

## Indo-U.S. collaborative studies on biocatalytic generation of novel molecular architectures\*

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*Abstract:* Biocatalytic polymerization reactions on a variety of substrates leading to value-added polymers have been summarized. The main focus of this review is on the control of molecular architecture during enzymatic polymerization reactions. Combined with chemical reactions, several extremely flexible chemo-enzymatic synthetic procedures are described to produce families of new polymeric materials with novel properties. The properties of the synthesized polymers and their applications in various fields, such as drug delivery and flame retardant materials, have also been studied and discussed.

### INTRODUCTION

Three years ago, the Departments of Chemistry at the Universities of Delhi and Massachusetts, Lowell developed a collaborative program in the area of biocatalytic synthesis of new materials. During this period, we exploited a variety of enzymes to synthesize polymeric materials of potential biomedical applications.

The ability to control molecular structure in reactions has been a goal of chemistry for a long time. Control of molecular architecture in polymerization reactions has been part of that expectation. Use of enzymatic and chemo-enzymatic 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. The stereo-, regio-, and chemoselectivity of enzymes observed in small molecule reactions have also been observed in the synthesis of polymeric materials [1,2]. Although the applications of biocatalysis in polymer science lag behind the use of biocatalysts in other areas, there is no shortage of opportunities either for fundamental research or for product development. In fact, materials found in nature, such as proteins, polysaccharides, and polynucleotides, are mostly polymers, and potentially countless enzymatic and microbial reactions are available to carry out the biotransformations. In the past 10 years, there has been a fair amount of research activity to develop new methodologies, reactions,

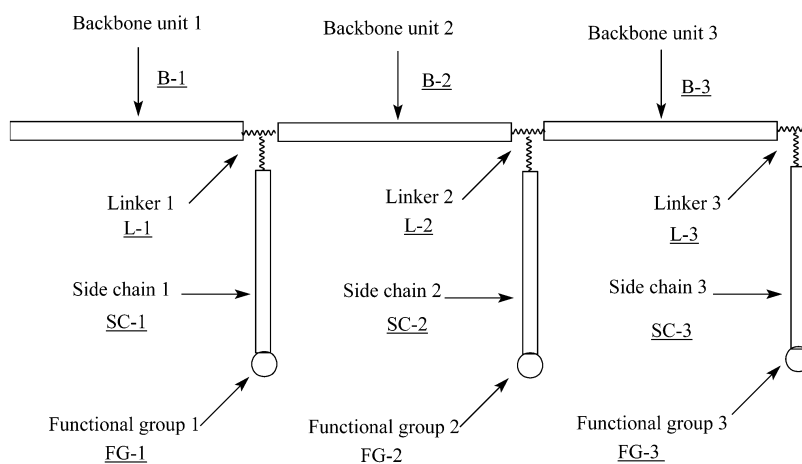
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and processes in order to exploit this exciting technology in the polymer synthesis area, both in academia as well as in industry [3]. With these advances, developments toward the generation of the diversity of enzymes and substrates have been of high interest in the scientific community.

Recently, we have developed an extremely flexible chemo-enzymatic synthetic methodology to prepare well-defined structures [4] illustrated in Fig. 1. This synthetic platform is designed to vary any of the parameters indicated in Fig. 1. The simplest of these variations would have all backbone units (Fig. 1, B-1 = B-2 = B-3, etc.), and linkers (Fig. 1, L-1 = L-2 = L-3, etc.) having the same structures. If the linker is bifunctional, the reaction is a standard condensation polymerization. In some cases, the same polymers may be prepared by chemical methods [5], although many of the advantages of enzymatic reactions, such as energy reduction with lower temperature of reaction, reduction of toxic solvent use, reuse of catalyst, are lost with these methods.



**Fig. 1** General schematic representation of polymer structure.

The enzymatic method, however, can be exceptionally useful where such reactions are not easily feasible via chemical methods. Based on this, we have synthesized a large number of polymers from a variety of starting materials.

