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Research Article

Environmental Applications of Immobilized Peroxidase onto Epoxy Bearing Cryogels

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Abstract

In this presented work, poly(2-hydroxyethyl methacrylate-glycidyl methacrylate) [poly(HEMA-GMA)] cryogels were prepared by using the free radical cryopolymerization technique. These cryogels were then modified with peroxidase enzyme by covalent bonding. These peroxidase immobilized cryogels were successfully used for the removing of phenol and bisphenol A and decolorization of a model dye molecule.

Key words: Peroxidase; cryogel; phenol removal; decolorization

1. Introduction

Peroxidase is a very valuable enzyme, which oxides aromatic compounds in the presence of hydrogen peroxide to form polymeric insoluble products. Due to their high molecular weight, polymeric products can be separated easily and thus, aromatic compounds can be depleted from the polluted waste waters [1]. Horseradish peroxidase (HRP) is one of the intensively used and applied industrial enzyme. This enzyme is highly available from various sources, its extraction and purification steps are very easy, and have been found unique application areas for the waste water treatment studies, decolorization processes and so on. HRP is a single chain protein, which glycosylated at eight specific regions. Active HRP contains two Cu(II) ions, and one heme group, which contains the Fe-protoporphyrin IX [2, 3]. HRP has been practically used for the removal of phenolic compounds and dyes from polluted waste waters [4, 8], synthesis of various organic compounds [9-12] and analytical purposes [2, 12]. Peroxidase enzymes have been immobilized onto various supports such as magnetic poly(GMA-MMA) beads [1], chitosan-halloysite hybrid-nanotubes [13], poly(MMA-CEA) microfibrous membranes [14], nanoporous copper [15], nonwoven polyester fabric coated with chitosan [16] and mesoporous silicas [17], in order to improve the re-usability behaviour of the enzyme, to increase the stability and to reduce the overall cost.

As a new polymeric material, cryogels have been found many biotechnological and industrial applications, and have been preferred as a support material for enzyme immobilization [18]. Preparation of a cryogel is a very simple and easy process; the main step for synthesis of a cryogel is the medium temperature: cryogels are synthesized at sub-zero temperatures [19]. Solvent for polymerization process is generally water, and at this cold conditions most of the water is frozen by creating the ice crystals. Thus, the dissolved monomers are concentrated in a small non-frozen water medium. In this cryopolymerization process, ice crystals act as a porogen, and create large, interconnected pore networks within the all cryogel system [20]. This pore structure allows a unhindered transport of the solution (even whole blood) through the cryogel system [21].

In this presented study, poly(HEMA-GMA) cryogels were synthesized to use as an immobilization support for peroxidase, and phenols removal efficiency and decolorization ability of the peroxidase immobilized cryogels were also investigated.

2. Materials and Methods

2.1. Materials

Peroxidase (EC 1.11.1.7; from horseradish), 2-hydroxyethyl methacrylate (HEMA), N,N,N',N'-tetramethylene diamine (TEMED), ammonium persulfate (APS), N,N'-methylene

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bisacrylamide (MBAAm), glycidyl methacrylate (GMA), phenol, bis-phenol A and Congo Red were obtained by Sigma (St. Louis, USA). The other chemicals were of analytical grade and supplied from Merck (Darmstadt, Germany). Water for preparation of all solutions was prepared by Millipore Simplicity© system (18.2 mΩcm).

2.2. Preparation and Characterization of the Cryogel

In this presented study, poly(2-hydroxyethyl methacrylate-glycidyl methacrylate) [poly(HEMA-GMA)] cryogels were synthesized by using free radical cryocopolymerization technique. For this purpose, monomers of HEMA and GMA were preferred as a structural monomer and functional monomer, respectively. Details of the whole polymerization process and the characterization studies of the poly(HEMA-GMA) cryogels were explained in detail in our previous study [22].

