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# Preparation of nanoparticles which contains histidine for immobilization of *Trametes versicolor* laccase

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ABSTRACT

Poly(hydroxyethyl methacrylate-N-methacryloly-(L)-histidinemethylester) [PHEMAH] nanospheres with an average size of 158.2 nm were prepared by surfactant free emulsion polymerization conducted in an aqueous dispersion medium. MAH was synthesized using methacryloyl chloride and L-histidine methyl ester. Then, Cu(II) ions were attached as a metal ligand because of its affinity towards proteins. Specific surface area of the PHEMAH nanospheres was calculated to be 1454 $m^2/g$. The PHEMAH nanospheres were characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Average particle size, size distribution, and surface charge measurements were also performed. Elemental analysis of MAH for nitrogen was estimated as 0.42 mmol/g nanosphere. Then, PHEMAH-$Cu^{2+}$ nanospheres were used for laccase adsorption in a batch system. The maximum laccase adsorption was observed at pH 6.0. Laccase could be repeatedly adsorbed and desorbed with PHEMAH-$Cu^{2+}$ nanospheres without noticeable loss in the adsorption capacity.



## 1. Introduction

Nanotechnology is an enabling technology that deals with nanometer sized objects [1]. It is expected that nanotechnology will be developed at several levels: materials, devices and systems. The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties [2]. Now, they have entered a commercial exploration period [3]. Only limited work has been published on the application of nanoparticles in the separation of proteins. Because of their nanoscopic size, nanoparticles can produce larger specific surface area and, therefore, may result in high binding capacity for proteins. Therefore, it may be useful to synthesize nanoparticles and utilize them for the purification of proteins [4].

Enzyme adsorption has been a popular strategy for most large-scale applications due to the ease in catalyst recycling, continuous operation, and product purification [5]. Poor biocatalytic efficiency of adsorbed enzymes, however, often limits the development of large-scale bioprocessing to compete with traditional chemical processes [6]. Improvements of biocatalytic efficiency can be achieved by manipulating the structure of carrier materials for enzyme adsorption. The most important advantages of this method are that the stability of enzyme activity after adsorption and reuse of the enzyme and support material for different purposes because of reversibility of the method [7].

Nonporous materials, to which enzymes are attached to the surfaces, are subject to minimum diffusion limitation while enzyme loading per unit mass of support is usually low. On the other hand, porous materials can afford high enzyme loading, but suffer a much greater diffusional limitation of substrate. Reduction in the size of enzyme-carrier materials can generally improve the efficiency of adsorbed enzymes. In the case of surface attachment, smaller particles can provide a larger surface area for the attachment of enzymes, leading to higher enzyme loading per unit mass of particles [8]. In the case of enzyme adsorption on porous materials, much reduced mass transfer resistance is expected for smaller porous particles owing to the shortened diffusional path of substrates when compared to large-sized porous materials. There have been extensive studies on the use of microparticles for the adsorption of biomolecules [9–11]. Recently, a growing interest has been shown in using nanoparticles as carriers for enzyme adsorption [12].

Laccases (EC 1.10.3.2) are polyphenoloxidases performing the reduction of oxygen to water while oxidizing organic substrates by a one-electron redox process. Laccase activity was first described in plants (Rhus vernicifera) by Yoshida in 1884 and more recently

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enzymes were evidenced in bacteria [13,14]. However, laccases are mainly produced by filamentous fungi (ascomycetes and mainly wooddecaying basidiomycetes) [15]. In these organisms, they are involved in several biological processes such as lignin degradation [16], morphogenesis and pathogenesis. In addition to lignocellulosic substrates, an extensive list of xenobiotics [pesticides, polycyclic aromatic hydrocarbons (PAHs), dyes, etc.] often containing phenolic or aromatic amino groups are transformed by laccases [17,18] either by bond cleavage or by oxidative coupling [14]. Owing to the great variety of reactions catalysed by laccase, this enzyme has attracted considerable interest in various fields of research, e.g. development of oxygen cathodes in biofuel cells, green biodegradation of xenobiotics, biosensors, organic synthesis, labelling immunoassays [19].