## SYNTHESIS OF AMPHIPHILIC POLYMERS FOR DRUG DELIVERY APPLICATIONS

The importance of polymers in medical applications has been well recognized. They are most widely used as pharmaceutical carriers in drug delivery, and a considerable amount of research has been directed toward the use of natural and synthetic polymers as polymeric drugs and drug delivery systems. The low-molecular-weight pharmaceutical surfactants, which are currently used, have low toxicity and high solubilization power. But these suffer from the drawback that they have relatively high critical micelle concentration (CMC) and are unstable upon high dilution. However, by using amphiphilic copolymers, these drawbacks can be eliminated as they have very high solubilization power and rather low CMC value, thus making them more stable in vivo [6].

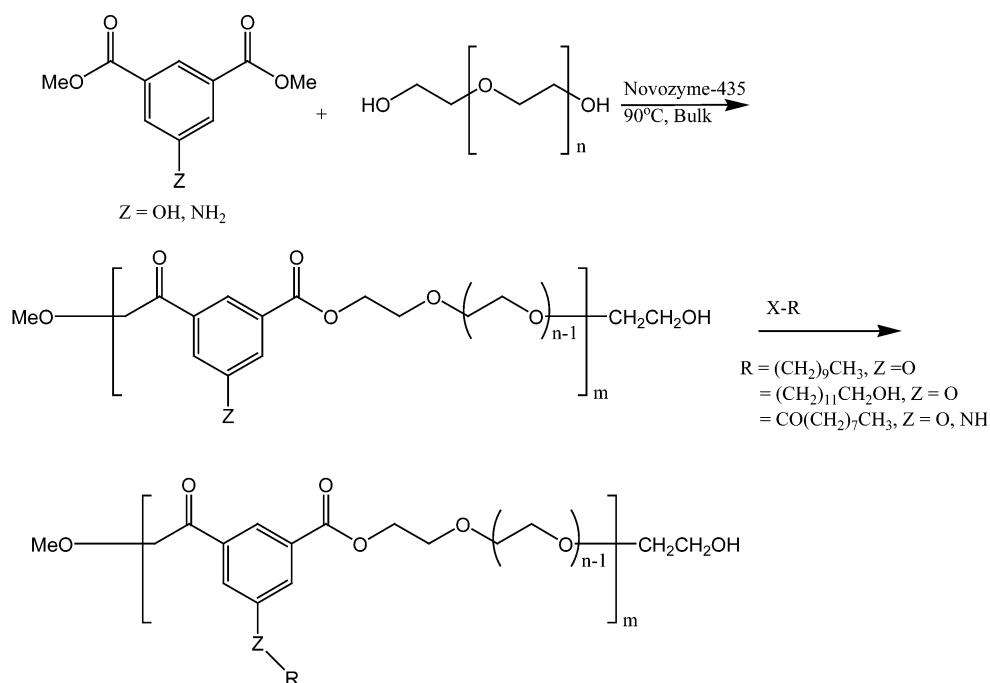
Taking the above advantages of drug delivery system into consideration, we have developed a chemo-enzymatic methodology for the synthesis of functionalized amphiphilic polymers (Scheme 1). We used poly(ethylene glycol)s, PEGs, because they are known to be biocompatible, nontoxic and, water-soluble. One of the unique properties of these amphiphilic polymers is their ability to self-assemble in specific solvents forming micelles, thus enabling them to encapsulate small molecules.

In order to demonstrate the tolerance of the Novozyme 435 enzyme system in the polymerization reactions, hydrophobic diols and diamines were utilized in the preparation of polyesters and polyamides

with these same linkers indicating that hydrophobic polymers may be prepared as well. The successful and easy preparation of these polymers allows the application of our synthetic method to many easily synthesized functionalized polymeric materials. As long as all of the units, backbone, linkers, and side chains are each equal to one another, complete control of the molecular architecture of the formed polymers is established.

