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Regulating Lipase Inhibition by Adjusting Composition of Binary Mixtures of 1-Ethyl-3 methylimidazolium Ethylsulfate and Propanol-1

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Abstract

This study investigates the regulation of lipase inhibition in binary mixtures of 1-ethyl-3-methylimidazolium ethylsulfate ($[C_2mim]C_2H_5SO_4$) and propanol-1, systematically examining solvent-composition-dependent effects on catalytic activity and kinetics. The hydrolysis of *p*-nitrophenyl butyrate (*p*-NPB) was monitored using UV–visible spectroscopy by following the formation of *p*-nitrophenolate (*p*-NP) at 410 nm, enabling quantitative determination of initial reaction rates and inhibition trends in aqueous, ionic liquid, and binary solvent systems. Results revealed that $[C_2mim]C_2H_5SO_4$ stabilized lipase structure and minimized enzyme denaturation, likely by attenuating unfavorable solvent–protein interactions at the active site, while propanol-1 acted as an inhibitor by disrupting the catalytic serine residue and hydrophobic substrate-binding regions. Maximum catalytic efficiency was observed at low propanol-1 mole fractions, whereas increasing alcohol content led to pronounced reductions in reaction rate, consistent with mixed-type inhibition and restricted substrate accessibility. These findings demonstrate, for the first time, how controlled binary ionic liquid–alcohol mixtures can be used to fine-tune lipase activity, highlighting the critical role of solvent composition in balancing enzyme stability and inhibition and providing practical design guidelines for optimizing biocatalysis in non-aqueous and mixed-solvent media.

Keywords: Lipase; Ionic Liquids; Inhibition; Kinetics; Biocatalysis

I. Introduction

Enzymes, the vital components of many industrial and biochemical processes, are composed mainly of proteins and are highly efficient and selective biocatalysts. Since, enzymes are designed naturally to operate in cellular environments that are enriched with water, enzyme-mediated processes have traditionally taken place in aqueous medium. However, there are a number of drawbacks of using aqueous systems, such as limited product recovery, restricted ability to regulate reaction selectivity, and the poor solubility of hydrophobic substrates.[1] The application of non-aqueous solvents like organic solvents, ionic liquids (ILs), and their combinations, to enhance enzyme performance has grown in popularity as a result of these concerns. Non-aqueous media, in particular, have the ability to regulate catalytic activity, enhance heat stability, and transform enzyme structure, extending the potential of biocatalysis beyond usual scenarios.[2]

ILs, a family of salts that are liquid below 100 °C, have attracted significant attraction in biocatalysis due to their substantially low melting temperature, non-flammability, minimal volatility, superior electrical conductivity, extended electrochemical stability range, exceptional thermal stability, expanded liquidous range, high solubilizing ability for diverse inorganic and organic compounds, and the capacity of tuning the structure of ions to get optimal physicochemical properties.[3,4] By providing a more appropriate environment than conventional solvents, ILs can increase enzyme activity and stability, hence increasing reaction efficiency. They can also affect the overall reaction route, substrate solubility, and enzyme selectivity.[5] Nevertheless, the toxicity of many ILs and the difficulty of enzyme compatibility continue to be barriers to their broad use, requiring additional studies for developing ILs that are less harmful and more enzyme-friendly.[6]

Co-solvents are frequently used in biocatalytic systems involving ILs in order to modify the physicochemical characteristics of the solvent and enhance enzyme activity. To improve mass transfer, fine-tune solvent polarity, and decrease the viscosity of ILs, common co-solvents like water, short-chain alcohols (like methanol and ethanol), and organic solvents (like acetonitrile and DMSO) are employed.[7] If the co-solvent is compatible with the biocatalyst, these changes may result in increased substrate solubility and enhanced enzyme stability.[4] Although water is the most commonly utilized co-solvent because it is crucial for preserving the structure and activity of enzymes, trace amounts of alcohols can also help stabilize the conformation of enzymes or improve their solubility.[7] On the other hand, high co-solvent concentrations may be harmful, changing reaction pathways or inactivating enzymes.[4] Thus, in order to achieve efficient and long-lasting biocatalysis in IL-based systems, the kind and concentration of co-solvents must be chosen and optimized.[8,9]

