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Surface functionalization of chitosan-coated magnetic nanoparticles for covalent immobilization of yeast alcohol dehydrogenase from *Saccharomyces cerevisiae*

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ABSTRACT

A novel and efficient immobilization of yeast alcohol dehydrogenase (YADH, EC1.1.1.1) from *Saccharomyces cerevisiae* has been developed by using the surface functionalization of chitosan-coated magnetic nanoparticles (Fe_3O_4/KCTS) as support. The magnetic Fe_3O_4/KCTS nanoparticles were prepared by binding chitosan alpha-ketoglutaric acid (KCTS) onto the surface of magnetic Fe_3O_4 nanoparticles. Later, covalent immobilization of YADH was attempted onto the Fe_3O_4/KCTS nanoparticles. The effect of various preparation conditions on the immobilized YADH process such as immobilization time, enzyme concentration and pH was investigated. The influence of pH and temperature on the activity of the free and immobilized YADH using phenylglyoxylic acid as substrate has also been studied. The optimum reaction temperature and pH value for the enzymatic conversion catalyzed by the immobilized YADH were 30 °C and 7.4, respectively. Compared to the free enzyme, the immobilized YADH retained 65% of its original activity and exhibited significant thermal stability and good durability.

1. Introduction

With the rapid development of nanotechnology, magnetic iron oxide (Fe_3O_4) nanoparticles have shown great potential applications in many biological and medicinal fields [1], such as bioseparation [2], tumor hyperthermia [3], magnetic resonance imaging diagnostic contrast agents [4], magnetically guided site-specific drug delivery agents [5,6], biomolecules (proteins, peptides and enzymes) immobilization [7,8] and so on. As the support of immobilized enzymes, magnetic nanoparticles have many advantages like higher surface area, lower mass transfer resistance, easier to recover by a magnetic field. Thus, there have been many reports on using magnetic nanoparticles to immobilize enzymes including proteases, lipase, penicillin G acylase, glucose oxidase and so on [9–13]. Increasing the loading amount of the enzymes on the magnetic particles could improve the stability of immobilized enzymes. Functionalized magnetic particles with water soluble, biocompatible and reactive functional groups are desired [11]. Chitosan and its derivatives are the most favorable micromolecules used to functionalize magnetic particles [14,15]. Wu and co-workers [14] used magnetic Fe_3O_4 -chitosan nanoparticles for lipase immobilization. The loading ability of the particles reached 129 mg/g and only lost 12% of enzyme activity after five batches.

Alcohol dehydrogenase, which catalyzes the oxidation of alcohols and the reduction of carbonyl compounds such as aldehydes and ketones, has attracted more attention because of its potential applications in preparing various starting materials and intermediates [16–19]. YADH is a special and important alcohol dehydrogenase that exists with large amounts in the microorganisms and livers of animals. Generally, *Saccharomyces cerevisiae* is a good source for this enzyme. In spite of the wide range of applications and interests, YADH is very sensitive and has poor stability in aqueous solutions, which limits its application [20]. YADH immobilization could offer many advantages with the possibility of continuous processing, reusing of the enzyme, and reduction of auto-digestion. To the best of our knowledge, YADH has already been immobilized on various supports such as platinum surface, silica nanotubes-doped alginate gel, agarose, magnetic nanoparticles, [13,20–25]. Till now, research conducted on YADH immobilization onto magnetic nanoparticles is notably scarce. Liao and Chen [23] immobilized alcohol dehydrogenase onto the magnetite nanoparticles, the bound yeast dehydrogenase retained 62% of its original activity and exhibited a 10-fold improved stability than the soluble enzyme using 2-butanone as

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substrate. Moreover, our previous study [13] dedicated to the direct immobilization of YADH onto the magnetic Fe_3O_4-chitosan nanoparticles via glutaraldehyde activation. The immobilized YADH retained 49% activity and exhibited significantly improved stability than the free enzyme.

Very recently, magnetic Fe_3O_4/carboxyl functional chitosan composite (Fe_3O_4/KCTS) nanoparticles have been prepared by covalently binding alpha-ketoglutaric acid chitosan (KCTS) onto Fe_3O_4 magnetic nanoparticles *via* carbodiimide activation in our group [26]. The resultant Fe_3O_4/KCTS nanoparticles are superparamagnetic with a diameter of about 26 nm, and a saturation magnetization of 24.8 emu/g. In the present study, the surface functionalization of chitosan-coated magnetic nanoparticles (Fe_3O_4/KCTS) was used for immobilizing YADH by electrostatic adsorption and covalent binding. The activity of immobilized YADH was measured using phenylglyoxylic acid as substrate and nicotinamide adenine dinucleotide (NADH) as coenzyme. The immobilization conditions were optimized and the effect of pH and temperature on the activity of free and immobilized YADH was determined. This study will prove that magnetic nanomaterials play an important role in enzyme immobilization/stabilization techniques, which will undoubtedly give rise to novel applications of immobilized enzymes in the fields of industry and biomedicine.