In this study, PHEMA based matrix containing MAH was prepared in the nanosphere form by surfactant free emulsion polymerization of 2-hydroxyethyl methacrylate (HEMA) and N-methacryloyl-(L)-histidine methyl ester (MAH). Then, Cu(II) attached as a metal ligand because of its affinity towards histidine groups in protein structure. The nanospheres were used for the adsorption of *Trametes versicolor* laccase via adsorption. The system parameters such as effect of the adsorption conditions (i.e., enzyme concentration, medium pH and temperature) and the reusability of the support were studied on the laccase adsorption.

## 2. Experimental

### 2.1. Materials

Crystallized and lyophilized laccase (from *Trametes versicolor* (EC 1.10.3.2)), histidine methyl ester and methacryloyl chloride were supplied by Sigma Chemical Co (St. Louis, MO, USA). 2-Hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) were obtained from Fluka AG (Switzerland). Commercial HEMA contains residual methacrylic acid and crosslinkers due to fabrication process. The polymerization inhibitor 4-methoxyphenol also needs to be removed. HEMA and EGDMA were purified and distilled under reduce pressure (0.01 mbar, 70 °C). Monomers were stored at 4 °C until use. Poly(vinyl alcohol) (molecular weight: 100,000, 98% hydrolyzed) was obtained from Aldrich (USA). All other chemicals were the guaranteed of analytical grade reagents commercially available and used without further purification. The water used throughout this work was produced using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead 3804 NANOPURE® organic/colloid removal and ion-exchange packed-bed system.

### 2.2. Preparation of N-methacryloyl-(L)-histidine methyl ester

Preparation of N-methacryloyl-(L)-histidine methyl ester (MAH) had been reported in our previous report [20]. Briefly, MAH (5.0 g) and sodium nitrite ($NaNO_2$; 0.2 g) were dissolved in potassium carbonate solution ($K_2CO_3$; 30 mL, 5% (w/v)). The mixture was transferred in a 100 mL round-bottomed three-necked flask fitted with a dropping funnel. The reaction chamber was cooled to 0 °C in an ice-water bath and stirred magnetically under a nitrogen atmosphere. Methacryloyl chloride (6.0 mL) was placed into the dropping funnel and introduced dropwise into the reaction mixture in 10 min. The reaction chamber was then removed from the ice-water bath and the reaction was maintained at room temperature for 2 h. After reaction, the pH of the mixture was adjusted to 3.0 and the product was extracted with chloroform (50 mL). After phase separation, the organic phase was dried with magnesium sulphate, and the chloroform was evaporated in a rotary evaporator. The product (i.e. N-methacryloyl-(L)-histidine methyl ester, MAH) was crystallized in an ether–cyclohexane mixture.

### 2.3. Synthesis of PHEMAH nanospheres

PHEMAH nanospheres were produced by surfactant free emulsion polymerization. Using the same procedure in a typical polymerization procedure may be summarized as follows: the stabilizer, poly(vinyl alcohol) (0.5 g), was dissolved in 50 mL deionized water for the preparation of the continuous phase. Then, the monomer and/or crosslinker mixture (0.6 mL/0.01 mL (HEMA/EGDMA)) was added to this dispersion phase which was mixing in an ultrasonic bath for about half an hour. Prior to polymerization, initiator was added to the solution and nitrogen gas was blown through the medium for about 1–2 min to remove dissolved oxygen. Polymerization was carried out in a constant temperature-shaking bath at 70 °C, under nitrogen atmosphere for 24 h. After the polymerization, the nanospheres were cleaned by washing with methanol and water several times to remove the unreacted monomers. For this purpose, the nanospheres were precipitated and collected with the help of a centrifuge (Zentrifugen, Universal 32 R, Germany) at 18,000 rpm for 1 h and resuspended in methanol and water several times. After that PHEMAH nanospheres were further washed with deionized water. PHEMA nanospheres were produced by same formulation without MAH comonomer [21].