The combination of ILs with alcohols in binary mixtures is a potential but underexplored method for regulating solvent characteristics in biocatalysis. While no significant efforts have been devoted to biocatalytic applications of these

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binary systems, the ability to tailor key solvent properties like polarity, viscosity, and lipophilicity via the selective mixing of ILs and alcohols is significant.[10,11] ILs are known for their capacity to alter physicochemical properties, and the addition of alcohols may improve enzyme stability, activity, and selectivity.[7]

ILs are environmentally friendly media for chemical and biological transformations since early research showed that they increase catalytic activity while also lowering the production of hazardous byproducts.[12] The synthesis of the dipeptide Z-aspartame catalyzed by thermolysin represented the earliest documented enzyme-catalyzed reaction in IL medium, demonstrating the suitability of enzymes for IL-based systems.[13] The most studied type of enzymes in ILs, according to later research, are lipases. ILs can enhance Michael addition reactions catalyzed by lipase for the production of warfarin, with hydroxyl-functionalized ILs improving both catalytic efficiency and enzyme reusability.[14] Reactions in hydrophilic ILs, including 1-methoxyethyl-3-methylimidazolium tetrafluoroborate, exhibit higher catalytic efficiency than those in conventional organic solvents.[15]

Further developments highlighted the application of IL-based microemulsions for lipase-mediated hydrolysis of *p*-nitrophenyl butyrate (*p*-NPB), demonstrating enhanced dispersion and activity of the enzyme.[11] Modifying the nature of the interfacial membrane has also been employed to regulate the activity of enzymes, demonstrating the significant impact of solvent microenvironments on biocatalysis.[16] Further research with double-salt ILs showed that catalytic stability and efficiency are controlled by physicochemical properties of the solvent medium as well as conformational changes in the enzyme.[17]

Early studies on binary IL-alcohol systems in different chemical reactions suggest that these mixtures may have synergistic effects on enzyme structure and catalytic performance.[18] However, these interactions have been scarcely explored in biocatalysis, highlighting a crucial knowledge gap that requires additional exploration to fully exploit the potential of binary IL-alcohol systems for enzyme optimization.

The regulatory mechanisms that control enzyme activity in binary mixtures of ILs and alcohols are complex and not well understood. However, various alternative mechanisms have been proposed based on the unique effects of ILs and alcohols on enzymes. ILs interact with enzyme structures via hydrogen bonding, electrostatic interactions, and solvation effects, influencing enzyme conformation and active-site accessibility.[19] The addition of alcohols may further alter the solvent environment, perhaps stabilizing the enzyme by increasing its solubility or, conversely, promoting denaturation at greater concentrations due to protein-solvent interactions.[20] Furthermore, the viscosity and polarity of the binary mixture might affect kinetic properties of the enzyme, such as substrate diffusion and catalytic effectiveness. The solvent microstructure, dielectric characteristics, and hydrogen-bonding network may all play important roles in determining enzyme stability and activity in mixed solvent systems.[7] ILs interact with enzymes through hydrogen bonding, electrostatic interactions, and solvation of charged and polar amino acid residues, which can either stabilize the native conformation or induce partial unfolding at higher concentrations.[7,19] Also ions capable of forming hydrogen bonds with peptide backbones, potentially disrupting intramolecular interactions essential for maintaining lipase tertiary structure.[7]

Propanol affects enzyme conformation by penetrating the protein hydration shell and weakening hydrophobic interactions. At low concentrations, alcohols may enhance enzyme flexibility and activity; however, at higher concentrations, propanol can promote lipase denaturation through unfavorable protein–solvent interactions and destabilization of the hydrophobic core.[7,19] This study aims to investigate how varying proportions of propanol-1 in binary mixtures with $[C_2mim]C_2H_5SO_4$ influence catalytic activity, lipase conformational stability, and kinetic properties. Significant findings are expected to provide insights into optimizing enzyme performance and understanding the complex regulatory mechanisms of enzymatic activity in IL-alcohol systems. Understanding these interactions at the molecular level is critical for maximizing enzyme performance in biocatalytic processes, but more study remains to be carried out to determine the precise regulatory mechanisms at work in IL-alcohol systems.