2. Material and methods

2.1. Reagents

Strains (*S. cerevisiae*, strain no.3) were obtained from our culture collection. Yeast alcohol dehydrogenase (EC1.1.1.1) from *S. cerevisiae* was prepared as described in section 2.2. Phenylglyoxylic acid and carbodiimides (cyanamide, CH_2N_2) were purchased from Sigma Chemical Co., Ltd. Nicotinamide adenine dinucleotide (NADH) was procured from Roche Ltd. Chitosan (MW 4.9×10^5, degree of deacetylation 95%) was procured from Dalian Xindie Chitin Co. (Dalian, China). 25% Glutaraldehyde solution was purchased from Hunan Normal University Chemical Co. (Hunan, China). Alpha-ketoglutaric acid was purchased from Qianshan Science and Technology Development Company (Zhuhai, China). Ferrous sulphates heptahydrate and aqueous ammonia solution were procured from Tianjin No. 3 Chemical Plant (Tianjin, China). Sodium borohydride ($NaBH_4$) was supplied by Fluka Co. Other chemicals are analytic grade reagents and used without further purification.

2.2. Cultivation and purification of YADH from Saccharomyces cerevisiae

S. cerevisiae was cultivated in bottle using culture medium (g/L): glucose 50, peptone 3.0, yeast extract 2.5, K_2HPO_4 1.0, $MgSO_4\cdot7H_2O$ 0.5, NaCl 0.5, $Fe_2(SO_4)_3$ 0.01, $ZnSO_4$ 0.01, at pH 6.5. The cells were incubated at 30 °C and harvested after 24 h. And the cells were collected from culture medium by centrifugation at $6687.5\times g$ for 15 min at 4 °C. Cell pellets were suspended with 10 ml 0.9% sterile physiological saline per gram of cell pellet and disrupted using Ultrasonic vibra cell crusher KS-250F (power 400 W, 120 times, work 8 s, stop 8 s). The disrupted cell mixture was centrifuged at $6687.5\times g$ for 10 min at 4 °C. The supernatant was collected and precipitated with ammonium sulfate at a final concentration of 3 M. The precipitant was collected by centrifugation at $6687.5\times g$ for 15 min at 4 °C and then resuspended in 5 ml of phosphate buffer (0.2 M, pH 6.8) per 100 mg of precipitant. The resuspended solution was dialyzed overnight against 500 ml of phosphate buffer (0.2 M, pH 6.2). The dialyzed sample was clarified by centrifugation at $12000\times g$ for 20 min and the crude YADH solution was obtained in the supernatant.

2.3. Preparation of surface functionalization of chitosan-coated magnetic nanoparticles

KCTS was prepared according to reference [27]. Fe_3O_4 nanoparticles were prepared by a hydrothermal method with a ferrous complex using H_2O_2 as an oxidizer. Magnetic Fe_3O_4/KCTS nanoparticles were prepared according to the reported method [26]. Typically, Fe_3O_4 nanoparticles (0.2 g) were dispersed in a solution with 30 ml paraffin and 0.5 ml span-80, and then 15.0 ml solution of KCTS in acetic acid with a concentration of 2% was added. The mixture was ultrasonic dispersed for 30 min, and then stirred with 1 ml carbodiimide solution ($30\ g\ L^{-1}$ in 0.003 M phosphate buffer, pH 6.0, 1 M NaCl). After 4 h, the Fe_3O_4/KCTS nanoparticles were recovered from the reaction mixture by a permanent magnet with a surface magnetization of 6000 G, and washed three times with water and ethanol, dried at 50 °C under vacuum. Scheme 1 shows an illustration for preparation of magnetic Fe_3O_4/KCTS nanoparticles.

The resultant Fe_3O_4/KCTS nanoparticles are superparamagnetic with a diameter of about 26 nm, and a saturation magnetization of 24.8 emu/g.