### 2.4. Chelation of $Cu^{2+}$ ions

$Cu^{2+}$ ions with PHEMAH nanospheres were chelated as follows: 1.0 g of the nanospheres were mixed with 50 mL of aqueous solutions containing 50 ppm $Cu^{2+}$ ions, at constant pH of 5.0 (adjusted with HCl and NaOH), which was the optimum pH for $Cu^{2+}$ chelate formation at room temperature. A 1000 ppm atomic absorption standard solution (containing 10% $HNO_3$) was used as the source of $Cu^{2+}$ ions. The flask was stirred magnetically at 100 rpm for 1 h (sufficient to reach equilibrium). The concentration of the $Cu^{2+}$ ions in the resulting solution was determined with a graphite furnace atomic absorption spectrometer (Analyst 800/Perkin-Elmer, USA). The amount of adsorbed $Cu^{2+}$ ions was calculated by using the concentrations of the $Cu^{2+}$ ions in the initial solution and in the equilibrium. $Cu^{2+}$ leakage from the PHEMAH-$Cu^{2+}$ nanospheres was investigated with media pH (3.0–7.0), and also in a medium containing 1.0 M NaCl. The nanosphere suspensions were stirred 24 h at room temperature. $Cu^{2+}$ ion concentration was then determined in the supernatants using an atomic absorption spectrophotometer. It should be also noted that metal-chelated nanospheres were stored at 4 °C in the 10 mM Tris–HCl buffer (pH 7.4) deionized water.

### 2.5. Characterization of PHEMAH-$Cu^{2+}$ nanospheres

FTIR spectra of the nanospheres were obtained using FTIR spectrophotometer (Varian FTS 7000, USA). The dry nanospheres (about 0.1 g) were thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a tablet form, and the spectrum was then recorded. The average particle size, size distribution and surface charge were determined by Zeta Sizer (Malvern Instruments, Model3000 HSA, England).

The nanospheres were imaged in dry state by scanning electron microscope, SEM (Phillips, XL-30S FEG, Germany). For this purpose, the dispersion of nanospheres was dropped onto a formvar coated copper grid and dried at room temperature. The dried nanospheres were then imaged. The degree of MAH incorporation in the synthesized PHEMAH nanospheres was determined by elemental analyser (Leco, CHNS-932, USA).

The surface area of PHEMAH nanospheres was calculated using the following expression [22]:

$$N = \frac{6 \times 10^{10} S}{\pi \rho s d^3} \quad (1)$$

Here $N$ is the number of nanospheres per milliliter; $S$ is the % solids; $\rho s$ is the density of bulk polymer (g/mL); $d$ is the diameter (nm). The number of nanosphere in mL suspension was determined by utilizing from mass–volume graph of nanospheres. From all these data, specific surface area of PHEMAH nanospheres were calculated by multiplying $N$ and surface area of one nanosphere.

### 2.6. Adsorption of laccase on nanospheres

Adsorption of *T. versicolor* laccase on the PHEMAH-$Cu^{2+}$ nanospheres was studied at various pHs in either acetate (20 mL, 0.1 M, pH 4.0–5.0) or phosphate buffers (20 mL, 0.1 M, pH 6.0–8.0). The enzyme initial concentration was 0.5 mg/mL in each corresponding buffer. Adsorption experiments were conducted for 120 min at 25 °C with continuous stirring. The equilibrium adsorption time was determined to be 120 min with pre-experiments. The effect of temperature on laccase adsorption was carried out in phosphate buffer (20 mL, 0.1 M, pH 6.0) containing 0.5 mg/mL enzyme at 4–45 °C. The amount of adsorbed laccase on the PHEMAH-$Cu^{2+}$ nanospheres was determined by measuring the initial and final concentrations of protein within the adsorption medium using Coomassie Brilliant Blue [22]. A calibration curve was constructed with laccase solution of known concentration (0.05–0.50 mg/mL) and was used in the calculation of protein amount. Nanospheres washed with corresponding buffer solution after adsorption of laccase before activity assay and investigation of biocatalytic properties. The experiments were performed in replicates of three and the samples were analyzed in replicates of three as well. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error.