II. Materials and Methods

The IL, $[C_2mim]C_2H_5SO_4$ (≥95% purity), Type II porcine pancreas lipase, and the substrate *p*-NPB were purchased from Merck (formerly Sigma-Aldrich). Propanol-1 (≥99.5% purity) was supplied by Lab Scan. Tris (hydroxymethyl) aminomethane (Tris) was obtained from Carl Roth. Double-distilled, deionized HPLC-grade water (σ = 0.055 μS cm^{-1} at 298.15 K) from a water purification system (Model BOE 8082060, Germany) was used for cleaning and buffer preparation.

Hydrolysis of *p*-NPB was monitored using a UV–visible spectrophotometer (UVD-3500, Labomed Inc., USA) integrated with a Peltier temperature controller (Model PTC-2). A 1 cm path length quartz cuvette was used for all measurements. Reactions were carried out in 50 mM Tris-HCl buffer at pH 7.4 to maintain a consistent pH environment. Reactions were initiated by adding the substrate to the enzyme-containing mixture and incubating at 37°C. Hydrolysis of *p*-NPB was followed by measuring the formation of *p*-nitrophenol (*p*-NP) against a blank containing denatured enzyme. All assays were carried out in duplicate and repeated independently.

III. Results

The composition of reaction medium significantly influences enzyme-catalyzed reactions, and the lipase-catalyzed hydrolysis of *p*-NPB has been investigated in

aqueous, $[C_2mim]C_2H_5SO_4$, and mixed media. The product *p*-NP in aqueous solution is the *p*-nitrophenolate ion, that shows absorbance at 410 nm.[16] To prevent interference with the small 400 nm peak, **(Figure 1)** all kinetic studies were performed by monitoring absorbance at this wavelength.

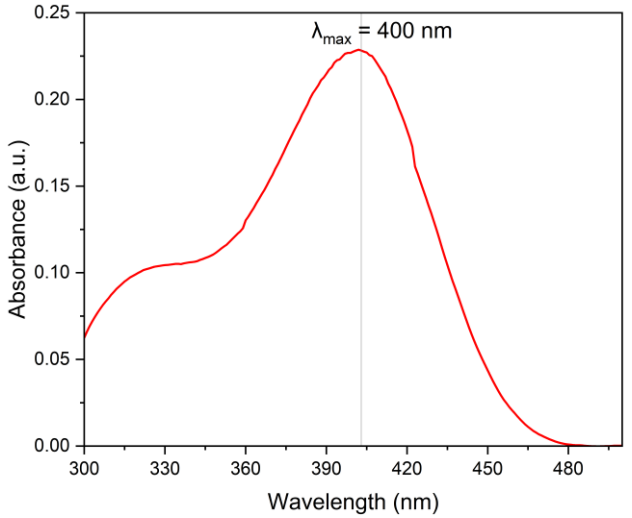

Fig. 1. UV-vis spectra of *p*-NP (0.01mM) in aqueous medium at constant pH of 7.4 maintained by 50 mM Tris-HCl buffer) at 310.15 K.

The absorbance-time profiles for the hydrolysis of *p*-NPB in aqueous medium by lipase at different substrate concentrations are shown in Figure 2. The initial slopes of the curves, when the reaction exhibits linear behavior, were used to find the initial reaction rates. Table 1 summarizes these initial rates, which correlate to various substrate concentrations and serve as an initial basis for further kinetic research.

