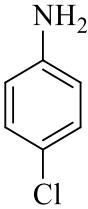
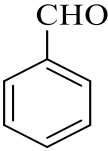
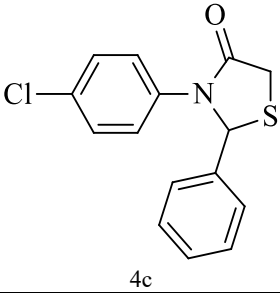
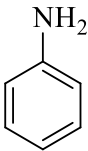
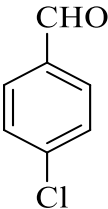
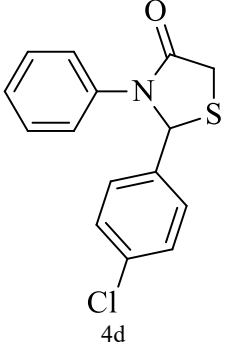
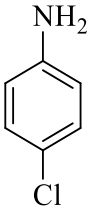
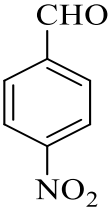
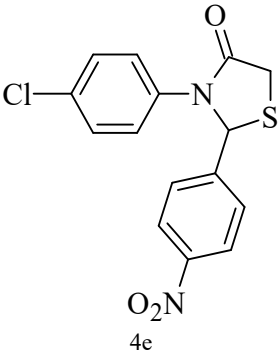
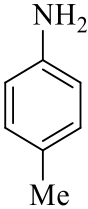
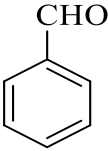
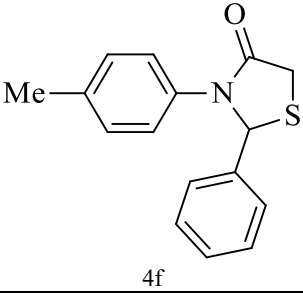
It indicates that the Baker's yeast plays a key role in the cyclocondensation of thiazolidinone in the presence of a eutectic solvent. The enzyme lipase which exists in Baker's yeast is likely to be responsible for enhancing the rate of imine formation and cyclisation, thereby facilitating product formation. The model reaction was carried out using the variable quantity of Baker's yeast from 1 g to 3 g. 2 g of Baker's yeast was found to be sufficient to carry out the reaction successfully giving an 80% yield of the desired product.

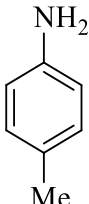
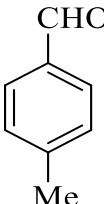
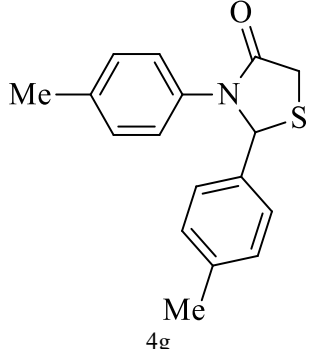
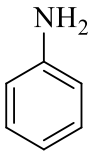
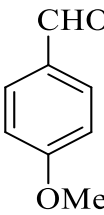
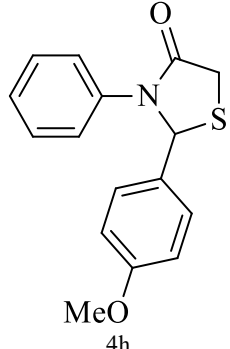
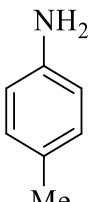
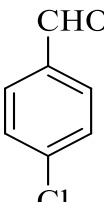
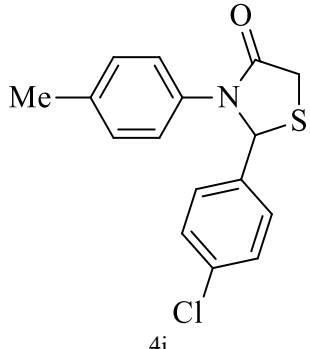
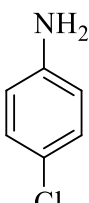
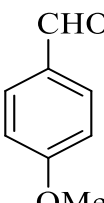
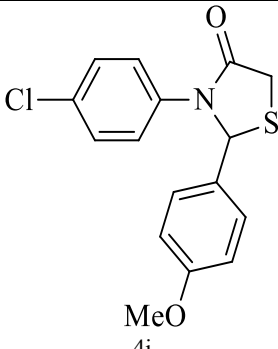
active and stable. The enzyme lipase available in Baker's yeast is responsible for the formation of the imine intermediate as well as cyclocondensation of the desired product, 4-thiazolidinone.

