ChemSusChem

Energy & Materials

Reprint

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ChemPubSoc Europe

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Walk around green approaches: Ecofriendly and efficient synthetic approaches involving chemoenzymatic methodologies based on the principles of “green chemistry” for waste reduction are discussed. These lead to the formation of unique nanomaterials and biomaterials for diverse applications, such as drug/gene delivery systems, flame retardant materials, conducting polymers, controlled release systems, diagnostic agents, and polymeric electrolytes for nanocrystalline solar cells.
Novozym 435-Catalyzed Syntheses of Polyesters and Polyamides of Medicinal and Industrial Relevance

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Dedicated to Professor Goverdhan Mehta, FRS on the occasion of his 70th birthday.

The adverse impact of chemical and biochemical waste on the environment and human health poses a serious challenge in today’s World. The best way to address these challenges is to reduce the waste by developing more efficient processes and technologies, based on the principles of “green chemistry”. Some of these synthetic approaches involving the chemoenzymatic synthetic methodologies are discussed herein. These lead to the formation of unique nanomaterials with diverse applications, such as drugs/gene delivery systems, flame retardant materials, conducting polymers, controlled release systems, diagnostic agents, and polymeric electrolytes for nanocrystalline solar cells.

1. Introduction

Polymeric nanoassemblies, such as micelles of various morphologies, toroidal assembled polymersomes, nanofibers, and nanoscale tubes have attracted considerable attention in recent years. These self-organized materials ranging from the nanoscale to the micro scale have found broad applications in areas such as bioengineering, biomedicine, cosmetics, materials science, and pharmaceutics.[1–6] Among these diverse applications, nanostructured polymeric assemblies for drug delivery and gene therapy are of special interest.[7, 8] Several formulations based on polymeric micelles have been extensively studied for cancer therapy and their efficacy has been well demonstrated.[9] Recent advances have made the delivery of therapeutic agents such as small molecules, peptides, proteins, plasmid DNAs and siRNAs possible in aqueous media.[10, 11] Furthermore, nanoparticle formation and their size can be controlled in an environment-friendly manner.[12] Also considering the chemical, economic, and social advantages of biocatalysis over traditional chemical approaches, biotechnology holds tremendous opportunities for realizing functional polymeric materials. Biocatalytic pathways to polymeric materials are an emerging research area with not only enormous scientific and technological promise but also with a tremendous impact on environmental issues. In recent years, some interesting reviews[13, 14] and books[15] have been published that give a useful introduction to the field of enzymatic polymerization. Among the enzymes used successfully for polymer synthesis, Candida antarctica lipase B (CAL-B) is by far the most well-known enzyme in literature. In most of the enzymatic polymerizations reported, it is used as an immobilized enzyme because of additional advantages, for example, ease of separation and its robust nature. Novozym 435 is a commercially available heterogeneous biocatalyst that consists of CAL-B physically immobilized within a macroporous resin of poly(methyl methacrylate) and is marketed by Novozymes.[16] Owing to its in vitro transesterification potential, Novozym 435 has been extensively utilized for synthesizing polyester architectures. In this Minireview, we have summarized some of the most useful enzymatic polymerization reactions forming amphiphilic polyesters and polyamides for various applications.

2. Nanomaterials for Drug and Gene Delivery

The search for new drug-delivery approaches and new modes of action are the major driving forces in polymer therapeutics. Pharmaceutical and biotech startup companies are engaged in the development of drug-delivery systems (DDSs) for new as well as already existing drugs.[17] Targeted and controlled DDSs ensure the commercial success of these bioactive molecules in terms of stability, absorption, easy metabolic inactivation, and need to cross cell and nuclear membranes to reach intracellular targets. Polymers that can self-assemble into micellar nanoparticles can be effectively used as vehicles for drug delivery.[17] Our interest has been in synthesizing amphiphilic polyesters and polyamides that aggregate in aqueous media and thus form nanospheric particles, the surface of which results in a nonimmunogenic response; therefore, these nanospheres
can be used as carriers for drug delivery. Initially we attempted to synthesize polyesters based on dimethyl 5-hydroxyisophthalate and polyethylene glycol (PEG) moieties through transesterification–polycondensation reactions using dibutyltin diacetate as catalyst (Scheme 1).[16]

According to control experiments, a C-5 hydroxyl moiety inhibits the reaction and its protection became necessary. However, prior protection of the phenolic moiety restricts the freedom of post polymerization modification. Also very harsh reaction conditions (330 °C, under vacuum, toxic tin-based catalyst) had to be adopted; dark colored oligomers were obtained that were contaminated with the tin catalyst, which was difficult to remove completely.