2.3. Immobilization of Peroxidase on the Cryogels

Immobilization process of the peroxidase was carried out according to the literature described [23]. Briefly, synthesized poly(HEMA-GMA) cryogel was rinsed with 100 mL of distilled water, and then equilibrated by 100 mL of phosphate buffer (pH 8.0, 50 mM) for 2h. Then, 20.0 mL of peroxidase solution (2.0 mg/mL) was passed through the cryogel column continuously by using a peristaltic pump for 18 h at room temperature. While this enzyme immobilization period, covalent bounds were created between the enzyme molecules and poly(HEMA-GMA) cryogel surface via reactive epoxy groups of the GMA monomers. Immobilization process of peroxidase onto the poly(HEMA-GMA) cryogel was schematically illustrated in Figure 1. Immobilized amount of peroxidase was determined by measuring the initial and final peroxidase concentrations in the immobilization medium by the Bradford method [24].

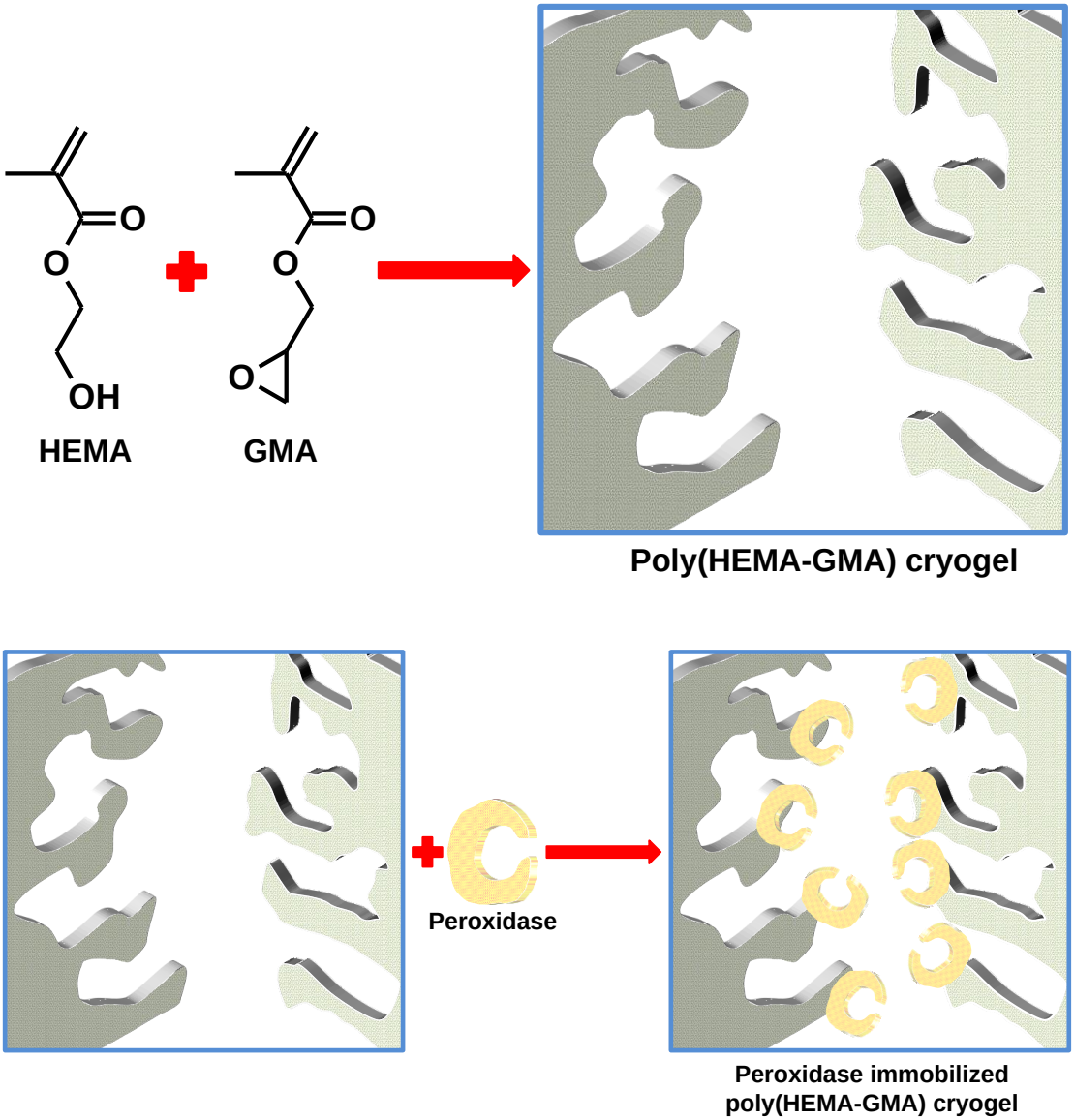

Figure 1. Schematic presentation for the synthesisof the peroxidase immobilized poly(HEMA-GMA) cryogels.

2.4. Activity of Peroxidase

Peroxidase activities were investigated by the method of Trinder [25], which uses 4-aminoantipyrine as hydrogen donor. Briefly, 1.4 mL phenol/aminoantipyrine solution (810 mg of phenol and 25 mg of 4-aminoantipyrine in 50 mL of reagent grade water) was mixed with 1.5 mL of hydrogen peroxide solution (0.0017 M). After adding of 0.1 mL of enzyme sample, absorbance of the resulting solution was recorded at 510 nm for 5 min. One unit of enzyme activity is defined as the decomposition of one micro mole of hydrogen peroxide per minute at 25 °C and pH 7.0 under the specified conditions.

2.5. Degradation of Phenols and Dye Decolorization Capability of Peroxidase Immobilized Poly(HEMA-GMA) Cryogels