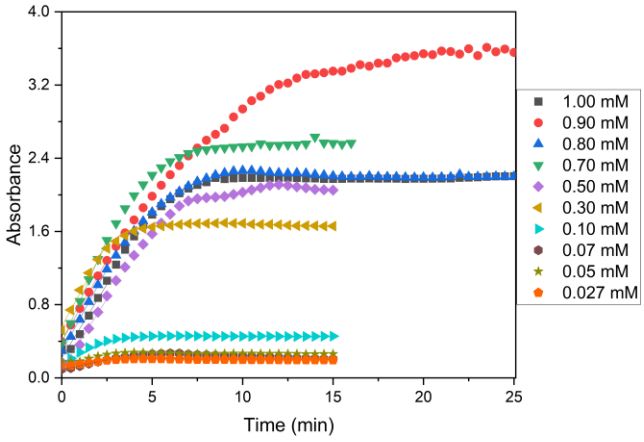

Fig. 2. Absorbance versus time profile for lipase catalyzed hydrolysis of *p*-NPB at varying concentrations of *p*-NPB (All the kinetic runs are taken at temperature 310.15 K, pH 7.4 (50mM tris-HCl buffer), and wavelength at 410 nm).

Table 1. The initial rate, *v* as a function of [*p*-NPB] for lipase-catalyzed hydrolysis of *p*-NPB in aqueous medium.

[*p*-NPB] (mM)	*v* (mg.mL^{-1}.min^{-1})
0.027	0.02594
0.05	0.0344
0.07	0.0428
0.1	0.1044
0.3	0.36029
0.5	0.30949
0.7	0.45326
0.8	0.36223
0.9	0.3564
1.0	0.32211

The initial rate increases with increasing *p*-NPB concentration at lower substrate levels, indicating substrate-dependent enhancement of enzymatic activity. A gradual rise in reaction rate is observed from 0.027 to 0.07 mM *p*-NPB, followed by a sharp increase at 0.1 mM and 0.3 mM, suggesting a strong acceleration of enzyme–substrate complex formation in this range. The maximum reaction rate is observed at 0.7 mM *p*-NPB. Beyond this concentration, the reaction rate decreases despite further increases in substrate concentration, indicating possible substrate inhibition or reduced catalytic efficiency at higher *p*-NPB levels. The decrease in enzymatic activity at higher substrate concentrations may be attributed to changes in the solvent microenvironment arising from strong heteromolecular interactions in the IL–alcohol binary mixture. the presence of strong hydrogen-bonding interactions and effective molecular packing between unlike species, which can alter local polarity, viscosity, and solvation dynamics around the enzyme, thereby influencing substrate diffusion, enzyme conformation, and catalytic efficiency.[21]

The rate marginally drops at very high concentrations of *p*-NPB, which indicates a mechanistic distinction from the typical Michaelis-Menten mechanism. Higher substrate concentrations can lead to the substrate binding to non-active site regions of the enzyme which prevents the substrate molecules from forming products.[22]

The initial rate was plotted against the concentration of *p*-NPB in **Figure 3.** The data points are fitted with Michaelis-Menten and Lineweaver-Burk equations. From the fitted parameters, the values of K_m, V_m, and K_{cat} are calculated (Table 2).

