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Synthesis and Characterization of Porous Polymer as a Support Matrix for Lipase Immobilization

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Abstract
Immobilization of lipase on solid supports has become a promising approach to improve enzyme stability, activity, and reusability in various industrial processes. This study focuses on the development of a porous polymer, poly(glycidyl methacrylate-co-trimethylolpropane trimethacrylate) [Poly(GM-co-TT)], as a functional support for covalent lipase immobilization. The experimental procedure included three main stages. First, Poly(GM-co-TT) was synthesized via free radical polymerization using a ternary porogenic solvent system consisting of 1,4-butanediol, 1-propanol, and water in a 4:7:1 (v/v) ratio to obtain a porous structure. Second, lipase was immobilized covalently through the reaction between epoxy groups of the polymer and amino groups of the enzyme, using a 0.2 M KCl–NaOH buffer (pH 11) at a final enzyme concentration of 10 mg/mL, incubated at room temperature for 120 minutes. Third, characterization was performed using FTIR spectroscopy and scanning electron microscopy (SEM) to verify successful immobilization. FTIR analysis revealed the presence of ester (C=O), ether (C–O–C), and epoxide (C–O) groups in the polymer. Post-immobilization spectra showed reduced epoxy band intensity (~910 and 840 cm^{-1}) and the appearance of amide bands (~1640–1660 cm^{-1}), indicating covalent bonding between polymer and enzyme. SEM images confirmed a porous, globular, interconnected morphology with well-distributed pores, ideal for enzyme anchoring. The open and rough surface increases surface area, enhancing immobilization efficiency. The novelty of this study lies in employing Poly(GM-co-TT) as a porous polymer that preserves epoxy functionality while effectively supporting covalent lipase immobilization.

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INTRODUCTION

Lipase is one of the most widely used biocatalysts in various industrial processes, including biodiesel production, pharmaceutical synthesis, food processing, and environmental applications (Sarmah et al., 2017).. However, the direct use of free lipase in catalytic systems faces several limitations due to its low operational stability, tendency to denature under extreme conditions, and difficulties in recovery and reuse (Giraldo et al., 2023; Mandari & Kumar, 2022; Xie & Huang, 2020). To address these issues, various enzyme immobilization techniques have been developed to improve the overall stability, reusability, and catalytic efficiency of lipase (Maghraby et al., 2023; Mandari & Kumar, 2022).

Common immobilization strategies, such as physical adsorption, entrapment, and encapsulation, have been widely applied to enhance the usability and stability of lipase (Lyu et al., 2021). Nevertheless, each of these methods presents certain drawbacks. Physical adsorption relies on weak interactions between the enzyme and the support, making the enzyme prone to leaching during the reaction process (Wan Osman et al., 2024). While entrapment and encapsulation offer better protection by enclosing the enzyme within a matrix or capsule, they

often restrict substrate and product diffusion, resulting in reduced reaction efficiency (Kawakami et al., 2009; Lam et al., 2010). To overcome these limitations, covalent bonding combined with crosslinking has been proposed as a more robust approach for enzyme immobilization (Miletic et al., 2009; Xie & Huang, 2020). In order to further enhance mass transfer efficiency on the polymer surface, Anggraeny et al., (2018) incorporated a ternary porogen system comprising 1-propanol, 1,4-butanediol, and water to prepare a porous polymer-based microreactor for trypsin immobilization.

Glycidyl methacrylate (GM) and its copolymer with trimethylolpropane trimethacrylate (TT) have attracted considerable attention as enzyme immobilization matrices due to their reactive epoxy groups, which facilitate covalent bonding with nucleophilic groups on the enzyme surface. TT acts as a crosslinking agent, forming a stable three-dimensional polymer network with good mechanical strength. Furthermore, the porous structure of the polymer can be tailored through the use of porogenic solvent mixtures. In particular, the ternary porogen system consisting of 1,4-butanediol, 1-propanol, and water has been demonstrated to generate a homogeneous, interconnected microglobular morphology. This porous structure not only enhances the specific surface area but also promotes uniform enzyme distribution and facilitates mass transfer (Amalia et al., 2021).

The central problem addressed in this study is that, despite numerous advancements in lipase immobilization techniques, limitations such as weak enzyme–support interactions, restricted mass diffusion, and poor operational stability remain significant challenges for the practical application of lipase as a heterogeneous biocatalyst. Therefore, the development of polymer-based immobilization systems with controlled structure and functionality is essential to create efficient and stable catalytic materials.