Our experience in using lipases to achieve chemo-, regio-, and enantioselectivity on a variety of substrates[19–26] encouraged us to develop an enzymatic route for the synthesis of polymers. We have successfully used Novozym 435 to synthesize copolymers of dimethyl 5-hydroxyisophthalate and PEGs of different sizes through transesterification (Scheme 2) under solventless conditions.[27] This greener approach has an added advantage of post-polymerization modification (Scheme 2) as the enzymatic polymerization is feasible without protecting the hydroxy moiety (C-5 OH) of the isophthalate moiety. Thus, polymers obtained by this biocatalytic method can be used for the delivery of drugs, proteins, or polysaccharides as these can be easily attached or en-

![Scheme 1. Copolymerization of PEG and dimethyl 5-hydroxyisophthalate.](image)

**Scheme 2.** Chemoenzymatic copolymerization of PEG and C-5-substituted isophthalates.

![Scheme 2.](image)

**Figure 1.** Functionalized PEG–(5-hydroxyisophthalate) copolymers.

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lation and delivery of equal doses of aspirin and naproxen have improved drug efficacy by a factor of 1.8–2.0 compared to their aqueous preparations (Figure 3). An encapsulation study of a series of aromatic guests with varied electron donor/acceptor properties demonstrated that electronic complementarity between polymer and encapsulated drug contributed significantly towards encapsulation. The effect of hydrophilic block, PEG length, linker, concentration, and temperature on these nanomicellar structures and interactions by static light scattering techniques has been studied. In addition, amphiphilic polymers (5) were synthesized by copolymerization of 5-aminoisophthalate/5-hydroxyisophthalate and PEG followed by conjugation of acyl chains as amide/ester linkages to the 5-NH$_2$/5-OH moieties, respectively (Figure 4).

2.1. Bio-catalytic synthesis of pluronic- and guanidine-based polymers

A poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO–PEO) tri-block copolymer family of pluronics with different numbers of hydrophilic and hydrophobic units were synthesized and characterized by us. Polymers 6 and 7 (Figure 5) could be used to encapsulate hydrophobic drugs, for example curcumin, in the range of 2.7–5.7% with regard to polymer weight, thus enhancing its aqueous solubility. Results from in vitro and animal studies suggested that curcumin has antitumoral, antioxidant, antiarthritic, antiamyloid, anti-ischemic, and anti-inflammatory properties. However, its low aqueous solubility delays its potential use. Thus, our study may be useful in enhancing the bio-availability of curcumin.

Arginine-based compounds have been reported to enhance cellular permeability and interaction with nucleic acids caused by the presence of a guanidine moiety. We have developed a guanidine-based polymeric system 8 (Scheme 3) and such systems may serve as gene/small interfering ribonucleic acid (siRNA) delivery vehicles.

2.2. Biocatalytic synthesis of nonproteinogenic amino acid/amino acid diesters and PEG-based copolymers

The high cellular permeability and chirality of amino acids prompted us to use them as building blocks for polymer synthesis. We have performed the copolymerization of nonproteinogenic amino dicarboxylic acids with PEG ($M_n$ = 600) in bulk. The free amino moiety of the copolymers was then functionalized by using acyl chloride, resulting in the formation of the amphiphilic polymers 9$^{[37]}$ and 10$^{[38]}$ (Figure 6).
2.3. Biocatalytic synthesis of glycerol-PEG co-polymers and polyglycerol esters

Glycerol exhibits good chemical stability and inertness under biological conditions.\[39\] Our initial efforts to utilize glycerol resulted in a highly efficient chemoenzymatic approach to synthesize aliphatic polyester/polyamide ester dendritic building blocks by using structured triacyl glycerol (TAG); this approach could be successfully applied to a variety of cores (Scheme 4). The synthesis of a unique class of structured TAG-based star-shaped and linear dendritic building blocks was achieved by using Novozym 435.\[40\] The carboxylic moiety of the TAG esters 12 and 13 was used to couple the esters with hydroxyl/amine functionalities of various core moieties (Scheme 5).

