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Immobilization of catalase onto chitosan and cibacron blue F3GA attached chitosan beads

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Abstract

In this study, chitosan beads (Ch-bead) and cibacron blue F3GA attached chitosan beads (CB-Ch-bead) were prepared. Their characteristics were investigated with experiments of swelling, thermogravimetric analysis and Fourier transform infrared (FTIR) spectroscopic analysis. Catalase (CAT) was immobilized onto these beads. The adsorption isotherms have a Langmuirian shape for Ch-beads and CB-Ch-beads. The CAT adsorption capacity of Ch-beads is higher than that of CB-Ch-beads, but CB-Ch-CAT showed better activity according to the Ch-CAT. The values of apparent K_m were found to be 18 and 41 mM for Ch-CAT and CB-Ch-CAT, respectively. However, V_{max} values were calculated as 4800 and 14,250 μmol (mg protein)$^{-1}$ min^{-1} for Ch-CAT and CB-Ch-CAT, respectively. Furthermore, various characteristics of immobilized catalase, such as the temperature profile, thermal stability, optimum pH, operational stability and storage stability were evaluated. Optimum temperature and optimum pH values were found as 35 °C and 7.0 for maximum activity of Ch-CAT and CB-Ch-CAT. It was observed that thermal, storage and operational stabilities of the enzyme were increased with immobilization.

Keywords: Chitosan beads; Cibacron blue F3GA; Catalase; Adsorption; Immobilization

1. Introduction

Dye-ligands have been considered as one of the important alternatives to natural counterparts for specific affinity chromatography. They are commercially available, inexpensive and can easily be immobilized, especially on matrices bearing hydroxyl groups. Dye-ligands are able to bind most types of proteins, especially enzymes, in a remarkably specific manner. The interaction between the dye-ligand and proteins can be by complex combination of electrostatic, hydrophobic and hydrogen bonding [1].

The triazine dye-ligands are usually smaller and simpler molecules than biospecific ligands. They have higher physical and chemical stability. Cibacron blue F3GA (CB) is a monochlorotriazine dye which contains three acidic sulfonate groups and four basic primary and secondary amino groups (Scheme 1). CB can interact group-specifically with proteins. CB can be immobilized onto the supports via the nucleophilic reaction between chloride located triazine ring and reactive groups of the supports, including hydroxyl and amino for protein adsorption [2–7].

Selection of the supporting matrix is the first important consideration in dye-affinity systems. The matrix must have functional surface groups (hydroxyl, carboxyl, amide, etc.) for further derivatization and immobilization of ligands. Chitosan (Ch) is a poly(aminosaccharide), normally obtained by alkaline deacetylation of chitin, the principal component of living organisms, such as fungi and crustacea. It is inexpensive, nontoxic and form gels readily. It has numerous potential application in biomedicine, biochemistry as well as in pharmaceutical, agricultural, cosmetic and chemical industries [8]. In our previous studies, chitosan were used for immobilization of catalase and bakers' yeast [9–11]. Its structure possess large number of reactive groups (hydroxyl and amino) which can be readily modified using different ligands to meet various needs.

Catalase (EC 1.11.1.6) is a heme containing metalloenzyme and regarded as one of the most common enzymes in plant and animal tissues. Catalase consists of four subunits, each of them involving ferriporphyrin as a prostethic group. Catalase which decomposes hydrogen peroxide to water and oxygen has been

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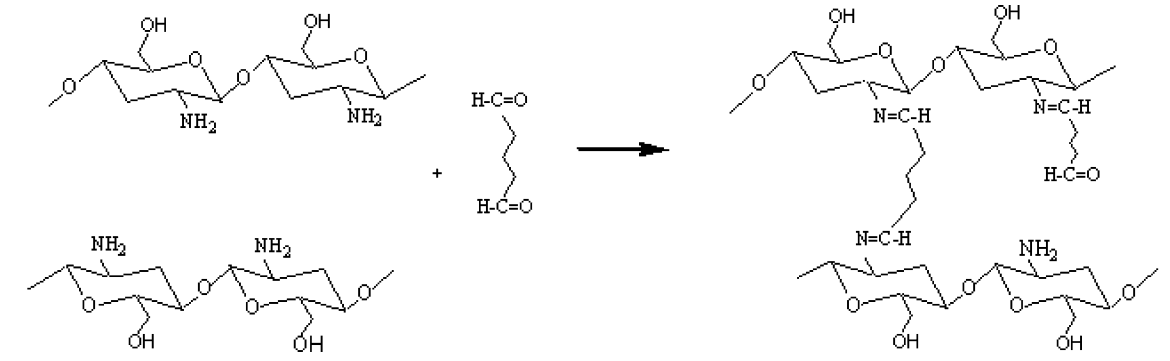