This study focuses on the synthesis and characterization of poly(GM-co-TT) as a matrix for lipase immobilization, with particular emphasis on the influence of porogenic system-induced morphology on the immobilization performance and its potential application in sustainable catalytic systems. The work also aims to validate the success of immobilization through comprehensive physicochemical characterizations and to evaluate the potential of the synthesized polymer as a platform for enzyme-based microreactors.

The novelty of this research lies in the use of a combination of the reactive monomer GM and the crosslinking agent TT within a ternary porogen system (1,4-butanediol/1-propanol/water) to produce a homogeneous, interconnected microglobular structure. This approach has rarely been applied to lipase immobilization and provides a new pathway for developing immobilization materials with enhanced mass transfer efficiency and improved enzyme stability.

METHOD

Material

The chemicals used in this study included trimethylolpropane trimethacrylate (TT, 98%, Merck, Germany), glycidyl methacrylate (GM, ≥97.0% (GC), Merck, Germany), 1,4-butanediol (≥99%, Merck, Germany), 1-propanol (≥99.5%, Merck, Germany), 2,2′-azobisisobutyronitrile (AIBN, 12 wt.% in acetone, Merck, Germany), pyridine (ACS reagent, ≥99.0%), sodium bicarbonate ($NaHCO_3$, ACS reagent, ≥99.0%, Merck, Germany), sodium carbonate (Na_2CO_3, ≥99.5%, Merck, Germany), and anhydrous sodium sulfate (≥99%, Merck, Germany) and Lipase made (99%, Shaanxi Fonde Biotech Co.Ltd).

Synthesis of Poly (GM-co-TT)

To prepare a suitable support for lipase immobilization, a methacrylate-based porous polymer was synthesized using a previously reported method with slight modifications (Amalia et al., 2021; Anggraeny et al., 2018; Tasfiyati et al., 2016). The polymer was prepared ex situ in a glass vial by combining glycidyl methacrylate (GM) as the monomer (%T 40), trimethylolpropane trimethacrylate (TT) as the crosslinking agent (%C 25), and a porogenic solvent mixture composed of 1,4-butanediol, 1-propanol, and distilled water in a 4:7:1 volume ratio (v/v). The mixture was first homogenized using a sonicator for 5 minutes. Subsequently, azobisisobutyronitrile (AIBN) was added as the radical initiator (1% w/v), and the mixture was sonicated again for another 5 minutes to ensure uniform dispersion.

The prepared solution was then sealed tightly and placed in an oven at 60 °C for 12 hours to complete the polymerization process. Once polymerization was complete, the solidified polymer was carefully removed from the vial and washed thoroughly with ethanol and distilled water under magnetic stirring for 30 minutes to remove unreacted components and porogenic residues. The final product, referred to as poly(GM-co-TT), was used as the support for lipase immobilization. The proposed reaction mechanism for the synthesis is shown in Figure 1.