2.4. Covalent immobilization of YADH on magnetic Fe_3O_4/KCTS nanoparticles

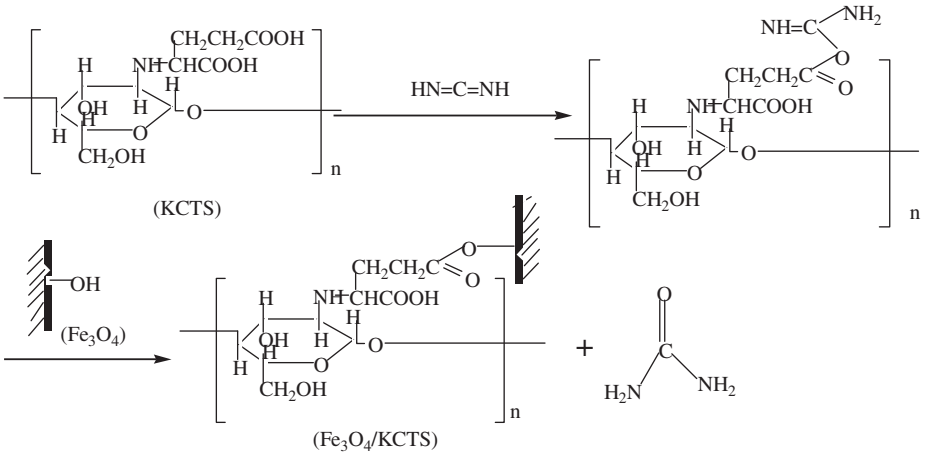
Magnetic Fe_3O_4/KCTS nanoparticles (50 mg) were redispersed in 30 ml 5% glutaraldehyde phosphate buffer solution at pH 6.8 and kept at room temperature for 10 h. The nanoparticles were separated by magnetic decantation and then washed three times with deionized water. Finally, 30 ml YADH diluent solution (5 ml 0.23 mg/ml YADH solution in phosphate buffer (0.02 M, pH 7.4) was added to the particles and continuously shaken at 25 °C at 150 rpm for 2 h. The immobilized YADH was recovered by a magnetic separation, washed with phosphate buffer at pH 7.4 to remove unbound YADH. The obtained immobilized YADH was directly used for the measurements of activity and stability. The supernatant solution was used to detect the amount of unbound enzyme.

2.5. Assay of the amount of YADH immobilized on the magnetic Fe_3O_4/KCTS nanoparticles

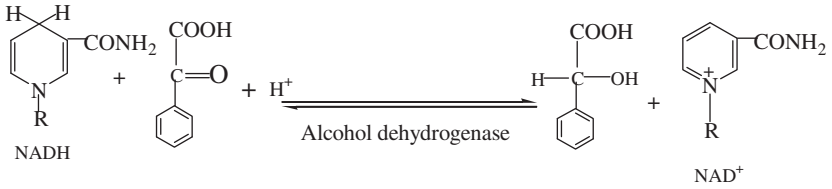
The amount of protein in the YADH solution and in supernatant after immobilization was determined by a Lab Tech UV 2100 spectrophotometer (absorption at 280 nm) with bovine serum albumin as the standard. The amount of protein immobilized on the magnetic nanoparticles was calculated by mass balance as the following formula:

$$Q=\frac{(C_i-C_f)\times V}{W}$$

where Q is the amount of bound YADH onto supports (mg/g), C_i and C_f are the concentration of the YADH protein initial and supernatant solution after immobilization (mg/ml), V is the volume of the medium (ml) and W is the weight of the supports (g). All data used in this formula are the average of duplicated experiments.



Scheme 1. An illustration for preparation of Fe_3O_4/KCTS nanoparticles.



Scheme 2. YADH catalyzed resolution of phenylglyoxylic acid to (R)-mandelic acid.

2.6. Determination of enzymatic activity

The kinetic of YADH catalyzed reduction of phenylglyoxylic acid to (R)-mandelic acid coupling with the oxidation of NADH to NAD^+ was studied (Scheme 2). According to the reference [21–25], the enzyme activity was determined by measuring the initial reduction rate of phenylglyoxylic acid by YADH following the decrease of NADH concentration at 340 nm at the desired temperature. Generally, 4 ml phosphate buffer solution (0.02 M, pH 7.0), 1 ml 1 mM NADH solution and 1 ml 0.1 M phenylglyoxylic acid solution were mixed to the test tube for 5 min, then 10 mg YADH-bound magnetic nanoparticles(5 ml 0.23 mg protein/ml YADH solution immobilized on 50 mg magnetic nanoparticles)was added and mixed for several minutes; the solution was separated from the magnetic nanoparticles *via* a permanent magnet and the decrease was measured in absorbance of NADH at 340 nm in 3 min. The concentration of NADH was calculated from ΔA_{340nm}. Unless specially stated, the amount of YADH to 10 mg magnetic nanoparticles was 1 ml 0.23 mg protein/ml YADH solution. The activity of YADH was determined in phosphate buffer (0.02 M, pH 7.0) at 25 °C, and the concentrations of NADH and phenylglyoxylic acid in the reaction mixture were 1 mM and 0.1 M, respectively.

For free YADH, the activity measurement was done following similar procedures and conditions for the immobilized YADH, except for free YADH solution used.

One unit of YADH activity was defined as the amount of YADH, which used 1 nmol NADH per minute at the assay conditions.

The activity recovery (%) was the ratio between the activity of immobilized YADH and the activity of free YADH.