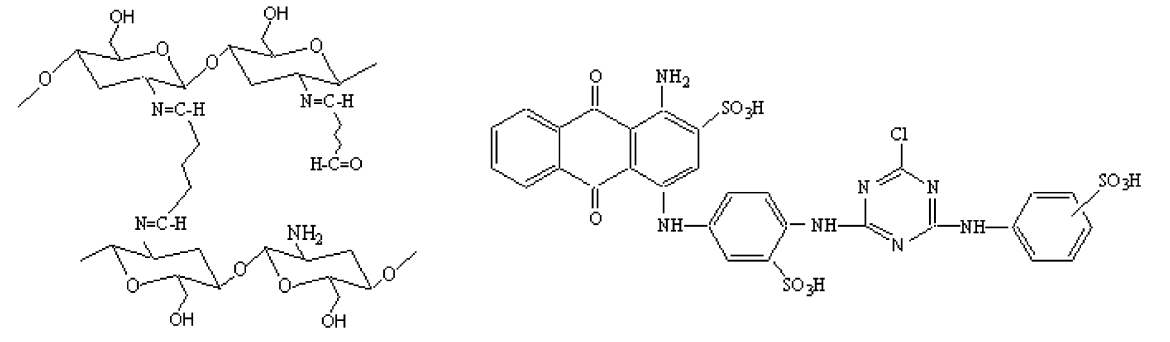

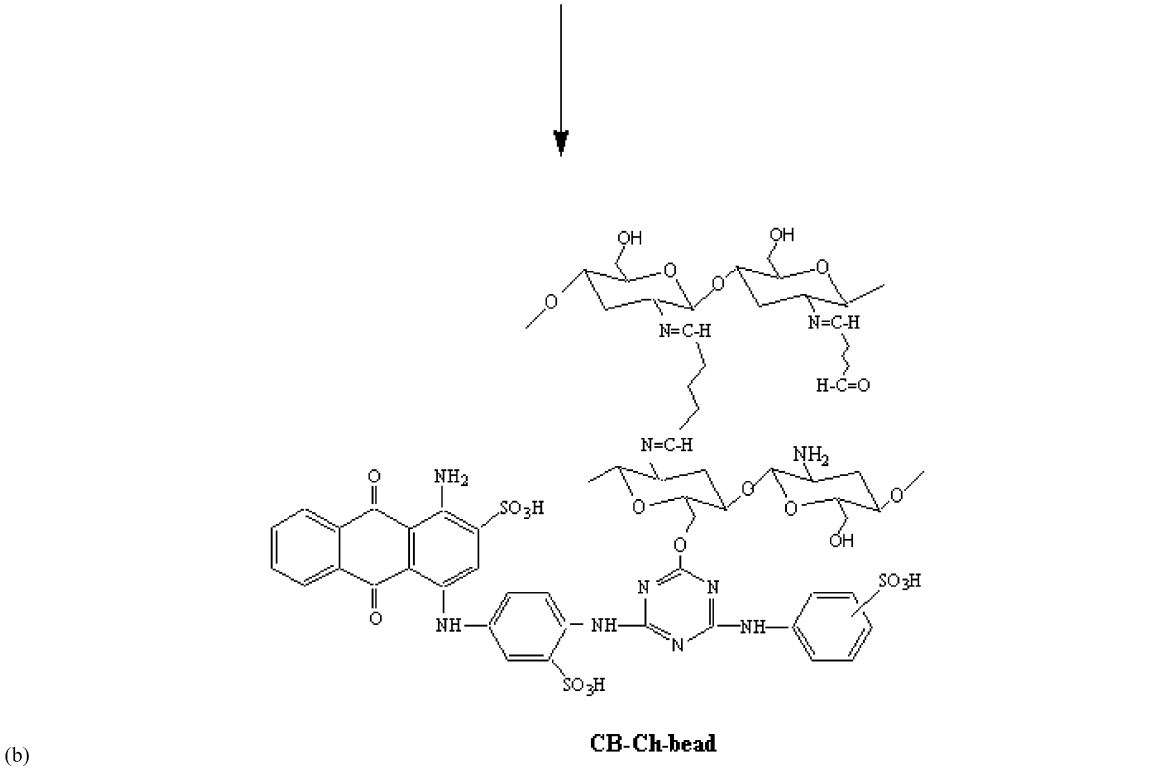

Scheme 1. Possible synthesis mechanisms of Ch-bead (a) and CB-Ch-bead (b).

used in industry for a long time. Immobilized catalase has useful applications in various industrial fields in removal of hydrogen peroxide used as oxidizing, bleaching or sterilizing agent and in the analytical field as a component of hydrogen peroxide or glucose biosensor systems [12–14]. With immobilized enzymes, improved stability, reusability, continuous operation, possibility of better control of reactions, high purity and product yields and hence, more favorable economic factors can be expected [15].