### 2.7. Activity assays of free and adsorbed laccase

Standard assays for laccase activity were performed by measurement of enzymatic oxidation of ABTS at 420 nm ($\varepsilon = 3.6 \times 10^4\ cm^{-1}\ M^{-1}$ for the oxidation product). The reaction mixture contained 100 μL concentrated extracellular fluid (prepared as described above) and 900 μL ABTS (1 mM) in acetate buffer (0.1 M, pH 5.0) at 30 °C. One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 mmol ABTS in 1 min.

### 2.8. Repeated use studies

In order to determine the reusability of the PHEMAH-$Cu^{2+}$ nanospheres, the laccase adsorption and desorption cycle was repeated five times using the same group of nanospheres. Laccase desorption from the nanospheres was carried out with 0.1 M KSCN (pH 8.0). The desorption medium was stirred magnetically at 100 rpm at 25 °C for 120 min. The equilibrium desorption time was found to be 120 min with pre-experiments. The enzyme concentration in desorption medium was determined by the Bradford method [23]. The desorption ratio of laccase was calculated by using the following expression:

$$\text{desorption ratio} = \left(\frac{\text{enzyme released}}{\text{enzyme adsorbed on the nanospheres}}\right) \times 100 \quad (2)$$

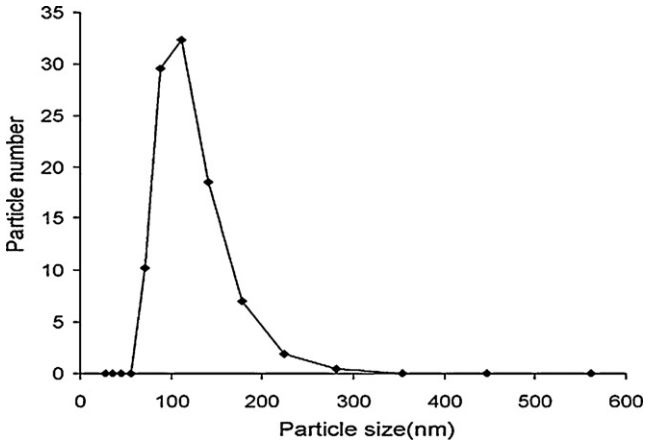


**Fig. 1.** Size analysis of PHEMAH nanospheres.

### 2.9. Thermal and storage stability

The thermal stability of free and adsorbed laccase was determined by measuring the residual enzymatic activity at 55 °C in a phosphate buffer (0.1 M, pH 6.0) for 2 h. After every 15 min time interval, a sample was removed and assayed for enzymatic activity as described above. The results were given as activity %. The storage stability experiments were conducted to determine the stabilities of free and adsorbed laccase preparations after storage in phosphate buffer (50 mM, pH 6.0) for 12 weeks. The residual activities were then determined as described above. Activity of each preparation was expressed as a percentage of its residual activity compared to the initial activity.

## 3. Results and discussions

### 3.1. Properties of PHEMAH-$Cu^{2+}$ nanospheres

Nanoparticles provide an ideal remedy to the usually contradictory issues encountered in the optimization of adsorbed enzymes: minimum diffusional limitations, maximum surface area per unit mass, and high enzyme loading. In addition to the promising performance features, the unique solution behaviors of the nanospheres also point to an interesting transitional region between heterogeneous and homogeneous catalysis. Theoretical and experimental studies demonstrated that particle mobility, which is governed by particle size and solution viscosity, could impact the intrinsic activity of the particle-attached enzymes [24]. The enzyme supports are the most frequently obtained after chemical modification of natural and synthetic polymers such as chitin, chitosan, cellulose acetate, acrylic polymers, poly(vinyl alcohol) or inorganic glass hollow fibres [25]. We suggest here a novel method of introducing a metal-chelated group into the polymeric nanospheres via polymerization of HEMA and MAH. The distinctive feature of this method is the elimination of the activation and ligand coupling steps during the preparation of the affinity matrices. Some other advantages over other methods include the use of a known amount of ligand in the polymer preparation mixture and the good reproducibility of the affinity matrix [25].