If the linker is trifunctional, with two identical functional groups and the other different, one may take advantage of the chemoselectivity of the enzyme to control the structure of the polymer as an alternating structure. As an example, in the reaction of PEG units with 5-hydroxy- or 5-amino-dimethyl isophthalate using Novozyme 435 (*Candida antarctica* lipase), the reaction selectively occurs at the aromatic ester sites with no products formed by reaction with the hydroxy or amino group as shown in Scheme 1.

The main advantage of this reaction is that the hydroxyl/amino substituents at the C-5 position of the isophthalate moiety may be further exploited for attaching different groups (e.g., alkyl, substituted alkyl, aryl, etc.) and thus not only the hydrophobic/hydrophilic balance of the polymer may be achieved, but also its physical and chemical properties can be fine tuned to design and synthesize polymers for different applications. Our interest has been to synthesize polymers that can aggregate in aqueous medium forming nanospheric particles that in turn can be used as carriers for drug delivery. For controlled aggregation, the balance between hydrophilic and hydrophobic groups is very critical. We found that the polymer that lacks any hydrophobic substituents linked at the C-5 amino/hydroxyl group is unable to aggregate in an ordered manner, implying the need for a hydrophobic group at this position. These results encouraged us to synthesize a series of polymers by attaching alkyl chains of different length (C-3 to C-12). Light-scattering data suggest that the alkyl chains of C-9 to C-12 confer enough hydrophobicity to the polymeric system to self-assemble in aqueous medium forming nanospheric particles with a hydrophobic core surrounded by hydrophilic PEG units. The inherent core-shell structure of our polymeric assemblies is ideally suited for targeted drug delivery. The hydrophobic core can serve as a nonaqueous reservoir for the drug, which is also protected, to some degree, against in vivo degra-



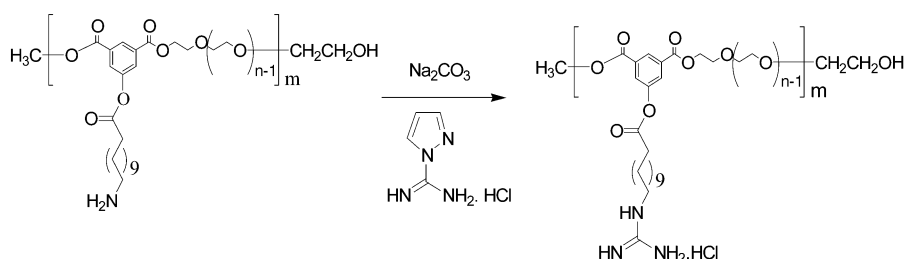
**Scheme 1** Chemo-enzymatic synthesis of amphiphilic polymers.

dation (e.g., metabolism). The hydrophilic shell is composed of nontoxic, biodegradable PEG units that have a dual role in stabilizing the micellar assembly while also dictating the pharmacokinetics and biodistribution of the carrier [7]. Furthermore, hydrophilically stabilized micelles can avoid phagocytosis and achieve prolonged circulation times [8].

Our study clearly established that by varying the length and functionality of the side chain (Fig. 1; SC-1, SC-2, SC-3, etc.) the relative hydrophobic and hydrophilic character may be varied over a wide range (Scheme 1;  $R_1$ ,  $R_2$ ,  $R_3$ ). With the capability of varying the side chains and functionality, the core of these nanospheres may also be varied to conform to the particular characteristics of the small molecule or drug. With this aim, we synthesized a variety of polymers varying not only the side chain, but also the PEG units of different molecular weight (average molecular weight 600, 900, 1500). All the polymers synthesized were well characterized on the basis of dynamic and static light scattering as well as NMR techniques [4,9].

We also investigated the ability of these nanospheres to encapsulate drugs and improve the efficacy of these drugs. Studies have been carried out *in vitro* with pancreatic, breast, and neuroblastoma cancer cells and found that the efficacy of known drugs such as doxorubicin and taxol was increased by a factor of 5–1000 times. Encapsulated anti-inflammatory drugs such as aspirin and naproxen were studied via a transdermal route [10].