Following this, we subsequently developed a biocatalytic method to synthesize polymeric systems using glycerol and PEG dimethyl ester\[41\] regioselectively through reaction of the primary hydroxyl moieties of glycerol, thus leaving the secondary hydroxyl moieties available for post-polymerization chemical modifications through attachment of alkyl chains simply by acylation (Scheme 6). The amphiphilic polymers 18a–c aggregated in aqueous media to form nanosized particles. We successfully attempted the encapsulation of vitamin E in these amphiphilic polymers (18a–c) up to 22% with regard to polymer weight. This study shows promise in enhancing the bioavailability of vitamin E, a well-known lipophilic antioxidant; its limited aqueous solubility severely hampers its antioxidant efficiency.

Recently, a new class of non-ionic dendronized multiamphiphilic polymers have also been prepared by us starting from a biodegradable (AB)n-type diblock polymer synthesized from 2-azido-1,3-propanediol (azido glycerol) and polyethylene glycol (PEG-600) diethyl ester using Novozym 435 as a biocatalyst (Scheme 7). These polymers are functionalized with dendritic polyglycerols (G1 and G2) and octadecyl chains at different functionalization levels through click chemistry.\[42\] Surface tension measurements and dynamic light scattering studies revealed that all of the multiamphiphilic polymers spontaneously self-assemble in aqueous solution. Cryogenic transmission electron microscopy further proves the formation of multiamphiphiles as monodisperse spherical micelles of about 7–9 nm in diameter. The evidence from UV/Vis and fluorescence spectroscopy suggests the effective solubilization of hydrophobic guests such as pyrene and 1-anilinonaphthalene-8-sulfonic acid within the hydrophobic core of the micelles.\[42\]

We have also explored the chemoenzymatic modification on dendritic hyperbranched polyglycerol (dPG) that led to amphiphilic polymeric architectures with easily hydrolysable ester
The Novozym 435-catalyzed pegylation of dPG occurred regioselectively at the primary hydroxyl moieties in the presence of secondary hydroxyl moieties (Figure 7). The remaining hydroxyl moieties were acylated to obtain amphiphilic dPG architectures. These architectures were studied for Nile Red solubilization, which showed a capacity of up to 5.6 mg L\(^{-1}\) at 0.1 wt% polymer concentration. The release of Nile Red from these polymers was observed with a half-life of 8 h at pH 5.0, where-as no release was found at pH 7.4. The cell viability studies of our polymeric architectures showed them to be relatively nontoxic.\[^{[43]}\]

We have developed a highly efficient temperature-dependent chemoenzymatic methodology for the regioselective synthesis of glycerol esters, G1 triglycerol dendrons and related esters for the first time using 4-nitrophenyl 2-[(tert-butoxycarbonyl) acetate (Boc-gly-Ph-pNO\(_2\)) as the acylating agent. The immobilized lipase Lipozyme TL IM in dioxane was the most efficient biocatalyst for the regioselective transesterification on glycerol and afforded the mono- and di-esterified products 29 and 30 (Figure 8).\[^{[44]}\] The regioselectivity achieved in case of glycerol was then extended to bifunctional G1 glycerol dendrons bearing four hydroxyl moieties (two primary and two secondary hydroxyl moieties). It was demonstrated that glycine loading occurred selectively on the primary hydroxyl moieties and that the secondary hydroxyl moieties remained unaffected (Figure 8).\[^{[44]}\]