FTIR spectra of HEMA and PHEMAH have the characteristic stretching vibration band of hydrogen bonded alcohol, O–H, around 3440 $cm^{-1}$. The FTIR spectrum of PHEMAH has the characteristic amide I and amide II adsorption bands at 1650 and 1550 $cm^{-1}$, respectively.

Average particle size and size distribution of the PHEMAH nanospheres were measured by Zeta Sizer. Particle size is about 158.2 nm with 0.171 polydispersity (Fig. 1). The average particle

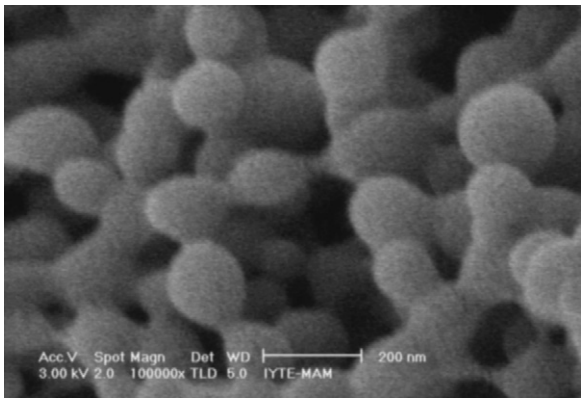


**Fig. 2.** SEM micrographs of PHEMAH nanospheres.

size was an average of minimum 30 measurements, and the size distribution was recorded automatically by the software of these repeated measurements.

The amount of MAH in the PHEMAH nanospheres was determined by elemental analysis. The MAH content of the nanospheres was calculated from the nitrogen analysis result and it was found to be 0.42 mmol/g.

Nanosized particles can produce larger specific surface area and may result in high enzyme loading. Therefore, it may be useful to synthesize nanosized particles with large surface area and utilize them as suitable carriers for the adsorption of enzymes. The specific surface area was calculated as 1415 $m^2/g$ for PHEMA and 1454 $m^2/g$ for PHEMAH nanospheres.

The scanning electron micrographs (SEMs) clearly showed the spherical character of PHEMAH nanospheres (Fig. 2). In addition, the total monomer conversion was determined as 96.2% (w/w) for PHEMAH nanospheres.

### 3.2. Adsorption of laccase onto PHEMAH nanospheres

#### 3.2.1. Effect of pH and temperature on laccase adsorption

The important parameters pH and temperature should be controlled during adsorption of enzyme to obtain reproducible results since these parameters influence the stability and conformational structure of proteins. Fig. 3 shows the amount of enzyme adsorbed onto PHEMAH-$Cu^{2+}$ nanospheres at different pH values. In this study, the maximum laccase adsorption value was obtained at pH 6.0. Significantly, a lower laccase adsorption was obtained for the nanospheres in all other tested pH regions.

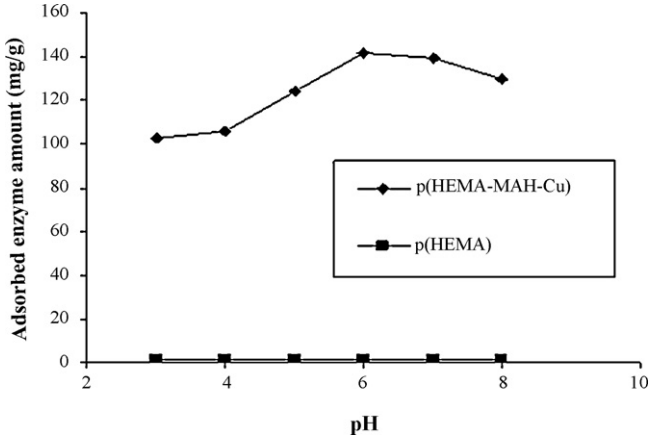


**Fig. 3.** Effect of pH on laccase adsorption on PHEMAH-$Cu^{2+}$ nanospheres MAH content: 0.42 mmol/g; laccase concentration: 0.5 mg/mL; *T*: 25 °C.