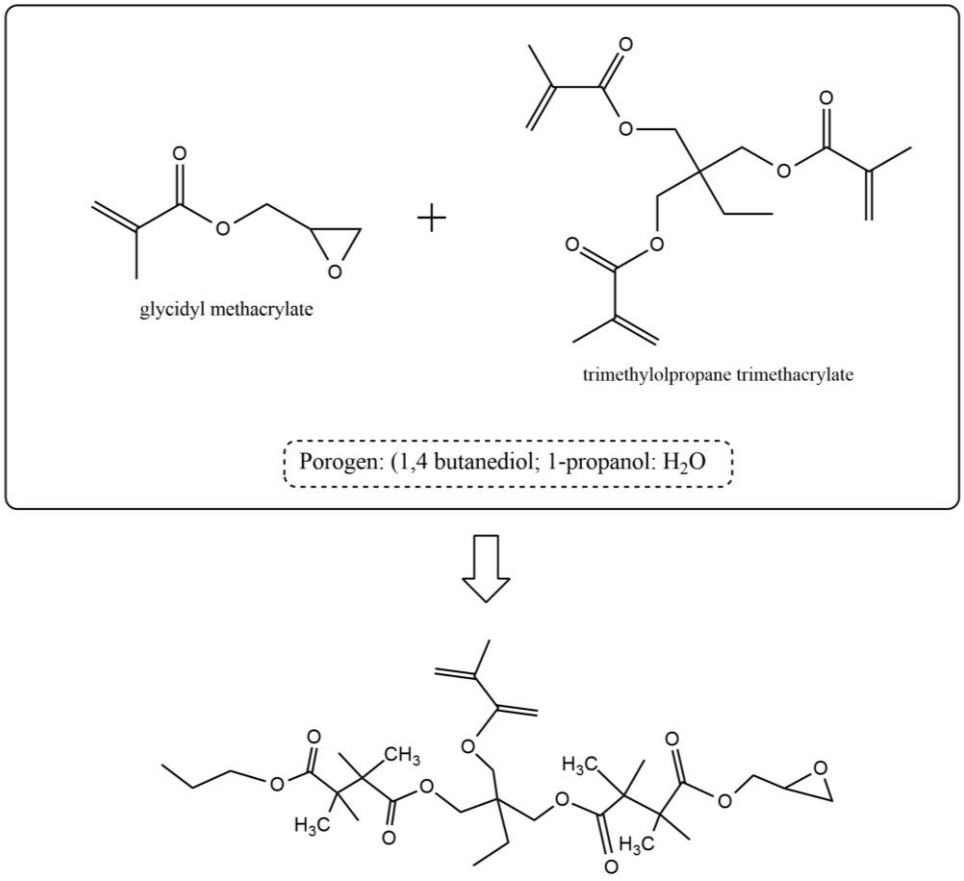

Figure 1. Illustrates the proposed reaction scheme for synthesizing Poly(GM-co-TT).

Immobilization of Lipase

Lipase immobilization was achieved through a covalent reaction between the epoxy groups present on the methacrylate-based polymer poly (GM-co-TT) and the amine groups of the enzyme. This reaction was carried out under basic conditions, allowing the formation of stable secondary amine linkages. To begin the process, lipase was dissolved in a 0.2 M KCl–NaOH buffer solution at pH 11 to a final concentration of 10 mg/mL. The enzyme solution was then

mixed with the synthesized polymer. The immobilization reaction was allowed to proceed at room temperature for 120 minutes. To evaluate the effect of the polymer-to-enzyme ratio on immobilization efficiency, various mass ratios of poly(GM-co-TT) to lipase (1:1, 1:2, and 2:1) were tested. The final material obtained from this process is referred to as lipase immobilized on poly(GM-co-TT), as illustrated in the proposed reaction scheme shown in Figure 2.