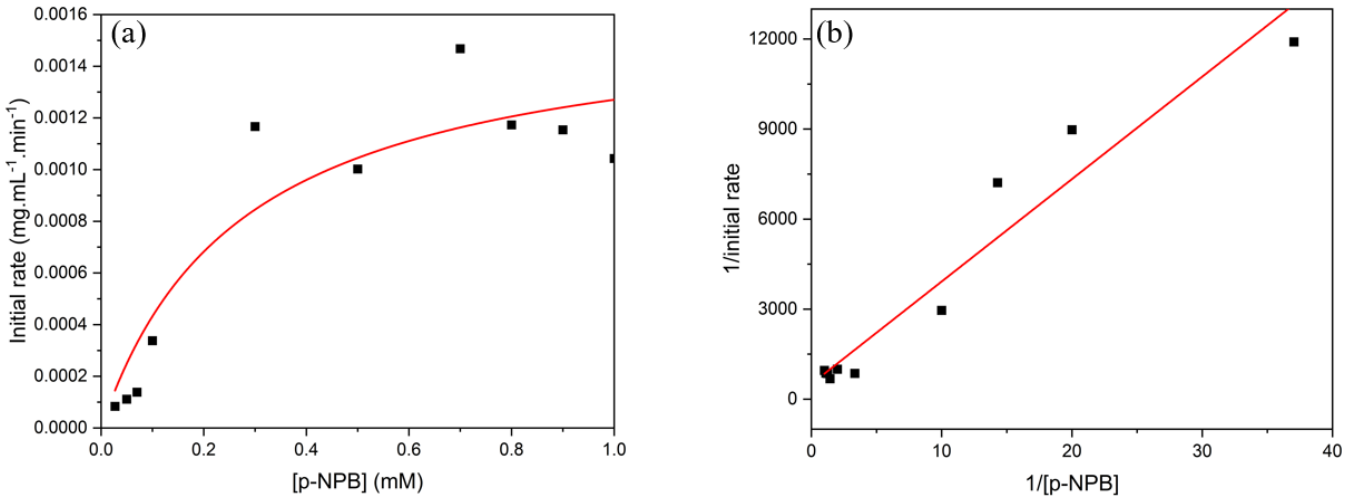

Fig. 3. (a) Initial rate versus *p*-NPB concentration (Michaelis–Menten fit) and (b) Lineweaver–Burk plot (1/*v* vs 1/[*p*-NPB]). Conditions: lipase = 2 mg·mol⁻¹, pH 7.4, T = 310.15 K.

Table 2. Kinetic parameters of lipase-catalyzed hydrolysis of *p*-NPB in aqueous medium.

Plots	K_m (mM)	V_m (mg.mL^{-1}mol^{-1})	R^2
Michaelis-Menten plot	0.2749±.1509	0.2253±0.0410	0.8574
Lineweaver-Burk plot	0.6750±0.0601	0.2750±0.2393	0.9330

IV. Discussion

The mechanism of interaction of lipase with the substrate has been systematically investigated.[23-25] By exposing hydrophobic areas of the lipase molecule, which causes irreversible conformational changes, the hydrophobic interaction between the lipase and the substrate is now well established. Although there are 446 amino acids in pancreatic lipase, serine, glycine, and aspartic acid make up the predominant region of the active site of enzyme. Serine plays the primary role in enzymatic activity involved in the binding of substrates to the lipase enzyme.[20] Two distinct isoforms of the enzyme, porcine pancreatic lipase, have been proposed. Lipase activity is associated with the shielding of the active site from the reaction environment by a polypeptide lid in its closed conformation. The enzyme is in its active state (open-form) when the lid is displaced (interfacial activation) and the active site is accessible to the reaction medium.[21] Lipase is in equilibrium between these two forms in an aqueous or homogeneous media, with the equilibrium shifting in direction of the closed form. Due to the interaction of substrate and enzyme during the reaction, the open form ends up being the most stable. As the substrate binds with the open form of the enzyme, the modification of serine happens as a result of the creation of the enzyme-substrate complex, which results in an apparent irreversible inactivation and may indicate the enzyme denaturation.[25]

Enzymatic reactions proceed at a reduced rate in the IL medium. This may be due to the steric hindrance created by larger cations and anions of $[C_2mim]C_2H_5SO_4$. The -OH group in serine at the active site may become affected by the supramolecular interaction with cation and anion. Hence denaturation of enzyme is less probable in the system. Despite steric hindrance the kinetics is thus not affected much.[13]

Figure 4 shows the initial rate at different medium. It is evident that initial rate is maximum in aqueous medium. The initial rate decreases in binary mixture medium.

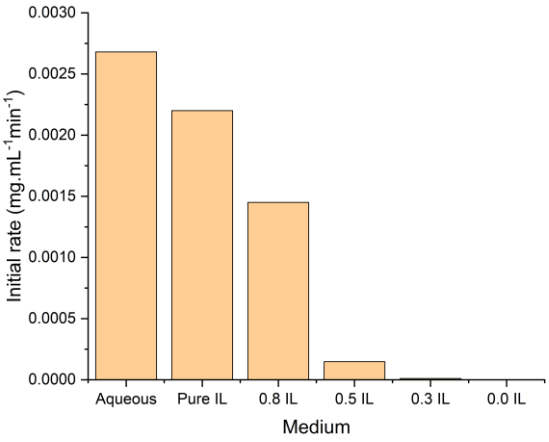

Fig. 4. Initial rates in aqueous medium and binary mixture media at different mole fraction of $[C_2mim]C_2H_5SO_4$.