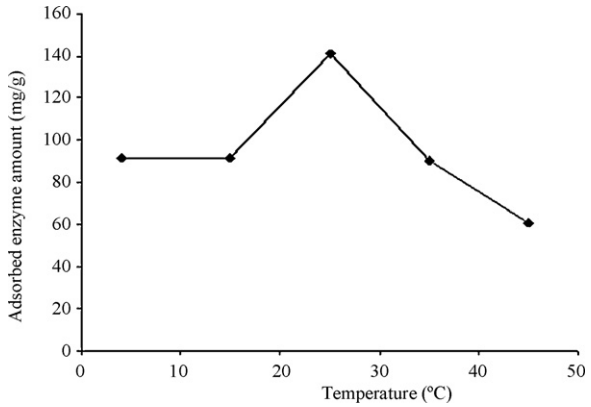


**Fig. 4.** Effect of temperature on laccase adsorption on PHEMAH-$Cu^{2+}$ nanospheres MAH content: 0.42 mmol/g; laccase concentration: 0.5 mg/mL; pH 6.0.

A point worth noting that, there was a low non-specific laccase adsorption (1.5 mg/g) onto PHEMA nanospheres. There are no reactive binding groups or binding sites onto PHEMA nanospheres which interact with laccase molecules. Hence, this non-specific adsorption may be due to weak interactions (van der Waals interaction and hydrogen binding) between laccase and hydroxyl groups on the surface of PHEMA nanospheres. On the other hand, much higher adsorption value was observed when the PHEMAH-$Cu^{2+}$ nanospheres were used (141.9 mg/g) due to the MAH-$Cu^{2+}$ reactive groups on the nanospheres.

The effect of temperature on laccase adsorption were studied in the range of 4–45 °C. The equilibrium adsorption of laccase onto the PHEMAH-$Cu^{2+}$ nanospheres significantly decreased with increasing temperature and the maximum adsorption was achieved at 25 °C (Fig. 4). A possible explanation for this behavior is the exothermic nature of the adsorption process and another explanation can be weakening of van der Waals interactions with increasing temperature.

#### 3.2.2. Effect of pH and temperature on the catalytic activity

The effect of pH on the activity of free and adsorbed laccase by measurement of enzymatic oxidation of ABTS at 420 nm ($\varepsilon = 3.6 \times 10^4\ cm^{-1}\ M^{-1}$ for the oxidation product) was determined in the pH range of 3.0–8.0, and the results are presented in Fig. 5. The maximum activity was obtained at pH 4.0 and pH 5.0 for free and adsorbed laccase. This observation could be explained by the creation of conformational limitations on the enzyme movements as a result of ionic interactions between the enzyme and the $Cu^{2+}$ chelated support. The effect of temperature on enzyme activity was

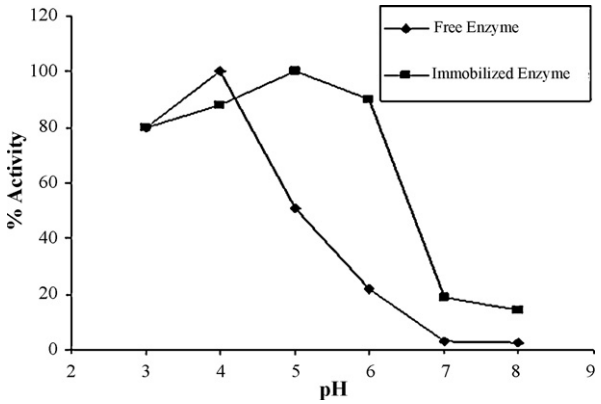


**Fig. 5.** Effect of pH on the free and adsorbed laccase activity MAH content: 0.42 mmol/g; laccase concentration: 0.5 mg/mL; *T*: 25 °C.

