Chirality has become a major theme in the design, discovery, development and marketing of new drugs. The role of chirality in efficacy and safety of drugs has been thoroughly identified and implicated globally by pharmaceutical industries as well as concerned regulatory agencies. Thus, in order to accomplish efficacious and safe medication and also compliance with the guidelines set by the regulatory agencies, pharmaceutical industries are compelled to move towards manufacturing and marketing of chiral drugs essentially in single-enantiomer dosage forms. The worldwide sales of chiral drugs in single-enantiomer dosage forms continued growing at about 13% annual rate over past few years. To respond the rising industrial demand of enantiopure drugs, search of new efficient methods of asymmetric synthesis and the strategic development of the available methods have been the center-stage of academic as well as industrial pharmaceutical research over the recent years.

Employing enzymes (biocatalysts) for enantioselective organic synthesis in last decade, proved to be a valuable alternative to conventional chemical methods. Enzymes quite often display high chemo-, regio-, and enantioselectivity, which make them attractive catalysts especially for pharmaceuticals, where the demand for enantiomerically pure molecules is continuously rising. Enzyme-catalyzed reactions are carried out generally under mild conditions (with respect to temperature, pressure and pH) that minimize problems like isomerization, racemization or epimerization of product. The biocatalytic processes are less hazardous, polluting, proceed with low energy consumption than conventional chemo-catalytic processes, especially those
making use of heavy-metal catalysts. Enzymes being efficient catalysts are capable of increasing reaction rates up to $10^{12}$ times. Moreover, Use of heterogeneous biocatalysts (immobilized enzyme or cross-linked enzymes) offer several advantages such as improved stability, ease of catalyst separation from the reaction mixture, repeated use in continuous processes, enabling greater control over catalytic processes and process economics.

The biocatalytic enantioselective synthesis of highly versatile chiral entities having prospective as drugs or drug intermediates is attempted in the present thesis. The work was undertaken with following research objectives: (i) To study enantioselective synthesis of unnatural amino acids (namely: phenylglycine, 3,4-dihydroxy phenylalanine, homophenylalanine and 2-naphthylalanine) using immobilized enzymes (viz. lipases, amidase and aminoacylase) and (ii) To study preparative scale enantioselective synthesis of vicinal diols (namely: phenylethane diol and $m$-chloro-phenylethane diol) using immobilized Solanum tuberosum epoxide hydrolase. The present thesis comprises eight chapters. A brief outline of each chapter is given:

**Chapter 1: Introduction**

This chapter outlines an overview on topics regarding importance of chirality in drugs, role of enzymes in chiral separation, development of heterogeneous biocatalysts, unnatural amino acids and vicinal diols as drug intermediates which will be followed by the scope of the Thesis and specific research objectives.

**Chapter 2: Enantioselective synthesis of unnatural amino acids using covalently immobilized lipase on porous beaded polymers**

Five commercial lipases from different sources were screened for chiral resolution of unnatural amino acid esters. The Candida rugosa lipase (CRL) and porcine pancreatic lipase (PPL) were immobilized on epoxy activated functional polymers. More than 50 functional polymers of different monomer-cross-linking agent compositions were screened for lipase immobilization. The effect of cross-link density and porogen on lipase immobilization was evaluated. The acrylic functional polymers containing allyl glycidyl ether (AGE) monomer units synthesized by using lauryl alcohol as a porogen, gave higher lipase binding and therefore thoroughly analyzed for their catalytic performance, stability and reusability. Under the optimum
conditions, AGE-(L)-100 gave 96.64% activity recovery for CRL binding and 74.35% activity recovery for PPL binding. The immobilized CRL and immobilized PPL were employed for the kinetic resolution of unnatural amino acid ethyl esters.

Chapter 3: Chiral resolution of unnatural amino acid esters using immobilized lipase in membrane bioreactor

The *Candida rugosa* lipase (CRL) and porcine pancreatic lipase (PPL) were immobilized on poly(urethane methacrylate -co- glycidyl methacrylate)-supported-polypropylene biphasic membrane. A polypropylene membrane was hydrophilized by coating followed by UV curing of a blend of 2-hydroxyethyl methacrylate terminated polyurethane prepolymer and glycidyl methacrylate. This allows formation of a hydrophobic membrane with increased surface hydrophilicity, biocompatibility and stability. Immobilized membranes were treated with 5% glutaraldehyde as a cross-linking agent for post immobilization stabilization of enzyme on membrane. Under the optimum conditions, the biocatalytic membranes retained >90% of initial lipase activity. The biocatalytic membrane was characterized for its catalytic performance, stability and reusability. The immobilized membranes were placed in membrane reactor where enantioselective synthesis of unnatural amino acids was studied.

