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"Green"-enzymatic synthesis of pegylated phenolic macromer and polymer

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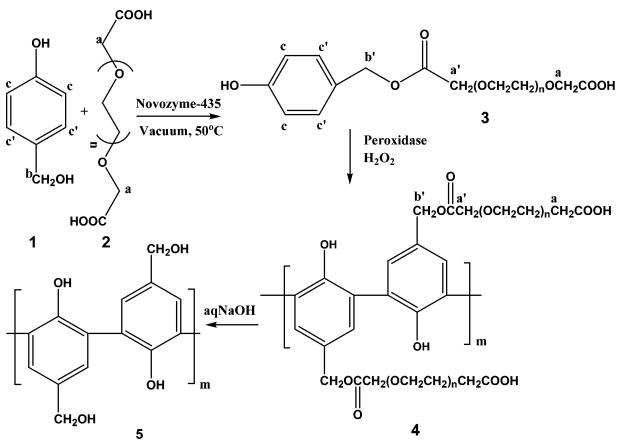
Environmentally benign synthesis of novel pegylated polyphenolics, by combining the extraordinary selectivities of a lipase and an oxidase to develop polymeric electrolytes for applications in dye sensitised solar cells.

Since the discovery that π-conjugated polymers can be electrically conductive,[1] this aspect of polymer chemistry has grown enormously.[2] The Nobel Prize for Chemistry in 2000 was awarded to its pioneers.[3] Several applications based on conducting polymers have been commercialized, current interest is in the development of small, lightweight, powerful, safe, environmentally benign, and portable energy sources.[4] The development of liquid-free rechargeable batteries that contain a polymeric electrolyte is a particular challenge. These could potentially yield batteries that are smaller, lighter, easier to manufacture, and contain less toxic compounds.[4a] Numerous reports have been published on the advances in the field of solid-state batteries. However, the available solid polymer electrolytes do not yet possess the required combination of properties. Therefore recent efforts have been focused on the design and synthesis of polymeric electrolytes.[5] Polyethylene glycol (PEG) is the most commonly used ion-conducting polymer matrix. However, PEGs can undergo crystallization in the presence of salts resulting in reduced mobility of the ions. In order to avoid the crystallization of polyethylene glycol and to increase the ionic conductivity, research has emphasized the development of amorphous materials, which provide facile ion transport and show good mechanical and thermal stability.

Various redox-active polymers, such as polyaniline, polyacetylene, polypyrrole and polyphenolics, have been investigated for applications in rechargeable batteries, sensors, electrocatalysts and as semiconductors in electronic microdevices.[6] We have designed phenolic monomers/macromers containing PEG segments in the side chains that can be polymerized to give a polymeric electrolyte, *i.e.* pegylated polyphenlics. Polyphenolics are typically prepared *via* chemical[7] or electrochemical methodologies,[8] and invariably involve complex processes that are difficult to scale up and which often produce large amounts of byproducts. An alternate synthetic strategy is to use enzymes to catalyze polymerizations of phenolic monomer. Peroxidases are highly effective phenol oxidizing catalysts.[9] Unfortunately, the direct peroxidase-catalyzed oxidation of phenolic monomers leads to minimal polymerization due to the lack of the solubility of monomer in aqueous media. Also the addition of co-solvents decreases enzyme activity and selectivity leading to a highly crosslinked product insoluble in common organic solvents.[10] Our group has earlier developed a template-assisted polymerization of phenols and anilines to obtain water-soluble polyphenolics and polyanilines.[11] However, the separation of template after the polymerization to obtain the polymers is difficult and limits the chemical and physical characterization of these materials, which in turn restrict the tailorability and fine-tuning of these materials for improved applications.

Here we report on a novel biotechnological route that incorporates a multi-enzymatic approach (combination of a lipase and an oxidase) to overcome these limitations. Specifically, we utilize an enzyme to catalyze the highly chemoselective mono-acylation of the alcoholic hydroxyl group of 4-hydroxymethylphenol (**1**) with polyethylene glycol diacid (**2**) under solvent-less conditions. The free phenolic moiety of pegylated hydroxymethylphenol, **3**, is then a substrate of peroxidase for the formation of the pegylated poly(hydroxymethyl phenol), **4**. The entire process (depicted in Scheme 1) does not require the use of any organic solvents during the reaction and was performed either in solventless conditions or using water as a solvent, thus it can be termed a "green process".

Numerous enzymes, especially lipases, are capable of acylation of a hydroxyl group; however, to get the pegylated macromer **3**, the reaction must be chemo- and regiospecific and block only one of the carboxyl groups of the PEG and that too by the alcoholic hydroxyl group. To that end, a number of lipases were screened for their ability to catalyze the synthesis of pegylated 4-hydroxymethylphenol (**3**): most of the lipases were able to catalyze the reaction with varying levels of selectivity and yields. However, *Candida antarctica* lipase B catalyzed the efficient monoacylation of the aliphatic hydroxyl group of 4-hydroxymethylphenol (95% conversion) with PEG diacid (determined by ^{1}H NMR) under solventless conditions.† During the acylation reaction less than 2% acylation at the phenolic hydroxyl was observed, thus making the reaction highly specific where in one step one can get a highly regiospecific and chemoselective product. This is not possible with chemical routes which often require 5–6 steps. The product **3** was completely identified from its various spectral data. Porcine pancreatic lipase and lipase AY from *Pseudomonas* species also showed very good conversion and selectivity but they also resulted in diacylation of PEG-diacid (around 5–7%). The acylation



Scheme 1 Biocatalytic synthesis of pegylated macromer **3** and polymers **4** and **5**.