However, in the presence of propanol-1 in the medium, the reaction kinetics slow down, and at higher concentrations of propanol-1, the reaction becomes more difficult. The role of propanol-1 in this reaction is as an inhibitor. The -OH group of propanol-1 interacts with -OH of serine and the hydrophobic chain of it masks the hydrophobic interaction sites which hampers the approach of *p*-NPB towards active sites of lipase. The schematic diagram of the mechanisms of all these processes is depicted in **Figure 5.**

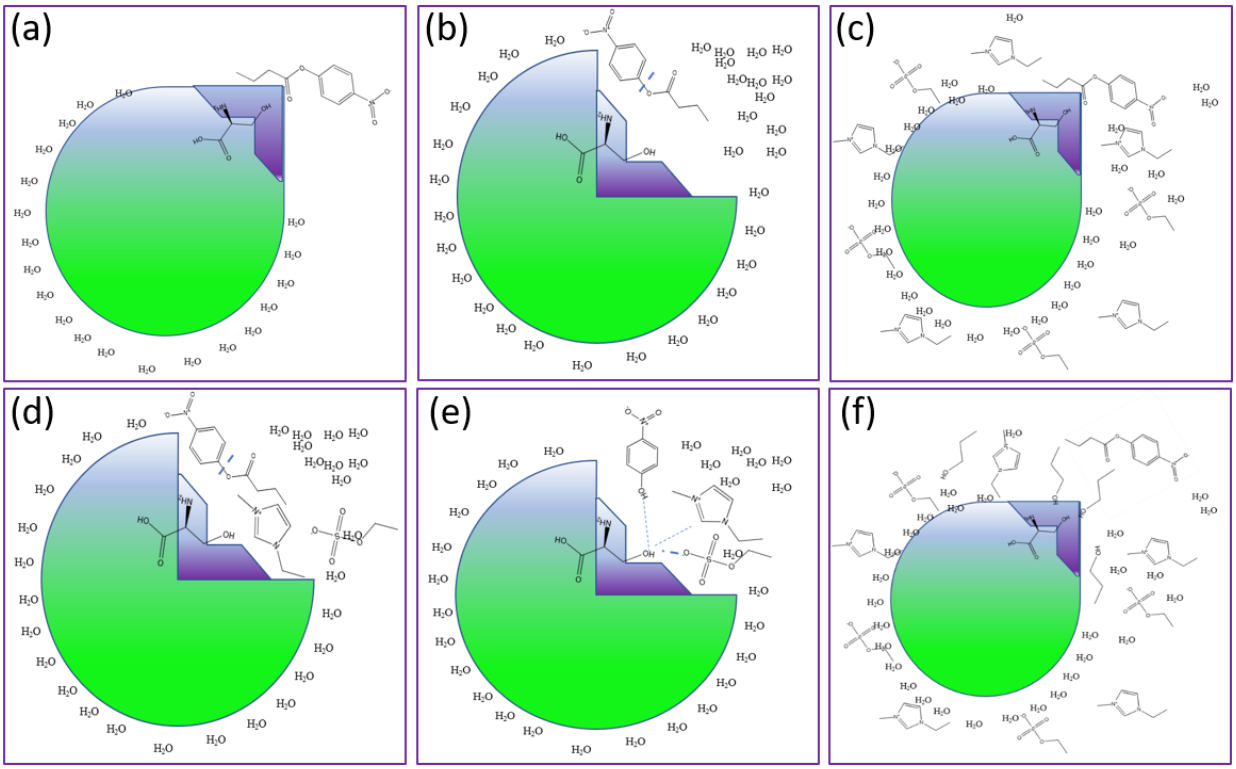

Fig. 5. Proposed mechanism of lipase activity in different media: (a,b) substrate binding and catalysis in aqueous medium; (c–e) competitive interactions and denaturation effects of $[C_2mim]C_2H_5SO_4$; and (f) inhibition of lid opening and substrate access by propanol-1 in binary mixtures.