We have extended our study to develop polymeric systems for gene delivery [11,12]. In this direction, the synthesis of cationic polymers may be useful as they have the capacity to interact with negatively charged DNA/RNA as well as with the phosphate groups at the cell surface. Our polymeric system is composed of amino/guanidine functional groups and PEG units (Scheme 2) [13,14]. The guanidine group is an important structural component in many biologically active compounds. Because of its strongly basic character ( $pK_a$ :12.5), it remains protonated over a wide pH range. The positive charge thus imposed on the molecule forms the basis for specific interactions between ligand and receptor or enzyme and substrate. The PEG units may form a characteristic micelle structure with a hydrophilic shell layer surrounding the core of the polyion complex (PIC) formed between DNA and the cation segments (PIC micelles).

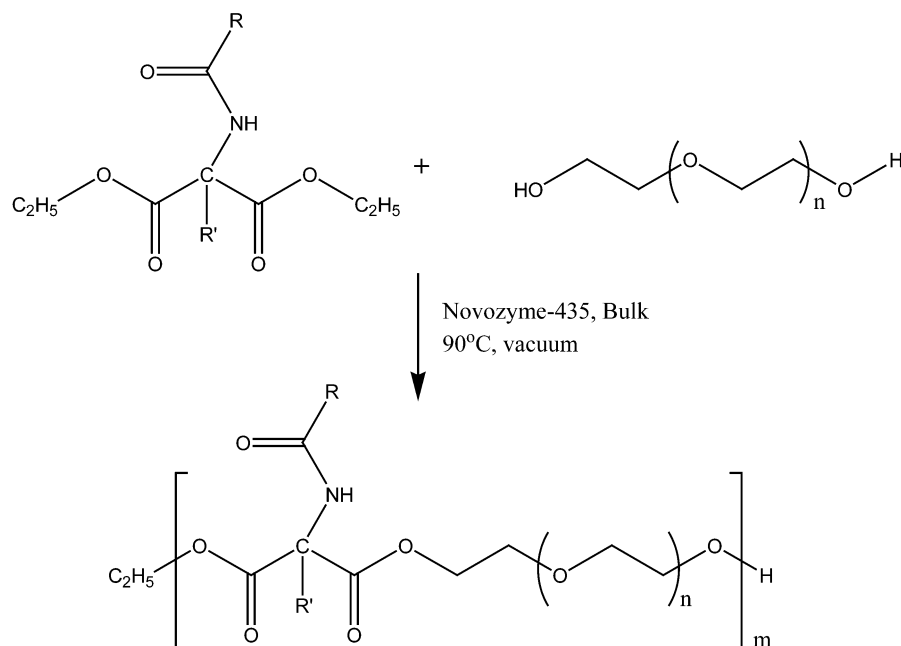


**Scheme 2** Extension of chemo-enzymatic synthesis.

## POLYMERS CONTAINING AMINO ACID DERIVATIVES

Amino acids are well known for their wide natural occurrence and are one of the five major classes of natural products [15–17]. Among them, dicarboxylic amino acids have recently drawn attention because of their specific functions in biological systems. Aminomalonic acid (Ama) is the first representative example of the homologous  $\alpha$ -amino dicarboxylic acid series, including important proteinogenic acids such as glutamic and aspartic acids. Recently, peptide derivatives of Ama have invoked significant interest because of their possible physiological activities as enzyme inhibitors, i.e., of rennin and HIV-1 protease [18]. The Ama molecule has a prochiral center and all of the derivatives, having different substituents at the two carboxyl groups, should be chiral. Obviously, the chiral moiety of the Ama derivatives might become an important stereocontrolling element if the Ama residue was incorporated in the peptide chain.