In this study, we aimed to prepare cibacron blue F3GA attached chitosan beads and immobilization of catalase onto this matrix.

2. Material and methods

2.1. Materials

Catalase (hydrogen peroxide oxidoreductase; EC.1.11.1.6) from bovine liver, glutaraldehyde, glyoxal (trimer; dihydrate), cibacron blue F3GA were obtained from Sigma Chemical Co. Chitosan (high molecular weight $M_W \sim 600{,}000$) was obtained from Fluka Chemie AG (Buchs, Germany). Hydrogen peroxide, tetrasodiumpyrophosphate and all other chemicals were obtained from Merck AG.

2.2. Preparation of cibacron blue F3GA attached–chitosan beads

Chitosan beads (Ch-beads) were prepared as reported in our earlier article [10]. Ch (3.0 g) was dissolved in 1.0% acetic acid (100 mL) and extruded dropwise through a syringe equipped with a hypodermic needle into crosslinking solution (glyoxal hydrate solution 4.0% (w/v) mixed with an equal volume of 3.0% (w/v) tetrasodium pyrophosphate solution, pH 8.0). Thereafter, the beads were washed with 50 mM phosphate buffer solution, pH 7.0. For reinforcement of Ch-beads, they were incubated in cold 0.05% (w/v) glutaraldehyde solution for 1 h. The brownish reinforced beads were washed several times by 0.05 M cold phosphate buffer (pH 7.0) and dried on air and then in vacuum.

Cibacron blue F3GA (CB) was used as the affinity ligand. CB immobilization was carried out in alkaline solution to induce covalent binding between the hydroxyl groups of the Ch and the triazine ring of the CB. For preparation of cibacron blue F3GA attached-chitosan beads (CB-Ch-beads), 4.0 g of Ch-beads was immersed at a constant temperature of 80 °C for 4 h with 100 mL of the CB (10 mg mL^{-1}) aqueous solution containing 4.0 g NaOH. Under these experimental conditions, a nucleophilic substitution reaction took place between the chlorine containing group of CB and the hydroxyl group of Ch-beads with the elimination of hydrochloric acid, resulting in covalent attachment of CB to the Ch-beads. After incubation, the CB-Ch-beads were removed from the solution and washed with distilled water, then washed with methanol several times until all the physically attached CB molecules were removed. Then the beads were dried in air and vacuum.

2.3. Immobilization of catalase via adsorption

Immobilization experiments were conducted for 5 h at 20 °C in a rotary shaker. Two hundred and fifty milligrams of chitosan beads (Ch-beads and CB-Ch-beads) were immersed in 50 mL of catalase solution (0.2 mg mL^{-1}) in 0.1 M citric acid, 0.2 M Na_2HPO_4 buffer pH 5.0. After immobilization period, catalase immobilized chitosan beads (Ch-CAT-beads) and cibacron blue F3GA (CB-Ch-CAT-beads) were removed from each solution and washed several times with 50 mM phosphate buffer solution (pH 7.0). Total concentration of catalase in the solutions was determined by the method of Bradford with a UV–vis spectrophotometer [16,17].

2.4. Characterization of Ch-beads and CB-Ch-beads

The swelling behaviour of beads was determined in distilled water and buffer solutions in various pH values. Dry beads were placed in distilled water/buffer solution and kept at a constant temperature, 25 °C. Swollen beads were periodically removed, dried superficially with filter paper and weighed.

Thermogravimetric analysis (TGA) thermograms of chitosan beads were measured using a thermogravimetric analyzer (Shimadzu Model 50). The samples of 10 mg were accurately weighed into an aluminium pan. The measurements were conducted at a heating rate of 10 °C min^{-1} from room temperature to 600 °C under nitrogen purge.

Fourier transform infrared (FTIR) of the CB, Ch-beads and CB-Ch-beads were recorded between 4000 and 400 cm^{-1} on a Nicolet 520 FTIR spectrometer as KBr pellets.