Phenol's degradation efficiency of the cryogels were determined in a continuous system by using model phenols; phenol and bis-phenol A. For this, phenol solutions (0.1 M) were passed through into peroxidase immobilized cryogel column and the degradation capacity of phenols were determined by measuring the initial and final phenol concentrations in the degradation solution. A colorimetric method was used to determine the phenol concentrations [26]. Briefly, 2.4 mL of phenol solution was mixed with 0.3 mL of ferric cyanide solution (83.4 mM in 0.25 M sodium bicarbonate) and 0.3 mL of 4-aminoantipyrine solution (20.8 mM in 0.25 M sodium bicarbonate). After the 5 min of incubation, absorbance of the final solution was measured at 510 nm. In order to measure the concentrations, a calibration curve was established with different phenol concentrations.

Dye decolorization studies were also conducted in a continuous mode by using the model dye of Congo Red. For this, 10 mL of dye solutions (0.1 mg/mL) were passed through the peroxidase immobilized cryogel column for 10 min and the decolorization efficiency was measured by following the dyes spectrophotometrically at their own wavelength.

3. Results and Discussion

3.1. Characterization of Poly(HEMA-GMA) cryogel

Synthesized poly(HEMA-GMA) cryogels were elastic with opaque character and demonstrated sponge-like morphology and can lost all accumulated water by simple compressed by hand. These cryogels also exhibited fast swelling kinetics, dry cryogels swelled rapidly when submerged in water, and thus cryogels restored their original shape and size very fast. SEM characterizations have been explained at our previous study [18]. As mentioned there, synthesized cryogel had macroporous structure with sponge-like texture, and the pore size of the cryogel was found to be around 30–50 nm. The FTIR spectrum of the poly(HEMA-GMA) cryogels have been given in our recent paper [27] . As stated here, stretching band of $–CH_3$ and C=O stretching bands located around 3,400 and 1,720 $cm^{–1}$, respectively. C–O stretching band of carbonyl group was localized at around 1,270 $cm^{–1}$. Also, the whole ring stretching band at around 1,255 $cm^{–1}$ and terminal oxiran of the GMA at around 904 $cm^{–1}$ was the proof for the incorporation of GMA monomer onto polymeric HEMA structure.