Chapter 4: Chiral resolution of unnatural amino acid amides using immobilized resting cells of *Rhodococcus erythropolis* MTCC 1526

Statistical experimental methodology was used to enhance the production of amidase from *Rhodococcus erythropolis* MTCC 1526. *R. erythropolis* MTCC 1526 was selected through screening of seven strains of *Rhodococcus* species. The Placket–Burman screening experiments suggested that carbon source (sorbitol), nitrogen sources (yeast extract and meat peptone) and amidase inducer (acetamide) are the most influential media components. The concentrations of these four media components were optimized using face centered design of Response Surface Method (RSM). Use of RSM increased the production of amidase from *R. erythropolis* MTCC 1526 by 6.88 fold. The cells of *R. erythropolis* MTCC 1526 having enhanced amidase activity were immobilized by different entrapment methods. The immobilized cells of *R. erythropolis* MTCC 1526 were used for chiral resolution of unnatural amino acid amides.
Chapter 5: Use of immobilized *Aspergillus melleus* aminoacylase for enantioselective synthesis of unnatural amino acids

Macroporous functional polymers containing surface epoxy groups were synthesized for immobilization of *Aspergillus melleus* aminoacylase. The effect of cross-link density of polymer on enzyme immobilization was studied. The novel styrenated acrylic ter-polymers gave maximum aminoacylase activity recovery (75.47%). Immobilized polymers were characterized for pH, temperature and storage stability. Immobilization of aminoacylase on styrenated ter-polymers gave excellent thermal stability to the enzyme. A kinetic model of thermal inactivation was derived to quantify the extent of thermal stability conferred to aminoacylase by immobilization. Immobilized aminoacylase catalyzed enantioselective synthesis of unnatural amino acids was studied.

Chapter 6: Preparation of cross-linked enzyme aggregates of *Aspergillus melleus* aminoacylase for enantioselective synthesis of unnatural amino acids

The cross-linked enzyme aggregates (CLEA) of aminoacylase were prepared via co-aggregation of the enzyme with polyethyleneimine (PEI). The PEI-enzyme co-aggregates were stabilized by cross-linking between primary amino groups of the PEI and the primary amino groups of enzyme using glutaraldehyde. The method described gave physically stable CLEAs and no release of enzyme was found upon prolonged storage. The process parameters such as PEI:enzyme ratio, glutaraldehyde concentration and time of glutaraldehyde treatment necessary to form stable CLEA were optimized. Under the optimum conditions, PEI-aminoacylase CLEA expressed 74.90% activity recovery with 81.20% aggregation yield. The thermal inactivation kinetics of soluble enzyme and PEI-aminoacylase CLEA was studied. The results suggest that the co-aggregation gave excellent thermal stability to the enzyme. Finally, PEI-aminoacylase CLEA were employed for synthesis of enantiopure unnatural amino acids.

Chapter 7: Preparative scale enantioselective synthesis of vicinal diols using immobilized *Solanum tuberosum* epoxide hydrolase

The recombinant plasmid (pGEF-StEH) containing functional gene of *Solanum tuberosum* epoxide hydrolase was inserted in *Escheria coli* BL21(DE3) strain. The recombinant *E. coli* cells were grown in LB medium at 37°C for about 16
h in a 5L fermenter. The extracellular enzyme was isolated from the fermentation broth. The enzyme was immobilized by multipoint covalent attachment on glyoxyl-agarose support. Immobilized enzyme gave ~ 150 units epoxide hydrolase activity per gram of support. Further, the immobilized enzyme was characterized with respect to pH stability, temperature stability and miscible/immiscible solvent stability. The immobilized epoxide hydrolase was used for enantioselective production of two vicinal diols (namely: phenylethane diol and m-chloro-phenylethane diol) via hydrolysis of their corresponding epoxides (namely: styrene oxide and m-chloro-styrene oxide respectively). The effect of incorporation of ionic liquids and organic solvents in aqueous reaction medium on enzymatic hydrolysis of epoxides was studied. The ‘Regio-selectivity constants’ for enzymatic hydrolysis of styrene oxide and m-chloro-styrene oxide were calculated. The preparative scale production of (R)-phenylethane diol and (R)-m-chloro-phenylethane diol (on gram scale) was studied using stirred cell bioreactor. The bioreactor performance was evaluated over 10 repeated cycles for production of each diol.

Chapter 8: Conclusions
This chapter recapitulates the significant findings of the present work and delineates the concluding remarks.

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