After optimization of the reaction conditions, this methodology was successfully evaluated for the synthesis of 1,3-thiazolidin-4-ones by using different amines and benzaldehydes and thioglycolic acid. Results are summarized in table 1.

Table 1: Synthesis of 1,3-thiazolidin-4-ones using Baker's yeast.^a

Entry	Aniline	Aldehyde	Product	Melting point (°C)	Yield (%) ^b
1.			 4a	130-132 (lit(25) 129-131)	80

2.			 4b	158-160 (lit(26) 158-160)	89
3.			 4c	120- 122 (lit(25) 121-123)	77
4.			 4d	124-126 (lit(25)122-126)	88
5.			 4e	112-114 (lit(27) 112-115)	86
6.			 4f	105-107 (lit(25) 105-107)	83

7.				122-125 (lit(25) 121-123)	90
8.				98-100 (lit(26) 97-99)	82
9.				161-163 (lit(25) 162-164)	88
10.				104-106 (lit(26) 104-106)	77

^a Reaction conditions: aryl amine (5 mmol), aryl aldehyde (5 mmol), thioglycolic acid (5 mmol), Baker's yeast 1 g in 8 cm³ DES stir, 26 hrs at r.t.; ^b Isolated yield.

Recyclability of DES ChCl:Gly

Recovery and recyclability of this eutectic solvent used in this biocatalysis were also studied. We employed DES ChCl:Gly as a reusable solvent with Baker's yeast to synthesize thiazolidinones. Recyclability of DES was studied using the model reaction of aniline, benzaldehyde and thioglycolic acid under optimized conditions. After the completion of the reaction, the reaction mixture was neutralized, and the product was separated. After 26 hours the above reaction mixture was filtered by the Celite bed to remove the Baker's yeast. The water-soluble DES recovered from the previous reaction and was reused for the next successive batch without purification, with no substantial loss observed in the yield of the expected product. (Fig. 1,

Table 2). The recovered DES was reused for the next five cycles successfully.

Table 2: Recyclability of DES ChCl:Gly^a

ENTRY	RUN	TIME (Hrs)	YIELD (%) ^b
1	Fresh	26	80
2	1	26	80
3	2	26	80
4	3	26	80
5	4	26	76
6	5	26	75

Reaction conditions: aryl amine (5 mmol), aryl aldehyde (5mmol), thioglycolic acid (5 mmol), Baker's yeast 1 g in DES 8 cm³ at r.t.; ^b Isolated yield.

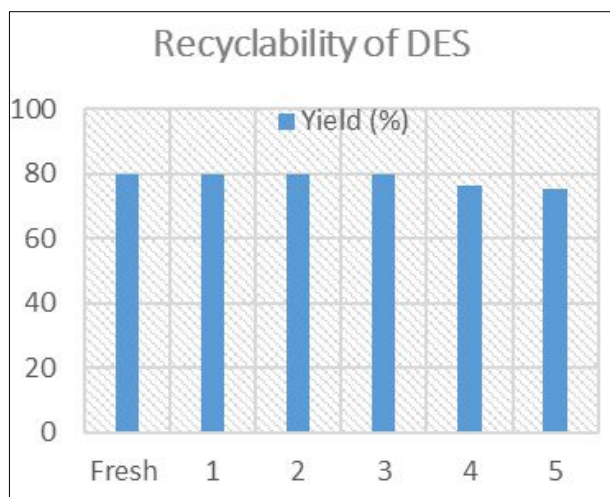


Fig 1: Recyclability of DES

Experimental Section

All the required chemicals were commercially available and purchased from Merck and Loba Chemie Pvt. Ltd. and used without further purification. All the reactions were monitored by TLC using silica-gel, 60F254 aluminum sheets. Melting points of all the thiazolidinone compounds were determined in open capillary tubes and are uncorrected. The FTIR spectra of the synthesized derivatives were recorded on Bruker 870 ATR alpha spectrometer, while their ^{13}C NMR and ^1H NMR spectra were obtained on Bruker Advance 600 MHz and 400 MHz spectrometer respectively in CDCl_3 as a solvent. Tetramethylsilane (TMS) was used as the internal standard and chemical shifts are reported in δ (ppm).