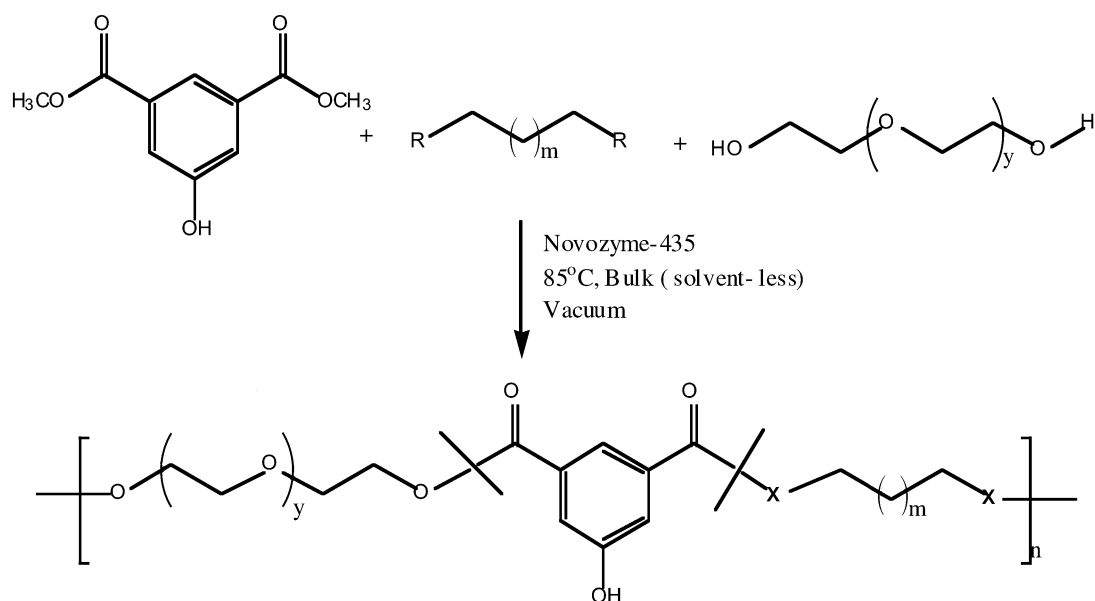
We have developed highly biocompatible compounds/polymers which can be utilized for such purposes. Scheme 3 represents the general strategy that was used for the synthesis of copolymers starting with nonproteinogenic amino acid derivatives and PEG 600 [19]. These monomers, which contain both the amide and ester functionalities, have been used for the first time in enzymatic polymerizations. *Candida antarctica* lipase was chosen to catalyze these copolymerization reactions, owing to its high catalytic activity for ester synthesis, high thermal stability, and the immobilization on the large surface area material.



**Scheme 3** Synthesis of copolymers of amino acid derivatives with PEG.

### Multicomponent polymerizations

A variety of materials based on multicomponent copolymers are being produced and used in various applications. Among these, aliphatic polyesters, e.g., poly-L-lactide (PLA) and polyglycolide, and polyethers, e.g., PEG, are the most important biodegradable polymers used clinically in wound closure [20,21], tissue repair and regeneration [22], and/or drug delivery [23]. In another approach, we altered the hydrophilic character of the polymeric backbone by incorporating the small hydrophobic units by either a terpolymerization reaction or by insertion into a ready-made polymer (Scheme 4). Both sequences worked quite well, although control of the molecular structure is less well defined. This resulted in multicomponent polyesters and mixed polymers having polyester and polyamide linkages under solvent-less conditions using *C. antarctica* lipase B. The effects of a third component, i.e., a series of 1,ω-alkanediols (1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, 1,12-dodecanediol, 1,14-tetradecanediol, and 1,16-hexadecanediol) on the copolymerization reaction of dimethyl 5-hydroxyisophthalate with PEG 600 has been studied. The effect of different functional groups (hydroxyl, amine, and thiol groups) on the terpolymerization reaction of dimethyl 5-hydroxyisophthalate with PEG by adding a third component having different functionalities (1,6-hexanediol, 1,6-hexanediamine, or 1,6-hexanedithiol) on the polymerization reactions have also been studied [24].