Encouraged by these results, multivalent polyglycerol-dendron-based amphiphiles with well-defined molecular structures expressing controlled glycine arrays on their surfaces were synthesized. The structure–activity relationships with respect to siRNA/DNA complexation, toxicity, and transfection profiles with the synthesized polycations were recorded. A second-generation amphiphilic dendrimer (G2-octaamine) with eight amine moieties on its surface and a hydrophobic C\(_{18}\) alkyl chain at the core acted as an efficient vector to deliver siRNA inside the cell and achieved potent gene silencing as demonstrated by the knockdown of normalized luciferase activity and also for glyceraldehyde 3-phosphate dehydrogenase in HeLa cells. The amphiphilic vector is nontoxic even at a higher ratio of N/P 100 both in vitro and in vivo.\[^{[45]}\]
2.4. Synthesis of amphiphilic polymers for magnetic resonance imaging and selective targeting in cancer therapy

The use of fluorescent dyes and perfluoro compounds for imaging and selective targeting is well known. To enhance the chemotherapeutic efficiency and selectivity for binding, we have attached a peptide to polymer 35 through a hydrophilic linker (triethylene glycol). Subsequently, fluorescent dyes, a perfluoroalkyl moiety (for FN MR imaging), and a hydrophobic side chain were attached by performing simple chemical modifications (Figure 9). Initial in vitro studies indicate cellular uptake of these nanocarriers; radioactive labeling and analysis have shown some selectivity for targeted (pancreatic) cancer cells over non-targeted cells.\(^{[46]}\)

Perfluorinated amphiphilic polymers were also synthesized by using a chemoenzymatic methodology (Scheme 8). The supramolecular organization of polymer 36 in aqueous and organic media was studied and observed to form nanomicelles (in the range of 50–60 nm at 25 °C) in aqueous media; however, no micellization occurred in organic media.\(^{[47]}\)

Recently, similar carrier molecules 37a–c and 38a–c were synthesized and studied for multiple applications, including drug encapsulation, drug delivery, and disease diagnosis (imaging) (Figure 10). These polymers showed 4–14% curcumin encapsulation in water.\(^{[48]}\)

2.5. Biocatalytic route to sugar-PEG-based polymers for drug delivery

Sugar-PEG-based polymers were synthesized by enzymatic copolymerization of 4-C-hydroxymethyl-1,2-O-isopropylidene-β-L-threo-pentofuranose/4-C-hydroxymethyl-1,2-O-benzylidene-β-L-threo-pentofuranose/4-C-hydroxymethyl-1,2-O-isopropylidene-3-O-penty1-β-L-threo-pentofuranose with PEG-600 dimethyl ester using Novozym 435 (Figure 11).

Results of aggregation studies on the copolymers revealed that in aqueous solution, those polymers bearing a hydrophobic pentyl/benzylidene moiety (39–41) spontaneously self-assembled into supramolecular aggregates. The polymeric aggregates were further explored for their drug encapsulation properties in buffered aqueous solutions of pH 7.4 (37 °C) using Nile Red as a hydrophobic model compound by means of UV/Vis and fluorescence spectroscopy.\(^{[49]}\)
3. Biocatalytic Synthesis of PEGylated Curcumin Block Copolymers

Curcumin is known as one of the Nrf2 activators, which is a central transcription factor regulating the antioxidant defense system and acts as a modifier for several inflammatory diseases. Curcumin is used as a dietary supplement, but its hydrophobic nature renders it ineffective.

To circumvent these issues, PEG–curcumin copolymers were synthesized and evaluated as potent Nrf2 activators. Among the copolymers 42a–d, copolymer 42a predominantly activated Nrf2-driven antioxidant gene expression (Figure 12). This study opens a new path for enhancing the efficacy of various existing hydrophobic drugs through their PEGylation.