Preparation of ChCl: Gly (1:2) DES

As per the literature, (28) 50 mmol of choline chloride (ChCl) and 100 mmol of glycerol were mixed together in a round-bottom flask. The reaction mixture was stirred and heated at 72°C until a clear liquid was formed. The atom efficiency of this reaction is 100%.

Preparation of 2, 3-diaryl 4-thiazolidinones using Baker's yeast

The mixture of aryl aldehyde (5 mmol), aromatic amine (5 mmol), thioglycolic acid (5 mmol) and Baker's yeast (active & dry, 1 g) added in 8 cm^3 ChCl:Gly (1:2) deep eutectic solvent and stirred continuously at normal room temperature. The progress of the reaction was observed by TLC (EtOAc:Hexane solvent system). After 26 hours the above reaction mixture was filtered by the Celite bed to remove the Baker's yeast. Under reduced pressure, the obtained filtration was concentrated and the resulting crude product thiazolidinone was recrystallized.

Physical constants and characterization of compounds

Spectral and physical data of all these synthesized thiazolidinones are in agreement with those discussed in the literature [29-32]. Representative spectral data is as follow.

4a: 2,3-diphenylthiazolidin-4-one: 4a was synthesized by cyclocondensation between aniline 1a, benzaldehyde 2a and thioglycolic acid 3 as a white solid; Yield: 80%; Melting point: $128-129^\circ\text{C}$ (lit(25)); ^1H NMR (600 MHz, CDCl_3) δ 7.30-7.34 (dd, 7H $J = 9.6, 4.9$ Hz), 7.14-7.17 (d, 3H, $J = 7.9$ Hz), 6.18 (s, 1H), 3.83-3.97 (dd, $J = 50.1, 2\text{H}, 15.8$ Hz); FTIR (cm^{-1}): 2893, 1678, 1588, 1488, 1338, 610; ^{13}C NMR

(400 MHz, CDCl_3) δ 34.35, 66.06, 124.70, 126.18, 127.22, 130.10, 130.27, 130.88, 137.66, 139.35, 171.41; LC MS (m/z): 256.09 $[\text{M}+\text{H}]^+$.

4b: 2-(4-nitrophenyl)-3-(p-tolyl) thiazolidin-4-one: 4b was synthesized by cyclocondensation between 4-methylaniline 1b, 4-nitrobenzaldehyde 2b and thioglycolic acid 3 as a Yellow solid; Yield: 89%; Melting point: $158-160^\circ\text{C}$ (lit(26)); ^1H NMR (600 MHz, CDCl_3) δ 8.15-8.17 (d, 2H, $J = 8.5$ Hz), 7.40-7.43 (d, 2H, $J = 8.2$ Hz), 7.02 – 7.12 (m, 4H), 6.0 (s, 1H), 3.87-4.09 (dd, 2H, $J = 36.7, 15.9$ Hz), 2.34 (s, 3H); FTIR (cm^{-1}): 2929, 1688, 1513, 1393, 1295, 630; ^{13}C NMR (400 MHz, CDCl_3) δ 21.07, 34.32, 64.50, 128.28, 125.21, 128.00, 131.14, 134.88, 137.68, 145.98, 148.87, 170.11; LC MS (m/z): 315.06 $[\text{M}+\text{H}]^+$.

4c: 3-(4-chlorophenyl)-2-(4-nitrophenyl) thiazolidin-4-one: 4c was synthesized by cyclocondensation between 4-chloroaniline 4a and 4-nitrobenzaldehyde 4b and thioglycolic acid 3 as a White solid; Yield: 86%; Melting Point: $112-115^\circ\text{C}$; (Lit(27)) ^1H NMR (600 MHz, CDCl_3) δ 7.33 (dd, $J = 9.6, 4.9$ Hz, 7H), 7.21 (d, $J = 7.9$ Hz, 3H), 6.00 (s, 1H), 3.98 (dd, $J = 50.1, 15.8$ Hz, 2H). FTIR (cm^{-1}): 3050, 1670, 1520, 1490, 1381, 825, 730, 702. LC MS (m/z): 335.02 $[\text{M}+\text{H}]^+$.

Conclusions

A simple and energy-efficient pathway for the development of biologically important functionalized thiazolidinones, like 1,3-thiazolidinon-4-one, has been reported using Baker's yeast in ChCl:Gly, cost effective and eco-friendly solvent. This catalyst-free methodology operates under mild reaction conditions and produces the products in good yields. Additionally, the recovered solvent could be recycled and reused for up to five runs.

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