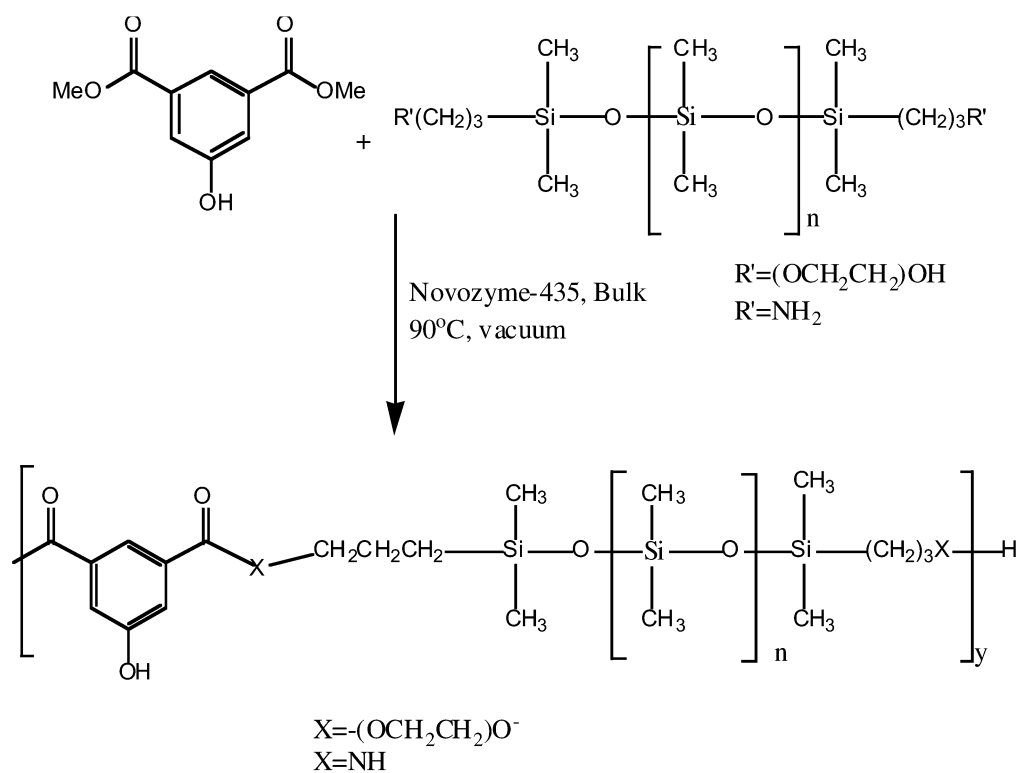
**Scheme 4** Terpolymerization reactions catalyzed by Novozyme 435.

### SILOXANE-BASED POLYMERS

Organosilicon polymers, specially polycarbosiloxanes, have been drawing attention [25] lately as potential substitutes for conventional flame retardant materials owing to their exceptional thermal stability and low flammability. Very few reports exist [26] on the chemical synthesis of polycarbosiloxanes reporting low-weight average molecular weights ( $M_w$ ) of the polymers in the range of 3000–10000 Dalton formed in 10–15 days. However, there are no literature reports on the formation of such polymers using biocatalysts. In view of their high thermal stability and attractive physical properties, tremendous incentive exists for their development as non-halogen containing flame-retardant materials. Traditionally, flame-retardant polymeric materials can be prepared by blending polymers with flame-retardant additives such as halogenated or phosphorus compounds. The most frequently used halogenated flame retardants are tetrabromobisphenol A, hexabromocyclododecane, PBBs, and PBDEs, which are produced globally at an estimated 150 000 tonnes a year [27]. However, these materials often generate toxic, corrosive, or halogenated gases during combustion and the materials are highly colored [28].

We have synthesized siloxane-based polymers using the enzymatic methods as shown in Scheme 5 [29]. The use of silicon substrates gave a new class of silicon polymers, which by traditional chemical methods require long reaction times and high temperature and give poor yields [30].

In summary, we believe that our synthetic methodology allows entry into a vast number of polymer families. Properties of polymers within these families may thus be varied over a wide range of values. The interactions of many of these polymers with small molecules such as drugs and/or the chemistry of attachment of these small molecules are exciting new areas of investigation, and we will continue to report on work in these areas and materials.



Scheme 5 Synthesis of silicon polymers.

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