The relative activity (%) was the ratio between the activity of every sample and the maximum activity of sample.

2.7. Optimization of the reaction conditions of enzymatic activity

To determine the optimum pH and temperature for the reduction of phenylglyoxylic acid to (R)-mandelic acid by free and immobilized YADH, the YADH activity was measured at various temperature (4, 20, 30, 35, 40 and 50 °C) in the presence of phosphate buffer (0.02 M, pH 7.4), and different pH of 5.8, 6.4, 6.8, 7.0, 7.4 and 8.0 at 30 °C, respectively. The highest activity under the optimum conditions was considered as 100% for both free and immobilized YADH, and the relative activity under other reaction conditions was defined as the proportion of the highest activity.

2.8. Thermal stability and reusability of immobilized YADH

For the thermal stability test, 1 ml of free enzyme (430 U/ml) and 10 mg immobilized enzyme (32.98 mg/g) were incubated in a water bath for 60 min at different temperatures ranging from 40 to 70 °C. After each incubation period, the enzyme was quickly chilled in crushed ice for 5 min. The enzyme was brought to room temperature and the residual enzyme activity was determined as described above. The residual activities were expressed as relative to the original activity assayed without heating.

The reusability of immobilized YADH was performed by conducting activity measurement of immobilized YADH at 25 °C at time intervals of 30 min. After each activity measurement, the immobilized YADH was separated by magnetization, washed three times with a phosphate buffer (0.02 M, pH 7.0). Then the fresh NADH and phenylglyoxylic acid solutions were added to the immobilized YADH in sequence and the next activity measurement was carried out and compared with the first run (activity defined as 100%).

3. Results and discussion

3.1. Effect of immobilization time on immobilized enzyme process

Fig. 1 shows the effect of immobilization time on the bound of YADH onto the magnetic Fe_3O_4/KCTS nanoparticles. It was found

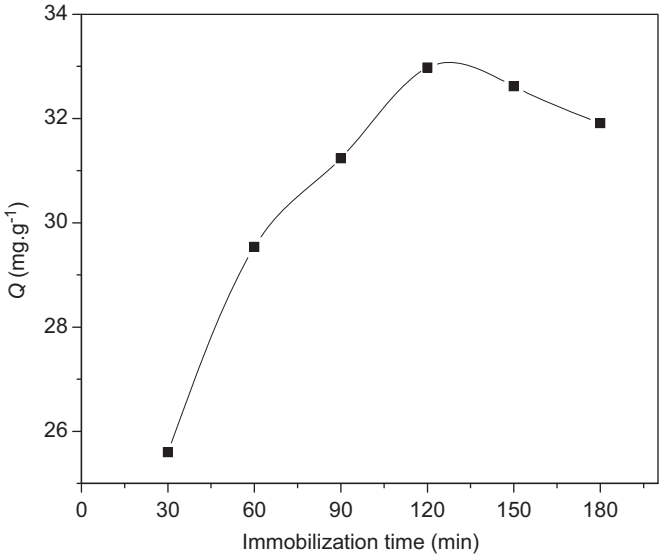


Fig. 1. The effect of immobilization time on immobilized enzyme process (Immobilization conditions: at 0.02 M phosphate buffer (pH 7.4) and 25 °C with 5.0 ml 0.23 mg/ml YADH protein on 50 mg supports).

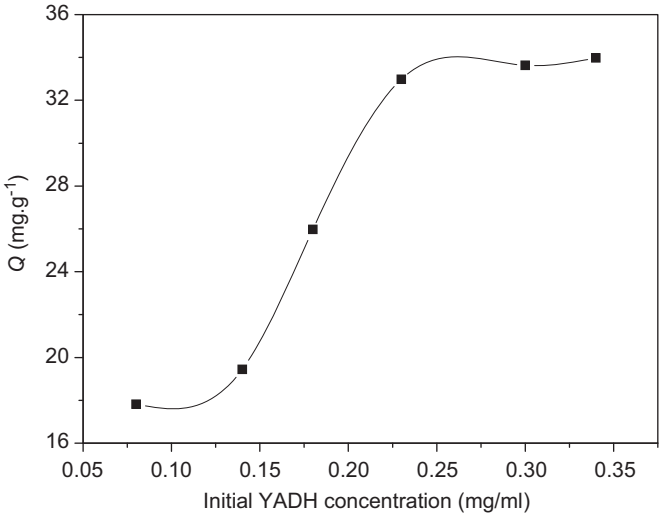


Fig. 2. The effect of concentration of YADH protein on immobilized enzyme process (Immobilization conditions: at 0.02 M phosphate buffer (pH 7.4) and 25 °C with 5.0 ml different concentration of YADH protein on 50 mg supports for 120 min).

that there was a gradual increase of immobilization YADH protein until equilibrium. When the immobilization time was lower than 120 min, the enzyme loading amount on the particles would keep pace with the immobilization time. But when the immobilization time was longer than 120 min, the enzyme loading amount on the particles would decrease. The time necessary to reach equilibrium was about 120 min and significant increases in the adsorbed amounts were not observed after the equilibrium stage. The possible explanation might be the interaction between the functional groups on the surface of particles and YADH enzyme. When these particles were added to the YADH solution, YADH molecule would be absorbed around the particles by electrostatic adsorption first, and then be immobilized onto the particles via a covalent reaction between carboxyl groups of the particles and the amino groups (or thiol, hydroxyl groups) of the YADH.