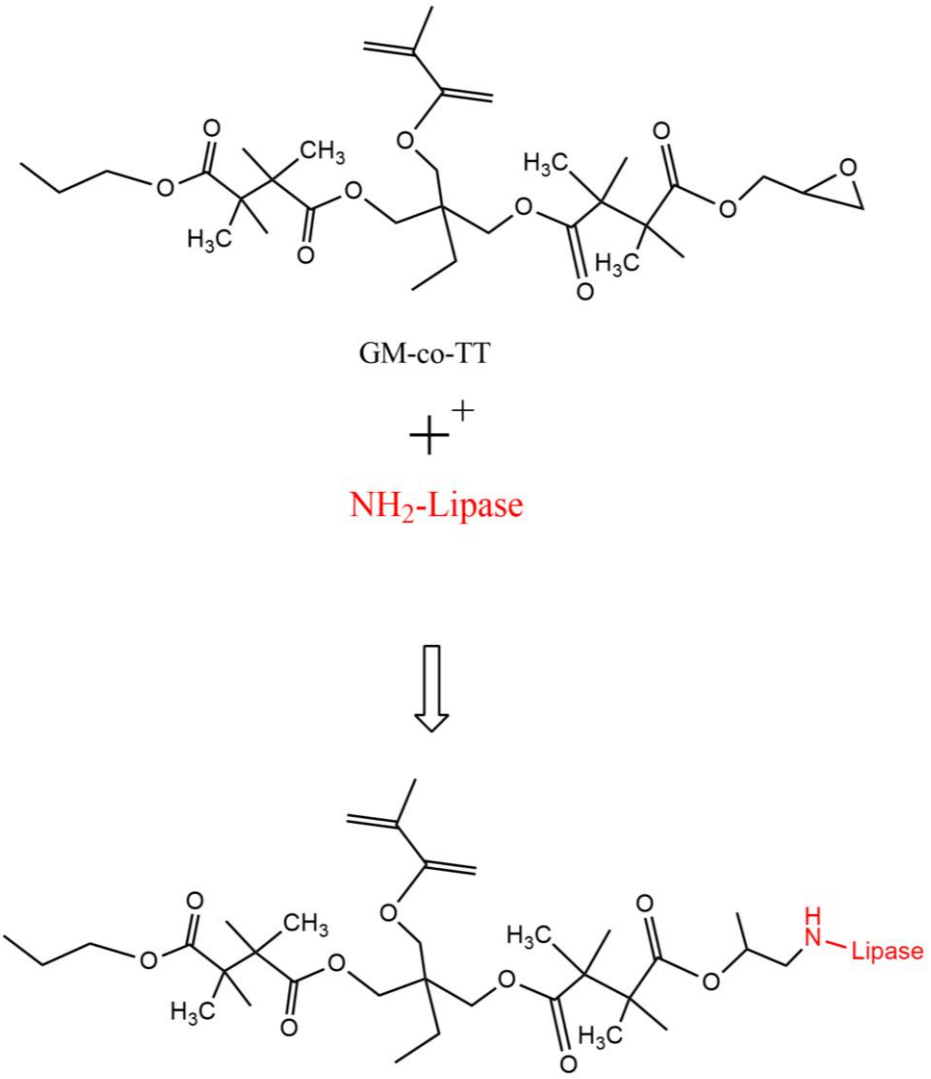

Figure 2 illustrates the proposed reaction scheme for the immobilization of lipase onto poly(GM-co-TT).

Characterization of Polymer Catalyst

To evaluate the immobilization of lipase onto the poly (GM-co-TT) support, several analytical techniques were used to assess the success of chemical bonding, surface morphology, pore structure, and the basic properties of the resulting catalyst. First, the presence and interaction of functional groups in the polymer structure were analyzed using Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR), performed on a Shimadzu IR Spirit-T instrument over a wavenumber range of 7800–350 cm^{-1}. This analysis aimed to confirm the formation of covalent bonds between the enzyme and the epoxy-functionalized polymer. Next, the surface morphology and microstructure of the catalyst were observed using a field emission scanning electron microscope (FESEM), model FEI Quanta FEG 650. This technique provided detailed images to examine the porosity and surface features of the polymer before and after enzyme immobilization.

RESULTS AND DISCUSSION

Chemical Characterization of the Polymer and Lipase Immobilization Based on FTIR Analysis

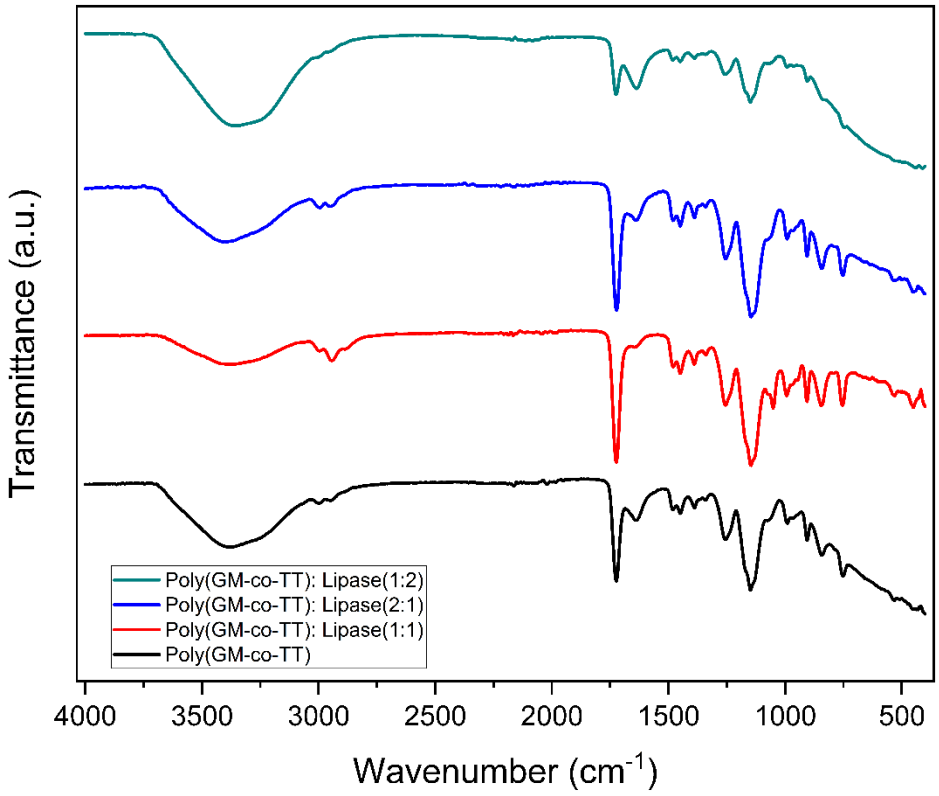

Figure 3. FTIR spectrum of Poly (GM-co-TT), Poly (GM-co-TT) immobilized Lipase (1:1; 1:2; 2:1)