Although the $[C_2mim]C_2H_5SO_4$ in the binary mixture minimizes the denaturation of lipase, the propanol-1 acts as inhibitor hence binary mixture of $[C_2mim]C_2H_5SO_4$ and propanol-1 works better at low content of propanol-1 but at higher content of propanol-1, the medium is not suitable for the catalysis.

V. Conclusions

This study demonstrates that the catalytic efficiency of lipase in binary mixtures of $[C_2mim]C_2H_5SO_4$ and propanol-1 is highly dependent on the alcohol content. While $[C_2mim]C_2H_5SO_4$ maintains the structural integrity of the enzyme, higher concentrations of propanol-1 significantly reduce enzymatic activity. Optimal catalytic performance is achieved at low propanol-1 levels, where enzyme denaturation and inhibition are minimal. In contrast, increasing alcohol concentrations enhance inhibitory effects, rendering the medium unsuitable for efficient catalysis. These results underscore the importance of carefully controlling alcohol content in IL-based solvent systems to preserve enzyme stability and maximize activity.

Conflict of interest

The authors declare no competing financial interest.

References

1. Klibanov, A. M. 2001. Improving Enzymes by Using Them in Organic Solvents. *Nature*, **409** (6817), 241–246. https://doi.org/10.1038/35051719.

2. Domínguez De P. María, and F, Hollmann, 2015. On the (Un)Greenness of Biocatalysis: Some Challenging Figures and Some Promising Options. *Front. Microbiol.* 6. https://doi.org/10.3389/fmicb.2015.01257.

3. Freemantle, M. 2010. An Introduction to Ionic Liquids; RSC Pub: Cambridge, UK.

4. Van Rantwijk, F. and R. A. Sheldon, 2007. Biocatalysis in Ionic Liquids. *Chem. Rev.* **107(6)**, 2757–2785. https://doi.org/10.1021/cr050946x.

5. Xu, P., S., Liang, M.-H. Zong, and W.-Y. Lou, 2021. Ionic Liquids for Regulating Biocatalytic Process: Achievements and Perspectives. *Biotechnol. Adv.* **51**, 107702. https://doi.org/10.1016/j.biotechadv.2021.107702.

6. Itoh, T. 2017. Ionic Liquids as Tool to Improve Enzymatic Organic Synthesis. *Chem. Rev.* **117(15)**, 10567–10607. https://doi.org/10.1021/acs.chemrev.7b00158 .

7. Moniruzzaman, M., K., Nakashima, N. Kamiya, and M. Goto, 2010. Recent Advances of Enzymatic Reactions in Ionic Liquids. *Biochem. Eng. J.* **48(3)**, 295–314. https://doi.org/10.1016/j.bej.2009.10.002.

8. Elgharbawy, A. A. M., M. Moniruzzaman, and M. Goto, 2020. Recent Advances of Enzymatic Reactions in Ionic Liquids: Part II. *Biochem. Eng. J.* **154**, 107426. https://doi.org/10.1016/j.bej.2019.107426.

9. Ali, Md. A. and Md. Susan, A. B. H. 2023. Molecular-Level Interactions in Binary Mixtures of 1-Ethyl-3-Methylimidazolium Ethylsulfate and Propane-1,2-Diol: The Interplay between Intermolecular and Intramolecular

Hydrogen Bonding. *ACS Omega.* **8(36)**, 32690–32700. https://doi.org/10.1021/acsomega.3c03457.