3.2. Effect of YADH concentration on immobilized enzyme process

In order to investigate the effect of enzyme concentration, initial YADH enzyme concentration was changed between 0.08 and 0.34 mg YADH protein/ml with 5 ml on 50 mg magnetic supports. As seen in Fig. 2, an increase in YADH enzyme concentration in the medium led to an increase in enzyme loading, when the initial YADH concentration of 0.23 mg/ml, the enzyme loading amount reached 32.98 mg/g, then the amount of YADH adsorbed was little affected when the YADH concentration is higher than 0.23 mg/ml. It is a well-recognized fact that the process of protein adsorption on a solid surface is a two-regime process [28]. At the initial stage, the solid surface is bare, and the kinetics of adsorption is governed by the diffusion of the molecules from the bulk solution to the surface. All of the molecules that arrive at the surfaces are assumed to be immediately adsorbed. At the later stage, a barrier of adsorbed molecules exists, and the molecules arriving from solution have to diffuse across this barrier. We can also know that the YADH activity declined after the YADH was immobilized. 65% of the activity of the free enzyme was kept after immobilization when 5 ml 0.23 mg/ml YADH was immobilized on 50 mg magnetic Fe_3O_4/KCTS nanoparticles.

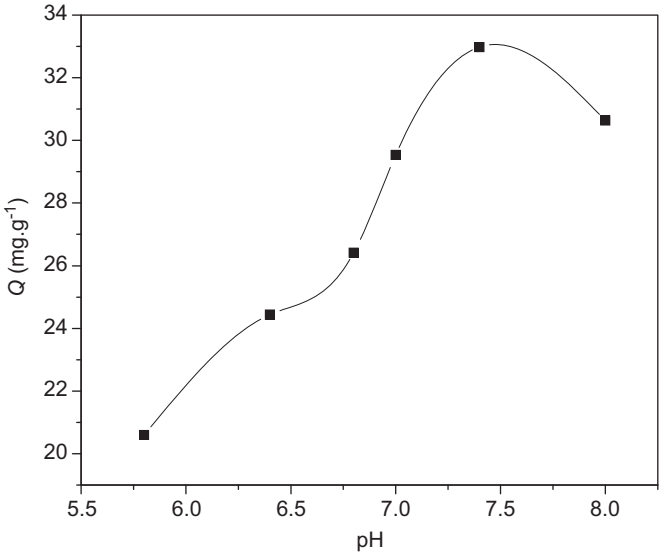


Fig. 3. The effect of pH on immobilized enzyme process(Immobilization conditions: at 25 °C with 5 ml 0.23 mg/ml YADH protein on 50 mg supports for 120 min).

3.3. Effect of pH on the immobilization process

Fig. 3 shows the effect of incubation pH on the immobilization process. The enzyme loading increased on increasing the pH of the solution (pH 5.8–7.4); the highest enzyme loading was gained at pH 7.4. The immobilized behavior showed that adsorption of protein onto magnetic nanoparticles was governed by hydrophobic interaction and by electrostatic interactions [29,30]. The influence of pH showed decreasing affinity with increasing electrostatic repulsion between protein and adsorbent and a maximum plateau value at pH 7.4.

In brief, the optimal conditions of immobilized YADH were: immobilization time 120 min, immobilization pH 7.4 and 5 ml 0.23 mg/ml YADH protein on 50 mg magnetic Fe_3O_4/KCTS nanoparticles. Under these conditions, the activity recovery of the immobilized YADH was 65% and the protein loading was 32.98 mg/g.

3.4. SEM micrographs of the immobilized YADH

The immobilization of YADH on the magnetic Fe_3O_4/KCTS nanoparticles was investigated by scanning electron microscopy (SEM). Fig. 4a and b shows the SEM images of Fe_3O_4/KCTS without and with bound YADH enzyme, respectively. Before enzyme immobilization, the particles remained discrete and had a similar size. After enzyme immobilization, it was observed in Fig. 4b that the particles were covered with a certain amount of enzymes and the particles became uneven with a rough surface. The SEM images confirmed that the YADH molecules have been substantially immobilized on the magnetic Fe_3O_4/KCTS nanoparticles by adsorption.