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reactions are very reproducible in terms of % conversion and stereo-selection even on a larger scale (50 g).

Polymerization of **3** was catalyzed separately by peroxidase from horseradish (HRP) in aqueous buffer over a wide range of pH values and resulted in water-soluble polymers with M_n's ranging from 2000 to 15000 (degree of polymerization up to 20 with polydispersities of 1.4–1.8, determined by gel permeation chromatography). HRP gave optimal molecular weight of pegylated polyphenolic **4** at pH 4.5–6.0 with isolated yields of *ca.* 80% following dialysis to recover the polymer. The pegylated polyphenolic **4** was fully characterized by its ^{1}H NMR (Fig. 1d), ^{13}C NMR, UV (Fig. 2) and IR spectra. The ^{1}H NMR spectrum of polymer showed the presence of a broad signal in the range of δ 3.3–3.6 for the polyethylene glycol moieties and the signal at δ 5.2 corresponding to methylene proton labeled as **b′** in Fig. 1d, further confirming that the PEG was attached to the polyphenol. The polymeric molecular weights obtained from this enzymatic polymerization reaction were highly dependent on the pH of the buffer solution. It was found that the molecular weight of the polymer obtained decreased with increasing the pH from 4.75 to 10.0. The highest number average molecular weight (M_n), 15000, was obtained at pH 4.75 whereas the lowest molecular weight (M_n), 2200, was obtained at pH 10.0. Fig. 2 shows the UV absorption spectra of the pegylated macromer **3** and polymer **4**, with tailing in the absorption spectrum of **4** to 450 nm as compared to **3**. This further confirms the presence of extended conjugation and polymer formation. Depegylation of the pegylated polyphenolic **4** was performed at room temperature using aqueous sodium hydroxide solution to give quantitative yields of the light yellow-colored solid **5** (M_n 2500).

In summary, we have shown that by combining the selectivities of two different enzymes, novel functionalized materials can be synthesized while circumventing much of the protection/deprotection chemistry. It is anticipated that this approach is not limited to the phenol based reactions and that many other natural or unnatural substrates can be used. It is important to note that lipases and peroxidases are among the most broadly used selective enzymes as they catalyze a tremendous variety of reactions on different substrates. We are presently investigating further the influence of reaction regioselectivity (controlled by the lipase) and the polymer size (controlled by the peroxidase). As such these materials will have applications as solvent free polymeric electrolytes in dye-sesitized solar cells.

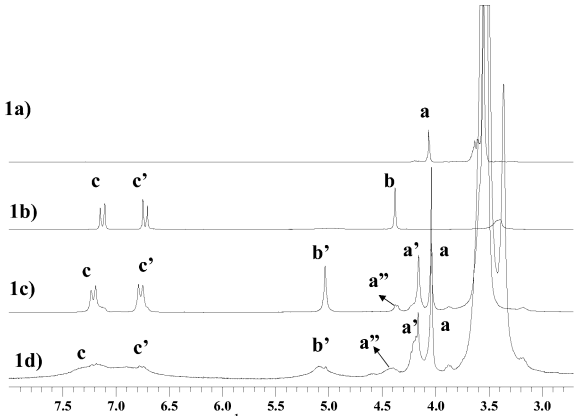

Fig. 1 Comparison of ^{1}H NMR (DMSO-d_6) spectra of a) polyethylene glycol diacid 600 (**2**); b) 4-hydroxymethylphenol (**1**); c) pegylated macromer **3**; d) pegylated polymer **4**.

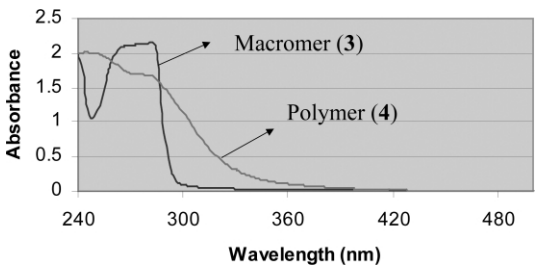

Fig. 2 UV Absorption spectra of pegylated macromer **3** and pegylated polymer **4**.

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Notes and references

† Equimolar amounts of 4-hydroxymethylphenol (**1**) and polyethylene glycol diacids (**2**, M_w 600) were mixed together in a round bottom flask and to that was added lipase (10% by weight with respect to total monomer weight). The reaction mixture was placed in an oil bath maintained at 50 °C and a vacuum was applied (for 5 min) and then it was closed. The progress of the reaction was monitored by thin layer chromatography using a gradient solvent system of methanol and chloroform. After completion (complete disappearance of 4-hydroxymethylphenol, 8 h), the reaction was quenched by adding chloroform and filtering off the enzyme. The filtrate was concentrated by removing the solvent under vacuum and the product obtained was purified by redissolving in water and filtering off traces of unreacted (if any) 4-hydroxymethylphenol, followed by column chromatography using a gradient solvent system of methanol in chloroform (to remove any unreacted PEG diacid) and identified as **3** by detailed spectral analysis.

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