Peroxidase enzyme was covalently attached on the surface of the poly(HEMA-GMA) cryogel by the help of the reactive epoxy groups of the GMA monomer. Activities of both free and immobilized form of peroxidase were also investigated, and the specific activities were found to be 0,945 U/mg and 0.796 U/mg, respectively. This also showed that, peroxidase enzyme was successfully immobilized onto the cryogel column. As also seen here, activity of peroxidase decreased only about 15.77 % after the immobilization process. This reduce in the enzymatic occurs in all general enzyme immobilization procedures. In this presented work, a reduced activity occurred due to the possible conformational changes of the enzyme during covalent immobilization process.

3.2. Degradation of Phenols and Dye Decolorization Capability

Phenol's degradation efficiency and dye decolorization capacity of the peroxidase immobilized poly(HEMA-GMA) cryogels were demonstrated in Table 1. As demonstrated here, peroxidase immobilized poly(HEMA-GMA) cryogels exhibited high phenol degradation properties with the degradation percentage of 40-45 %. Some recent phenol degradation studies demonstrated lower degradation yields such as; approximately 20 % degradation by Lai and Lin [28], and 51 % phenol removal capacity by Kalaiarasan and Palvannan [29]. As stated here it could be concluded that, peroxidase immobilized poly(HEMA-GMA) cryogels had a good degradation capability towards to phenol and Bis-phenol A, and showed comparable results with the recent works on the phenol removal.

Table 1. Degradation of phenols and dye decolorization capacity of the peroxidase immobilized poly(HEMA-GMA) cryogels

Phenolics		Dye
Phenol	Bisphenol A	Congo Red
45.21 %	40.35 %	61.25 %

As also demonstrated in this table that, immobilized peroxidase showed pleasant decolorization efficiency toward to tested model dye Congo Red and the decolorization capability was found to be around 61.25 % at the end of the 120 min incubation. Many researchers founded various decolorization efficiency's to many type of dye molecules such as Remazol Brilliant Blue R, Remazol Black B, Reactive Orange 122, and Reactive Red 251 dyes, and overall dye decolorization capacities have been found around 30 %-80 % with a long incubation periods as 2 to 6 h [30-34].

As demonstrated here, peroxidase immobilized poly(HEMA-GMA) cryogels showed good phenol degradation and dye decolorization capacities. However, applying the samples in a continuous mode, eventuating of no-blockage in pores of the cryogel due to the wide, interconnected porous structure, and fast process capabilities improve the usability of this peroxidase immobilized cryogels in waste water managing studies.

Another advantageous behaviour of this presented study was the very fast decolorization rates, and thus high decolorization yields were found within the short time periods like 120 min. It can be also concluded form these presented results that immobilized peroxidase was successfully applied for the decolorization of model dye molecule in a continuous system. Also it can be suggested that, this enzyme modified system can be adapted to the industrial waste water management system.

4. Conclusion

Phenols and dye effluents from various industrial plants cause serious environmental pollution. Removing and management of these effluents is very difficult because of their complex structures. Some techniques have been developed and used to remove these wastes. But the main drawbacks of these methods are their expensiveness and lac of efficiency. For these reasons, new management methods have been developed and used to reduce the accumulation of these products. For this point of view, peroxidase has been used intensively for the management process for both phenolics and dyes. Its immobilized form is also preferred due to the potential reuse for the next waste management. As a new generation polymeric matrix cryogels have been found unique place for the application for various biotechnological studies. In this presented work, peroxidase enzyme was successfully immobilized onto the poly(HEMA-GMA) cryogel, and the phenol's removing and dye decolorization potency of this immobilized preparation was showed by two model phenolics and a model dye molecule. In the light of the results of these study; it can be concluded that immobilized form of peroxidase can be proposed as an efficient tool for the applications of waste water treatment and management.

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