3.5. Effect of pH on the activity of immobilized YADH

The effect of pH on the activity of the free and immobilized enzyme for the reduction of phenylglyoxylic acid has been studied at different pH ranging from 5.8 to 8 at 30 °C, and the data are shown in Fig. 5. As seen from Fig. 5, the free and immobilized enzymes showed different behaviors of pH dependence, the maximum activity for free YADH can be clearly seen at pH 6.8, and the optimum pH of the immobilized form was found at pH 7.4. From pH 6.8 to 7.4, the relative activity of the immobilized enzyme kept high activity, while the native enzyme was more sensitive than the immobilized enzyme in the range of pH 7.0 to 8.0. Similar phenomena were also observed in previous reports [13,31]. It was presumed that the configuration of YADH fixed on the surface of carriers might lead to an increasing of the enzyme's tolerability to pH variance in surroundings, or the pH of the carriers' inner was changed because of the surface charge of the carriers. It would be ideal if immobilized enzymes could be functional at a wide range of pH values for many practical applications.

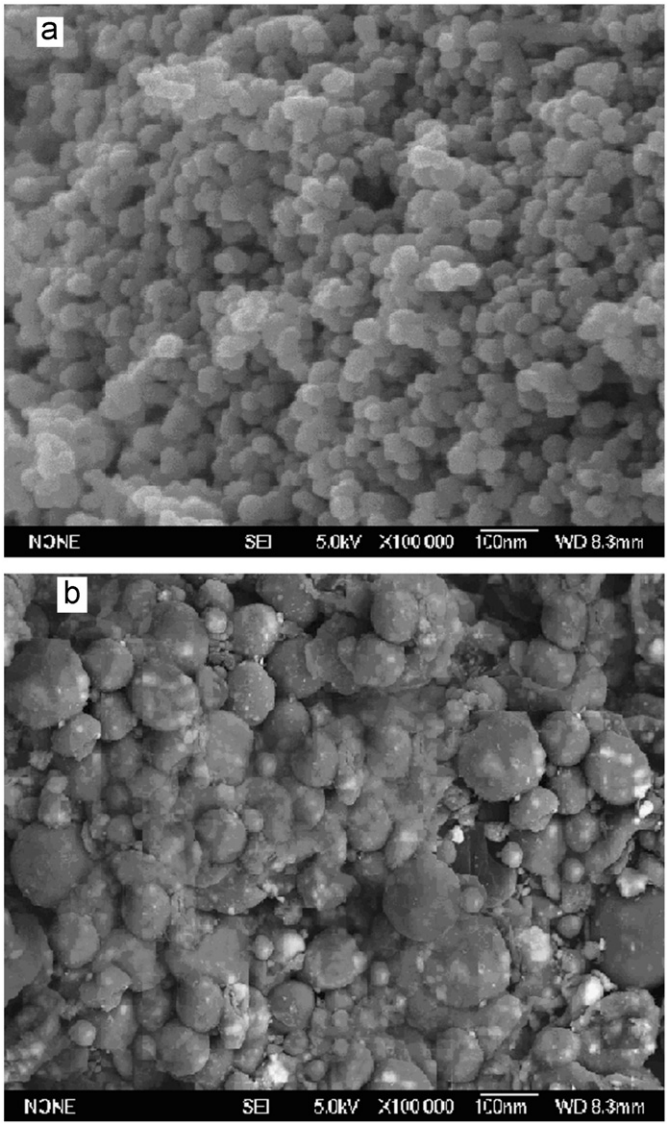


Fig. 4. SEM micrographs of the magnetic nanoparticles without (a) and with (b) bound YADH.

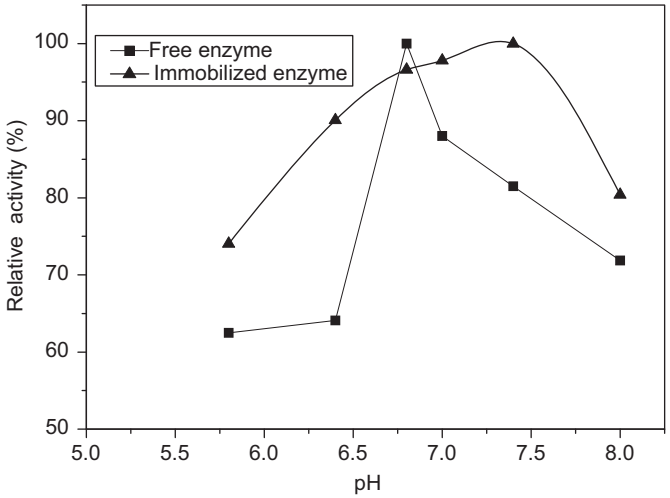


Fig. 5. Effect of pH on the activity of free and immobilized YADH for the reduction of phenylglyoxylic acid (Incubated in phosphate buffer (0.02 M, pH 5.8–8.0) at 30 °C for 5 min).