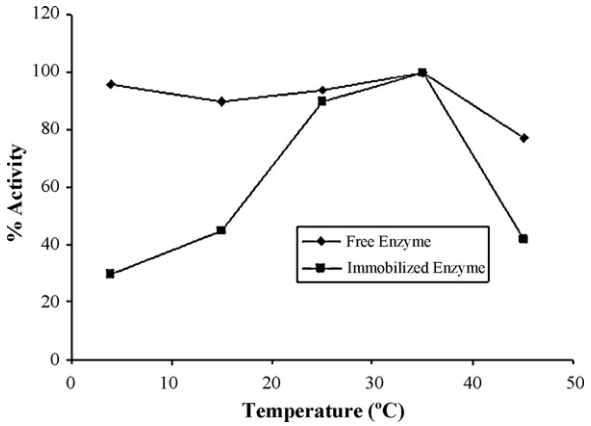


**Fig. 6.** Effect of temperature on the free and adsorbed laccase activity MAH content: 0.42 mmol/g; laccase concentration: 0.5 mg/mL; pH 5.0.

investigated in a acetate buffer (0.1 M, pH 5.0) in the temperature range 4–45 °C. The apparent temperature optimum for free and immobilized enzyme was about 35 °C (Fig. 6).

### 3.2.3. Thermal stability

Effect of temperature on the stability of free and adsorbed laccase is shown in Fig. 7. The pattern of heat stability indicated that a smaller rate of thermal inactivation was observed for the adsorbed laccase on the PHEMAH-$Cu^{2+}$ nanospheres than that of the free enzyme. At 45 °C, the free enzyme retained 26.5% of its initial activity after a 240 min of heat treatment, while the adsorbed enzyme showed significant resistance to thermal inactivation (retaining about 66% of its initial activity after the same period). These results suggest that the thermostability of adsorbed laccase becomes significantly higher at higher temperature. If the heat stability of enzyme increased upon adsorption, the potential application of the enzyme would be extended. Increased thermal stability has been reported for a number of adsorbed enzyme, and the polymer network and multi-point attachment in covalent adsorption method are supposed to preserve the tertiary structure of enzyme.

## 3.3. Kinetic parameters

Kinetic constants of free and adsorbed laccase, i.e. $K_m$ and $V_{max}$ values were determined by measurement of enzymatic oxidation of ABTS at 420 nm ($\varepsilon = 3.6 \times 10^4\ cm^{-1}\ M^{-1}$ for the oxidation product) One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 μmol ABTS in 1 min. The activities of free and adsorbed laccase for various concentrations of the substrate were plotted in the form of Lineweaver–Burk plots. $K_m$ and $V_{max}$ values were calculated from the intercepts on *x*- and *y*-axis, respectively. The apparent $K_m$ value of adsorbed laccase was higher than that of free laccase, and apparent $V_{max}$ value of adsorbed laccase was $4.76 \times 10^{-9}$ U/mg protein and while $V_{max}$ value of free laccase was $6.89 \times 10^{-9}$ U/mg protein. The change in the affinity of laccase to its substrate is probably caused by structural changes in the enzyme introduced by the adsorption procedure and by lower accessibility of the substrate to the active site of adsorbed enzyme [26].

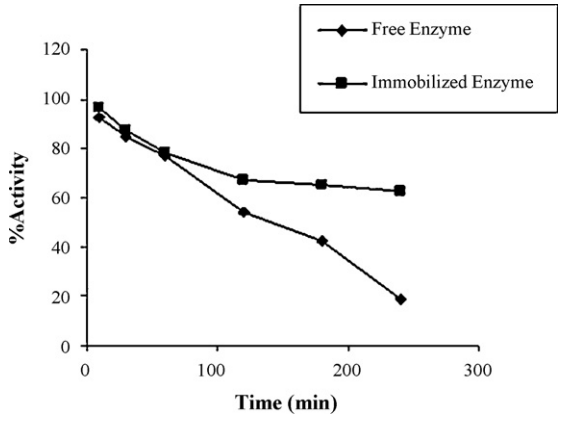


**Fig. 7.** Activity profiles of the free and adsorbed laccase at 45 °C MAH content: 0.42 mmol/g; laccase concentration: 0.5 mg/mL; pH 5.0.