Figure 3 presents the FTIR spectra of the poly (GM-co-TT) polymer before and after lipase immobilization, using various polymer-to-enzyme mass ratios of (1:1), (1:2), and (2:1). These spectra display noticeable changes in major absorption bands, indicating chemical modifications on the polymer surface due to interactions between the enzyme and the functional groups of the support matrix during immobilization.

The FTIR spectrum of poly(GM-co-TT) prior to immobilization exhibits a prominent absorption band near 1720 cm^{-1}, corresponding to the stretching vibration of ester carbonyl (C=O) groups. This confirms the formation of a polyester network resulting from the copolymerization of glycidyl methacrylate (GM) and ethylene glycol dimethacrylate (EGD). Furthermore, characteristic ether (C–O–C) stretching vibrations were detected in the 1140–1250 cm^{-1} region, indicating the formation of ester linkages that contribute to the three-dimensional polymer structure. These spectral features are consistent with previous reports on methacrylate-based porous polymers (Amalia et al., 2021; Septiana et al., 2018), thereby validating the reliability of the synthesis approach.

In addition, distinct absorption bands at approximately 910 cm^{-1} and 840 cm^{-1} were observed, corresponding to symmetric and asymmetric stretching vibrations of epoxy (oxirane) rings. This indicates the presence of reactive functional groups, making the polymer suitable for enzyme immobilization via covalent coupling. This observation aligns with findings from

Hasanah et al., (2020); Sabarudin et al., (2021); and Xie & Huang, (2020), who also reported epoxy-rich methacrylate polymers effective in biomolecule attachment.

Following immobilization, significant spectral changes were detected. Notably, the intensity of the epoxy bands at 910 cm^{-1} and 840 cm^{-1} decreased, indicating the reaction of epoxy groups with nucleophilic amine groups from the enzyme to form covalent secondary amine linkages. Moreover, a new or enhanced band around 1640–1660 cm^{-1} appeared, associated with the peptide backbone of the immobilized enzyme. A broad absorption near 3300–3400 cm^{-1} was also observed, suggesting N–H or O–H stretching from protein residues.

These spectral transitions strongly support the successful covalent immobilization of lipase. The reduction in epoxy signals, alongside the emergence of protein-specific bands, is in agreement with the spectral changes reported by Amalia et al., (2021) and Xie & Huang, (2020)Thus, this confirms that the synthesized poly(GM-co-TT) polymer contains appropriate chemical functionalities for stable enzyme anchoring. The spectroscopic evidence, supported by multiple studies, affirms both the structural integrity of the polymer and the covalent nature of the enzyme immobilization process.

Physical Characterization of the Polymer Based on Porous Structure

Figure 4. Photographic images of poly(GM-co-EGD) polymers after lipase immobilization at different polymer-to-enzyme ratios: (a) 1:1, (b) 1:2, (c) 2:1.

Figure 4. shows the physical appearance of the synthesized poly (GM-co-TT) polymer with and without porogen. The sample prepared with porogenic solvents (1,4-butanediol, 1-propanol, and water) appears as an opaque white solid with a visibly porous and rough surface texture. This visual evidence supports the assumption that pore structures were successfully formed during the polymerization process. The porogenic solvents play a critical role by inducing phase separation, forming microvoids that remain after the solvents are removed. In contrast, the polymer synthesized without porogens appears transparent and compact, with a smooth and dense morphology—indicating a non-porous internal structure due to the homogeneous polymerization environment. These observations are consistent with previous reports by Amalia et al., (2021) and Septiana et al., (2018), which showed that ternary solvent systems enhance porosity and produce a globular, interconnected structure suitable for biomolecule immobilization. This confirms that the approach used in this study effectively generated porous materials with potential for enzyme support applications.