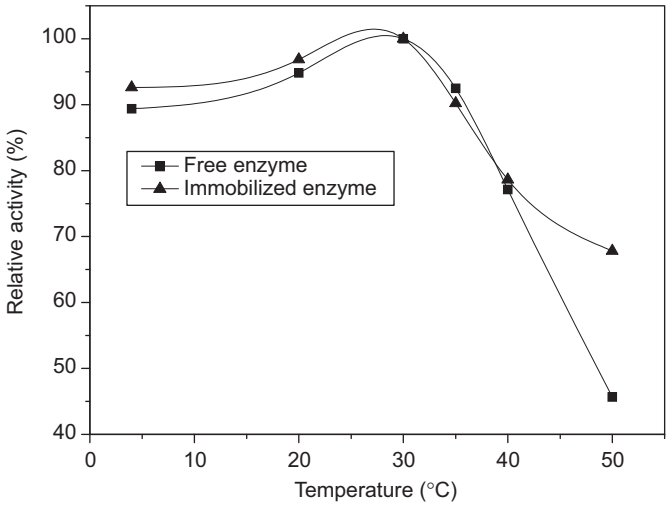


Fig. 6. Effect of temperature on the activity of free and immobilized YADH for the reduction of phenylglyoxylic acid (Incubated in phosphate buffer (0.02 M, pH 7.4) for 5 min in the range of 4–50 °C).

3.6. Effect of temperature on the activity of immobilized YADH

The effect of temperature on the free and immobilized YADH activities were investigated at five different temperatures between 4 and 50 °C in the presence of 0.02 M phosphate buffer (pH 7.4) by using phenylglyoxylic acid as the substrate, and the results

are given in Fig. 6. From Fig. 6, it was found that the optimal temperature for both free and immobilized YADH to achieve the highest activity was 30 °C, which was consistent with that of other research [24,9]. At low temperature section (from 4 to 30 °C), the catalytic activity of free and immobilized YADH increased with the rise of the temperature at first and, after a maximum for both enzyme at 30 °C, decreased at higher temperature. For free enzyme, activity drops dramatically with increasing temperature, which was only 46% relative activity at 50 °C. The activity of immobilized YADH was far less influenced by temperature, which remained 68% relative activity at 50°C. The effect of temperature on the activity of enzyme has two aspects: raising the temperature can speed up the enzymatic reaction and raising the temperature can also make the enzyme denatured. The optimum temperature for enzyme reaction is the comprehensive results of the reaction rate of enzyme and thermal stability. Usually after enzyme immobilization, the optimum temperature will increase with increase in thermal stability, which is a very beneficial result. Of course, the optimal temperature was reported unchanged or decreased [32].

3.7. Thermal stability of immobilized YADH

The thermal stability of the immobilized enzyme is one of the most important criteria with respect to applications. In order to test the thermal stability of immobilized YADH, the thermal stability of free and immobilized YADH was determined by incubation of the enzymes in different temperatures (shown in Fig. 7) for 60 min, and their residual activity was measured. The data in Fig. 7 showed that both free and immobilized YADH enzymes had diminished relative activities from 40 to 70 °C. YADH is a tetrameric metalloprotein of molecular mass 150 kDa and contains two zinc atoms per subunit, one in the active site (catalytic zinc) and one in a loop (conformational zinc). The presence of conformational zinc helps keep the conformation of the active site in a strained state [33]. Residues 40–60 in YADH may function as an intramolecular chaperone and prevent complete unfolding of YADH during thermal stress [34]. It has been reported that the thermal denaturation temperature T_d for YADH was 63 °C, which was caused by the irreversible thermo-inactivation of the protein such as thiol group oxidation, aggregation and deamidation of the protein [35]. From Fig. 7, the free YADH had more enzyme activity below 50 °C, but less activity above 65 °C. Due to the tetrameric nature of this enzyme,

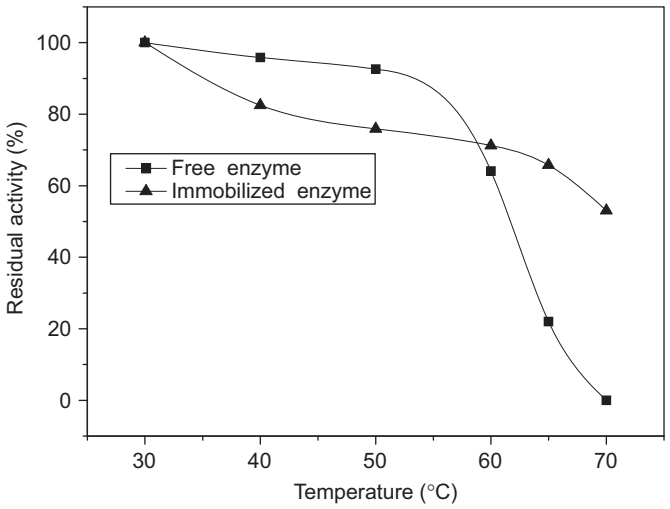


Fig. 7. Thermal stability of free and immobilized YADH (incubated in phosphate buffer (0.02 M, pH 7.0) for 60 min in a range of temperature 40–70 °C).