4. Biocatalytic Synthesis of PEGylated Coumarin Block Copolymers

Coumarins constitute an important group of natural products belonging to the flavonoid family. To enhance their bioavailability, potent antioxidant coumarins were encapsulated as well as covalently attached to base polymers. These new classes of polymeric materials have superior antioxidant properties in comparison to the starting monomers. We also synthesized coumarin-and-PEG-based block copolymers (Scheme 9). These PEGylated coumarin derivatives were evaluated for their anti-inflammatory activities with respect to their ability to inhibit the tumor necrosis factor
TNF-α induced intercellular cell adhesion molecule-1 (ICAM-1) expression on human endothelial cells. Both PEGylated 4-methyl- and 4,8-dimethylcoumarins have shown improved ability to inhibit the TNF-α induced ICAM-1 expression in comparison to the corresponding monomers. Coumarin derivatives have also been extensively investigated for electronic and photonic applications. We have used coumarins for sensor (fluorescence-quenching sensors) applications, that is, detection of nitro aromatics/explosive materials. However, coumarins themselves are not suitable for the preparation of solid devices because of their low molecular weight and aggregation in solid thin films, which reduces the fluorescence quantum yield. To overcome this problem, we have copolymerized the diester of 4,8-dimethylcoumarin with PEG and polydimethylsiloxane (PDMS; Figure 13). The obtained polymers 44 and 45 have good solubility in a wide range of solvents, thus making them suitable candidates for thin film fabrication.

5. Biocatalytically Generated Polysiloxane-Based Copolymers and their Nanocomposites as Flame Retardants

Flame retardants (FRs) comprise a diverse group of chemicals widely used at relatively high concentrations and have many applications, including in the manufacture of electronic equipment, textiles, plastics, and polymers as well as in the aviation and automobile industries. Owing to environmental concerns in the synthesis of nanocomposites and polymer/layered silicate composites, we used biocatalysts to synthesize FR materials. 5-Hydroxy- and 5-aminoisopthalates were polymerized with siloxane and evaluated for FR properties (Scheme 10). In addition to this, siloxane-based aliphatic polyamides were also synthesized (Scheme 11). The flammability of copolymers 46a and b is comparable to that of Kevlar or polyether ether ketone (PEEK), two commercial products of DuPont. Polymers 47b and c may be used for applications that require ultra-fire-safe polymers, as the heat-release capacity of these polymers is under 100 J g⁻¹ K⁻¹.

Furthermore, we have prepared composites of titanium dioxide (TiO2) nanoparticles and biocatalytically synthesized dimethyl siloxane co-polyamides and co-polysterers, and evaluated their thermal and FR properties. A number of other siloxane polymers, siloxane copolyimide (siloxime E) 48 (Scheme 12) and siloxane co-polyamides, with coumarin in the backbone were also synthesized (Figure 11) for FR applications.
In another study, cross-linking of enzymatically synthesized polydimethyl siloxane copolymers with aromatic dianhydrides yielded the cross-linked polymer 51 as cross-linked FR (Scheme 13).

6. Polymeric Electrolytes for Nanocrystalline Solar Cells

Dye-sensitized solar cells (DSSCs) offer the advantage of significant reduction in the cost of production of solar electricity owing to the inexpensive raw materials and simple fabrication process involved in the production of DSSC-based solar modules. An alternative approach focused on the development of polymeric or quasi-solid matrices for efficient operation of already well-known redox electrolytes for DSSCs. The chain mobility and ionic conductivity of the polymer electrolyte can be increased by adding organic solvents and polymer gelling agents to the liquid electrolyte to promote its solidification.

6.1. Biocatalytic approach for preparing quasi-solid electrolyte systems for DSSCs and their photovoltaic performance

Quasi-solid electrolytes prepared from the biocatalytically synthesized polymers 52a–c (Scheme 14) by adding at least 25 wt% polymer to an ionic-liquid-based electrolyte showed a photovoltaic (PV) efficiency of around 4.3%; the measured ionic conductivity of the formulation used in these devices was approximately $2 \times 10^{-5}$ S cm$^{-1}$.[72] Studying the intensity-dependent PV efficiency suggested that the best PV performance was achieved at around 0.5 Sun (50 mW cm$^{-2}$) intensity level. An efficiency of over 4.6% was achieved by a polymer 52c-based gel-incorporated flexible cell at 55 mW cm$^{-2}$, which is about 10% higher than the 1 Sun condition.[72] The solar conversion efficiency of solar cells incorporating quasi-solid electrolytes depended strongly on the polymer's microstructure used in formulating the redox electrolyte. Continuing this work further, we synthesized new polymers and these polymeric materials...
showed photovoltaic efficiency of up to 9.0% in the laboratory.[72]