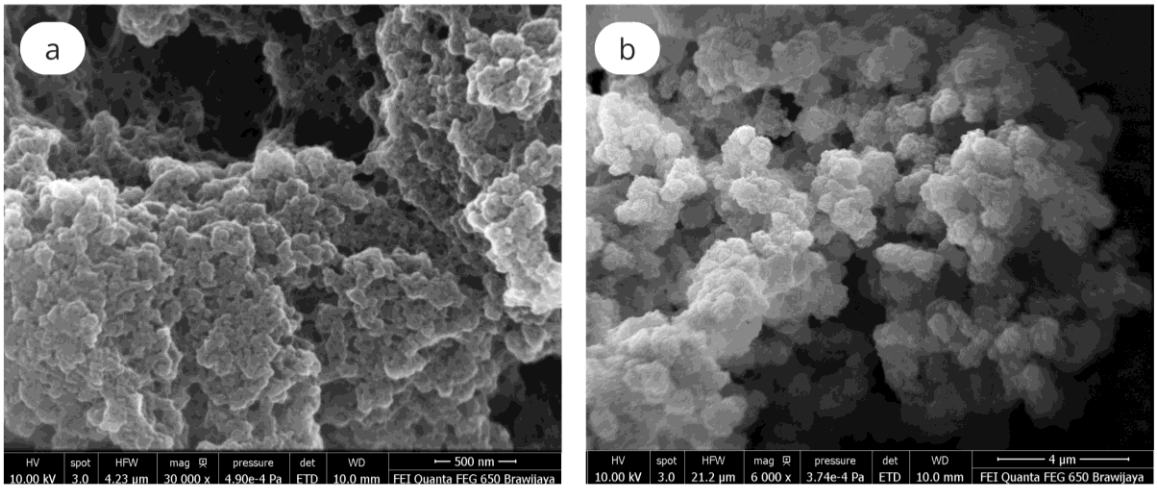

Figure 5. SEM images of a) Poly(GM-co-TT) and b) Lipase immobilized poly(GM-co-TT.

Figure 5. presents the surface morphology of the porous poly (GM-co-TT) polymer observed via Scanning Electron Microscopy (SEM). The image clearly reveals an interconnected globular network, with well-distributed pores of varying sizes, characteristic of polymers synthesized in the presence of porogens. The open and rough surface structure offers increased surface area, which is essential for efficient enzyme anchoring. Such morphology aligns well with earlier SEM studies by Amalia et al., (2021) dan Hasanah et al., (2020), who demonstrated similar microstructures in GMA-based polymers with porogenic phase systems. The uniformity and continuity of the pores also indicate good phase separation during polymerization, contributing to the mechanical robustness and diffusion performance of the material. Overall, the integration of porogenic solvents into the synthesis of poly (GM-co-TT) successfully results in a porous structure, both visually and microscopically. This physical characterization not only supports the FTIR findings but also reinforces the material's suitability for enzyme immobilization through enhanced mass transfer and active surface interaction.

CONCLUSION

This study demonstrated that a porous methacrylate-based polymer synthesized from glycidyl methacrylate and trimethylolpropane trimethacrylate, using a ternary porogenic solvent system, can serve as an effective support for lipase immobilization. The findings confirm that the designed poly(GM-co-TT) matrix possesses the essential structural and chemical characteristics—such as well-distributed microglobular porosity and reactive epoxy groups—needed to ensure stable and efficient enzyme attachment.

The impact of this study lies in advancing the design of polymeric immobilization supports that enhance enzyme stability and mass transfer, thereby offering significant potential for application in sustainable biocatalytic processes.

The novelty of the findings is reflected in the successful utilization of a ternary porogen system (1,4-butanediol/1-propanol/water) to produce a homogeneously interconnected porous structure in poly(GM-co-TT), which has not been widely explored for lipase immobilization. This structural innovation contributes to improved catalytic performance and operational stability of the immobilized enzyme, establishing a new approach for the development of robust enzyme-based microreactors.

RECOMMENDATIONS

Future studies are encouraged to explore the catalytic performance of the immobilized lipase using the synthesized poly (GM-co-TT) support in specific reaction systems such as transesterification or hydrolysis. Optimization of immobilization conditions—such as pH, temperature, and enzyme loading—should also be considered to enhance the enzyme's activity and reusability.

One potential obstacle encountered in this study is the limited control over pore size distribution, which may influence the diffusion of substrates and enzymes. Therefore, incorporating advanced porogen systems or templating agents may improve structural uniformity in subsequent research. Furthermore, complementary analyses such as surface area measurement (BET) and thermal stability testing (TGA/DSC) are recommended to better understand the physicochemical performance of the polymer matrix in various operational environments.

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