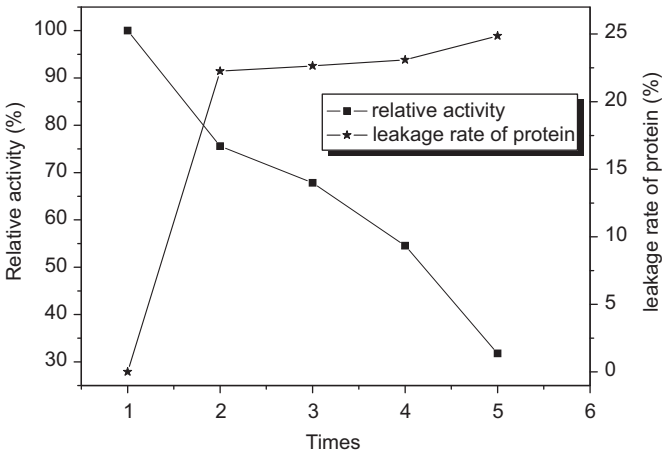


Fig. 8. Reuse of the immobilized YADH for the reduction of phenylglyoxylic acid (The initial activity of the immobilized YADH is 280.75 U).

it is possible that a very intense multi-interaction with the support may have some negative effect on the subunits assembly, reducing the enzyme stability even although each monomer can be more "rigid". Thus, from 40 to 50 °C, the residual activity of immobilized YADH decreased sharply than that of free YADH. However, when the incubation temperatures are above 60 °C, the residual activities of immobilized enzyme are consistently higher than that of free enzyme. Immobilized YADH holds approximately 54% at 70 °C, while free enzyme holds less than 22% at 65 °C and no activity at 70 °C. These observations were in agreement with the findings of some earlier investigators [9,11,12]. Clearly at high temperatures, loss in the YADH activity of soluble form was due to denaturation of enzyme. Thermal stability of YADH improved after immobilization on the magnetic Fe_3O_4/KCTS nanoparticles, which is due to either the creation of conformational limitation on the enzyme movement or a low restriction in the diffusion of the substrate at high temperature. The results indicate that the immobilized YADH enzyme had a good thermal stability.

3.8. Reusability of immobilized YADH

To investigate the reusability of immobilized YADH, several repetitive uses of immobilized YADH were operated. After each cycle, the immobilized YADH was recovered by magnetic separation and recycled for the reduction of phenylglyoxylic acid. The supernatant solution was used to detect the leakage of protein. The activity of the first batch was taken as 100% and the leakage rate of protein is 0. The assay condition remained the same for all batches. Fig. 8 shows variation of activity of the immobilized YADH and the leakage rate of protein after multiple reusing by magnetic separation. Within 5 cycles of usage, the relative activity is about 100%, 76%, 68%, 55% and 32% of the first run, while the leakage rate of protein is 0%, 22%, 23%, 23% and 25%. The decrease in activity may be caused by the leakage of protein from the carriers and the enzyme denaturation. Although the immobilized YADH activity decreased significantly, the immobilized YADH had a good durability and could be readily recovered by magnetic separation. This revealed the magnetic Fe_3O_4/KCTS nanoparticles could be practically used for the immobilization of enzymes.

4. Conclusions

The surface functionalization of chitosan-coated magnetic (Fe_3O_4/KCTS) nanoparticles was used to immobilize YADH by electrostatic adsorption and covalent binding. The process was

simple and effective. The optimal conditions of immobilized YADH were: immobilization time 120 min, immobilization pH 7.4 and 5 ml 0.23 mg/ml YADH protein on 50 mg supports. Under the conditions, the activity recovery of the immobilized YADH was 65% and the protein loading was 32.98 mg/g. For the reduction of phenylglyoxylic acid, the maximal activity of the immobilized YADH appeared at 30 °C and pH 7.4, and the immobilized YADH retained much of its activity in wider ranges of temperature and pH than that of the free form. In addition, the immobilized enzyme maintained a greater rigidity and was more resistant to unfolding at higher temperatures than its free form. The immobilized YADH had good durability and could be readily recovered by magnetic separation. Using magnetic Fe_3O_4/KCTS nanoparticles as an effective carrier for YADH immobilization will be helpful for the practical application of YADH.

Acknowledgements

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