6.2. Biocatalytic synthesis and ion-transport properties of PEGylated polyphenolics

We used Novozym 435 to catalyze the highly chemoselective monoacylation of the alcoholic hydroxyl moiety of 4-hydroxymethylphenol with PEG diacid under solvent-less conditions (Scheme 15). The resulting acyloxy macromer 53 was then polymerized using horse radish peroxidase (HRP) to form the PEGylated poly(hydroxymethylphenol) 54.[74]

7. Miscellaneous Applications

Various amphiphilic copolymers were also synthesized that self-assemble into nanomicellar aggregates in aqueous media and were used for the encapsulation and controlled release of carbofuran, a systemic insecticide–nematicide (Figure 14).[75–77] The chemoselectivity shown by biocatalysts was also utilized in synthesizing serinol-based surfactants[78] avoiding the protection/deprotection chemistry.

8. Conclusions and Outlook

Use of enzymatic and chemoenzymatic methods for the preparation of polymeric structures has expanded rapidly in recent years as the commercial availability of enzymes has increased dramatically in the same time period. In addition to selectivity, factors such as energy reduction with lower temperature of reaction, reduction of toxic solvent use, and reuse of catalyst are additional advantages of enzymatic reactions. The stereo-, regio-, and chemoselectivity of enzymes observed in small-molecule reactions have also been observed in the synthesis of polymeric materials. But the applications of biocatalysis in polymer science still lag behind the use of biocatalysts in other areas.

Our efforts to exploit the array of lipases to develop new methodologies, reactions, and processes in polymer synthesis have led to the identification of Novozym 435 being the enzyme of choice, with a wide versatility for the synthesis of polyesters and polyamides. The successful and easy preparation of these polymers allows the application of our method to the easy synthesis of many functionalized polymeric materials. However, for biomedical applications, the challenge still remaining is the optimization of biocatalytic synthesis of amphiphilic polymers that form aggregates with sizes of 20–100 nm, show a high targeting potential, and can be cleared by the kidneys (molecular weight cut-off ~40 kDa). Furthermore, new chemoenzymatic approaches to prepare polyether architectures need to be addressed in the future; this may open new arenas to enhance the versatility of polyether-based amphiphilic scaffolds. Thus, significant research is still needed to expand the possibility of using enzymes to modify and tailor polymeric architectures to fit future demands of the biomedical and industrial sectors.

Acknowledgements

We thank all PhD students and postdoctoral fellows, in particular Dr. Rajesh Kumar (who was an instrumental initiator that led to the foundation of the work included in all areas in this manuscript), Dr. Mukesh Pandey, and Dr. Ravi Mosurkal for their great contributions, inspiring and dedicated hard-work in obtaining all
the results in our laboratories in the past thirteen years. We would also like to thank all colleagues at various institutions worldwide included in the publications/research papers mentioned in the “Reference Section”, for their active collaborations and contributions, in particular Dr. Lynne Samuelson and Dr. Ashok Cholli at the University of Massachusetts Lowell (UML, MA, USA), Professor Ashok Prasad at the University of Delhi (India), and Professor Dr. Rainer Haag at the Free University of Berlin (Germany). S.K.S. and V.S.P. thank the Department of Biotechnology (DBT, New Delhi, India), Department of Science and Technology (DST, New Delhi, India) and the University of Delhi for financial assistance under the DU-DST PURSE Scheme and DBT-CREST award to S.K.S. A.K. thanks the Council of Scientific and Industrial Research (CSIR, New Delhi, India) for the award of Junior and Senior Research Fellowships.

**Keywords:** biocatalysis - micelles - nanomaterials -photovoltaic cell - polymerization

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