## 3.4. Adsorption isotherm

The Langmuir adsorption is expressed by Eq. (3). The corresponding transformations of the equilibrium data for laccase gave rise to a linear plot, indicating that the Langmuir could be applied in these systems and described by the equation:

$$\frac{C_{eq}}{Q} = \frac{1}{Q_{max}b} + \frac{C_{eq}}{Q_{max}} \quad (3)$$

where $Q$ is the adsorbed laccase concentration (mg/g), $C_{eq}$ the equilibrium laccase concentration in solution (mg/mL), $Q_{max}$ the maximum amount of adsorbed laccase per gram of adsorbent (mg/g), and $b$ is the Langmuir adsorption equilibrium constant (mL/mg).

The range of laccase concentration selected for the adsorption isotherm study was 0.05–2.0 mg/mL. The maximum capacity ($Q_{max}$) data for the adsorption of laccase was obtained from the experimental data. The plot of $C_{eq}/Q_{eq}$ versus $C_{eq}$ yielded a straight line, revealing the adsorption of laccase obeyed the Langmuir adsorption isotherm. From the slope and intercept, the values of $Q_{max}$ and $b$ might be found to be 149.6 mg/g and 2.48 mL/mg, respectively. The correlation coefficient ($R^2$) was 0.9949. The distribution of amino acid residues on the protein surface is very important factor in determining the interactions with the ligand on the matrix. In the aqueous medium, polar or charged residues tend to be on the surface and non-polar residues in the interior. However, each amino acid residue cannot be distributed independently owing to the primary structure of proteins, the distribution varies from one protein to another. Thus, the amino acid residues are by no means all buried.

## 3.5. Reusability of PHEMAH-$Cu^{2+}$ nanospheres

In order to demonstrate the reusability of the PHEMAH-$Cu^{2+}$ nanospheres; the adsorption–desorption cycle of laccase was repeated five times using the same group of nanospheres. The desorption of the adsorbed laccase from the PHEMAH-$Cu^{2+}$ nanosphere was studied in a batch system. The laccase adsorbed onto the nanosphere was desorbed with 0.1 M KSCN solution. The adsorption amount of the PHEMAH-$Cu^{2+}$ nanosphere decreased only 7% during the repeated adsorption–desorption operations. These findings showed that PHEMAH-$Cu^{2+}$ nanospheres can be repeatedly used in reversible enzyme adsorption without any noticeable changes in the initial adsorption amounts.

## 4. Conclusion

Recent developments in nanotechnology have made various nanomaterials more affordable for a broader range of applications. Various nanomaterials have been examined as hosts for enzyme adsorption via approaches including enzyme covalent attachment, encapsulation, and sophisticated combinations of methods [27]. Various nanomaterials, generally providing a large surface area for the adsorption of enzyme molecules, have been actively developed for enzyme stabilization. Only limited work has been published

on the application of nanoparticles in the adsorption of enzymes. Nanoparticles can produce larger specific surface area and, therefore, may result in high adsorption capacity for enzymes. Therefore, it may be useful to synthesize nanoparticles with large surface area and utilize them as suitable nanocarriers for the adsorption of enzymes. One of the most important aims of the enzyme technology is to enhance the conformational stability of the enzyme. The extent of stabilization depends on enzyme structure, adsorption methods, and type of support [28]. In this study, histidine containing nanospheres were prepared by polymerization of HEMA and MAH. The adsorption behavior of laccase onto the nanospheres was investigated using various experimental conditions. It has been shown that pH and temperature have important effects on the adsorption equilibrium and activity of free and adsorbed laccase. The histidine containing nanospheres revealed good adsorption properties as a nanocarrier and will be useful in the enzyme immobilization technology.

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