2.5. Activity assay of catalase

The activity of catalase was determined spectrophotometrically by direct measurement of the decrease in the absorbance of hydrogen peroxide at 240 nm due to its decomposition by the enzyme. Hydrogen peroxide solutions (2.5–10 mM) were used to determine the activity. 0.1 g of catalase immobilized chitosan beads (Ch-CAT-beads and CB-Ch-CAT-beads) were mixed with 10 mL of hydrogen peroxide solution in 50 mM phosphate buffer (pH 7.0) at 35 °C. After 5 min, the reaction was terminated by removal of the chitosan beads from the reaction mixture. The absorbance of the reaction mixture was determined and the immobilized catalase activity was calculated.

The activity assays were carried out over the pH range of 3.0–8.0 and temperature range of 25–55 °C to determine the pH and temperature profiles of immobilized enzyme. Activity of pH profiles was determined at various pH in 10 mM hydrogen peroxide solution at 35 °C. Activity of temperature profiles was determined at indicated temperatures in 10 mM hydrogen peroxide solution (pH 7.0).

2.6. The thermal stability of immobilized catalase

The thermal stability of immobilized catalase on chitosan beads was ascertained by measuring the residual activity of enzyme exposed to various temperatures (25–65 °C) in 50 mM phosphate buffer for 5 h. After incubation at indicated temperature, activities were assayed at 35 °C by using 10 mM H_2O_2 prepared in 50 mM phosphate buffer at pH 7.0.

2.7. The storage stability of immobilized catalase

The activity of free and immobilized catalase was measured as fresh and after stored in phosphate buffer (50 mM, pH 7.0) at 4 °C in a batch operation mode with the experimental conditions given above.

2.8. The operational stability of immobilized catalase

The retention of enzyme activity was tested in a batch system as described in Section 2.5. After each reaction period, Ch-CAT-beads and CB-Ch-CAT-beads were removed from reaction medium and washed with phosphate buffer (50 mM, pH 7.0) to remove any residual substrate within/on the beads. They were then reintroduced into fresh reaction medium and enzyme activities were detected at optimum conditions.

Each experiment was performed at least three times and average values were reported. The results of pH, temperature, reusability and storage stability of immobilized catalases were presented in a normalized form, with the highest value of each set being assigned the value of 100% activity.

2.9. Desorption and regeneration studies of Ch-CAT and CB-Ch-CAT

To investigate the regeneration of Ch-CAT and CB-Ch-CAT, catalase adsorption and desorption cycle was investigated by using the same beads. Catalase immobilized chitosan beads were treated with 0.1 M NaSCN (pH 8.0) at room temperature for 2 h. The beads were removed from the desorption medium, washed several times with phosphate buffer (50 mM, pH 7.0) and were then reused in enzyme immobilization. This adsorption–desorption process was carried out four times.

3. Results and discussion

3.1. Characteristics of the beads

Ch-beads and CB-Ch-beads were prepared as described in Section 2. The main features of the synthesis of the CB-Ch-beads may be divided into two distinct stages, which are illustrated in Scheme 1.

The first stage involves to the crosslinking process displayed in Scheme 1a. The free amine $-NH_2$ groups of chitosan are crosslinked by glutaraldehyde, through covalent interaction of both aldehyde $-COH$ groups present at the ends of this linear dialdehyde molecule. In the second stage showed in Scheme 1b, the attaching process takes places, forming CB-Ch-beads. The hydroxyl group of the Ch-beads might be attached with the triazine ring of CB by covalent binding.

Swelling behavior of Ch-beads and CB-Ch-beads were followed gravimetrically. Swelling degree ($S\%$) of the beads was calculated from the following relation:

$$S\% = \frac{(m_t - m_0)}{m_0} \times 100 \quad (1)$$

where m_t is the mass of swollen bead at time t and m_0 is the initial mass of the dry bead.

The swelling curves of the beads in distilled water are shown in Fig. 1a. As seen in the figure, the beads swell, approach the equilibrium-swelling value in distilled water rapidly within 70 min and then reached a constant value.

For extensive swelling of beads, following second-order kinetics, the following equation can be used:

$$\frac{t}{S} = A + Bt \quad (2)$$

where A is reciprocal of initial swelling rate, r_i or $1/k_s S_{max}^2$ and B is inverse of the degree of swelling at equilibrium.

To test the kinetic model, t/S versus t graphs were plotted (Fig. 1b). The initial swelling rate (r_i), the swelling rate constant (k_s) and the theoretical equilibrium swelling (S_{max}) of the beads are calculated and tabulated in Table 1 . These results observed that the swelling process of CB-Ch-beads is quicker than the swelling rate of Ch-beads.