10. Ali, Md. A. and Susan, Md. A. B. H. 2022. Volumetric and Spectroscopic Studies of 1-Ethyl-3-Methylimidazolium Ethylsulfate/Propane-1-Ol Binary Mixtures at Different Temperatures. *Spectr. Emerg. Sci.* **2(2)**, 17–28. https://doi.org/10.55878/SES2022-2-2-5.
11. Moniruzzaman, M., Kamiya, N. and Goto, M. 2010. Activation and Stabilization of Enzymes in Ionic Liquids. *Org. Biomol. Chem.* ***8*(13)**, 2887. https://doi.org/10.1039/b926130c.
12. Seddon, K. R., 1997. Ionic Liquids for Clean Technology. *J. Chem. Technol. Biotechnol. Int. Res. Process Environ. Clean Technol.* **68(4)**, 351–356. https://doi.org/10.1002/(SICI)1097-4660(199704)68:4%3C351::AID-JCTB613%3E3.0.CO;2-4.
13. Kragl, U., Eckstein, M. and Kaftzik, N. 2002. Enzyme Catalysis in Ionic Liquids. *Curr. Opin. Biotechnol.* ***13*(6)**, 565–571. https://doi.org/10.1016/S0958-1669(02)00353-1.
14. Madeira Lau, R., Van Rantwijk, F., Seddon, K. R. and Sheldon, R. A. 2000. Lipase-Catalyzed Reactions in Ionic Liquids. *Org. Lett.* **2(26)**, 4189–4191. https://doi.org/10.1021/ol006732d.
15. Kazlauskas, R. J. 1994. Elucidating Structure-Mechanism Relationships in Lipases: Prospects for Predicting and Engineering Catalytic Properties. *Trends Biotechnol.* **12(11)**, 464–472. https://doi.org/10.1016/0167-7799(94)90022-1.
16. Xue, L., Qiu, H., Li, Y., Lu, L., Huang, X. and Qu, Y. 2011. A Novel Water-in-Ionic Liquid Microemulsion and Its Interfacial Effect on the Activity of Laccase. *Colloids Surf. B Biointerfaces.* ***82*(2)**, 432–437. https://doi.org/10.1016/j.colsurfb.2010.09.016.
17. Yao, P., Yu, X. and Huang, X. 2015. Effect of the Physicochemical Properties of Binary Ionic Liquids on Lipase Activity and Stability. *Int. J. Biol. Macromol.* **77**, 243–249. https://doi.org/10.1016/j.ijbiomac.2015.03.048.
18. Beniwal, V. and A. Kumar, 2015. Synergistic Effects and Correlating Polarity Parameters in Binary Mixtures of Ionic Liquids. *ChemPhysChem* ***16*(5)**, 1026–1034. https://doi.org/10.1002/cphc.201402825.
19. Zhao, H. 2010. Methods for stabilizing and activating enzymes in ionic liquids—a review. *J. Chem. Technol. Biotechnol.,* ***85*(7)**, 891-907. https://doi.org/10.1002/jctb.2375.
20. Fehér, E., Illeová, V., Kelemen-Horváth, I., Bélafi-Bakó, K., Polakovič, M., and Gubicza, L. 2008. Enzymatic production of isoamyl acetate in an ionic liquid–alcohol biphasic system. *J. Mol. Catal. B Enzym.* ***50*(1)**, 28-32. https://doi.org/10.1016/j.molcatb.2007.09.019.
21. Ali, Md. A., and Md. A. B. H. Susan, 2025. Formation and Disruption of Hydrogen Bond in 1-Ethyl-3-methylimidazolium Ethylsulfate and Glycerol Binary Mixtures: A Molecular Perspective. *J. Phys. Chem. B,* ***129*(22)**, 5541–5549. https://doi.org/10.55878/SES2022-2-2-5.
22. Reed, M. C., A. Lieb, and H. F. Nijhout, 2010. The Biological Significance of Substrate Inhibition: A Mechanism with Diverse Functions. *BioEssays* ***32*(5)**, 422–429. https://doi.org/10.1002/bies.200900167.
23. Van Tilbeurgh, H., L., Sarda, R. Verger, and C. Cambillau, 1992. Structure of the Pancreatic Lipase–Procolipase Complex. *Nature* ***359*(6391)**, 159–162. https://doi.org/10.1038/359159a0.
24. Winkler, F. K., D' A. Arcy, and W. Hunziker, 1990. Structure of Human Pancreatic Lipase. *Nature* ***343*(6260)**, 771–774. https://doi.org/10.1038/343771a0.
25. Derewenda, Z. S. and A. M. Sharp, 1993. News from the Interface: The Molecular Structures of Triacyglyceride Lipases. *Trends Biochem. Sci.* ***18*(1)**, 20–25. https://doi.org/10.1016/0968-0004(93)90082-X.