The variation in swelling degree of beads with pH is presented in Fig. 2. As seen in this figure, the CB-Ch-beads swell much greater than the Ch-beads at various pH values.

The TGA curves of chitosan beads are presented in Fig. 3. The thermogram exhibits two distinct stages. One is in the range of 20–220 °C due to water loss, which is adsorbed both on the surface and in the pores of the beads, the other in the range of 220–420 °C is ascribed to a complex process, including dehydration of the sugar rings, depolymerization and decomposition of the chitosan. It can be observed from the profiles of the TG curves, both Ch-beads and CB-Ch-beads are practically similar.

Table 1
The swelling parameters of chitosan beads

Beads	$r_i \times 10^2$ (g (g bead)$^{-1}$ min^{-1})	k_s (g bead g^{-1} min^{-1})	S_{max} (g (g bead)$^{-1}$)
Ch-bead	11.1	62.05×10^{-3}	1.40
CB-Ch-bead	14.2	55.37×10^{-3}	1.60

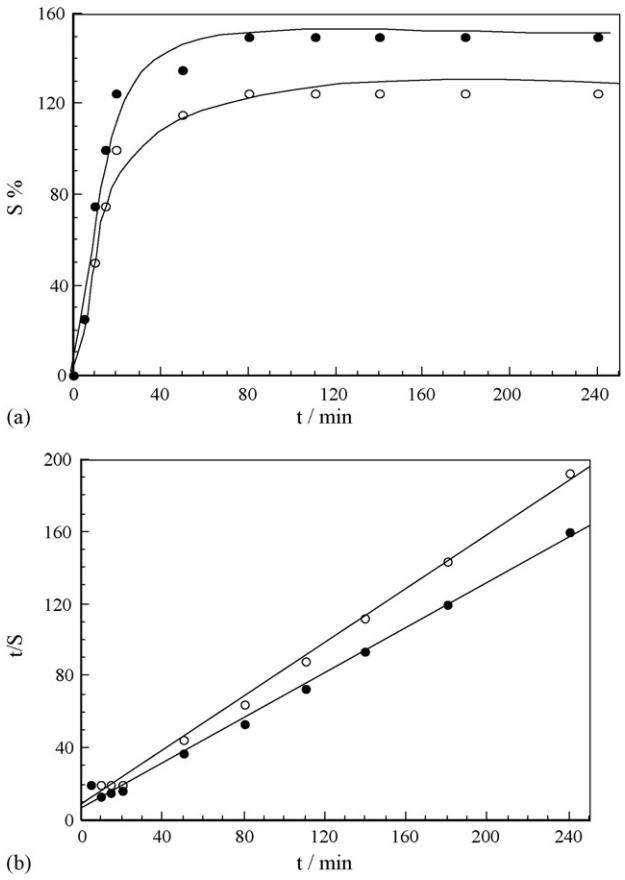

Fig. 1. Swelling (a) and second-order swelling kinetic (b) curves of beads: (●) CB-Ch-bead and (◯) Ch-bead.

To examine the nature of the interaction between the dye (CB) and the Ch-beads, FTIR spectra of plain CB, Ch-beads and CB-Ch-beads were obtained.

The spectrum of Ch-beads (Fig. 4.) shows the characteristic absorption bands at 1587 cm^{-1} (amide) and 1380 cm^{-1} ($-CH_2$) bending. The absorption bands at 1160 cm^{-1} (anti-symmetric stretching of the C–O–C bridge), 1075 and 1040 cm^{-1} (skeletal

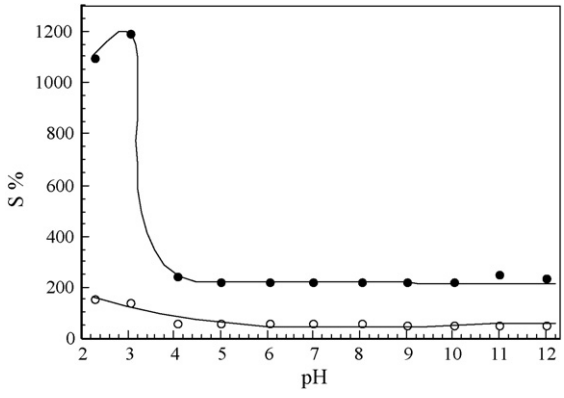

Fig. 2. The influence of pH to swelling of the beads: (●) CB-Ch-bead and (◯) Ch-bead.

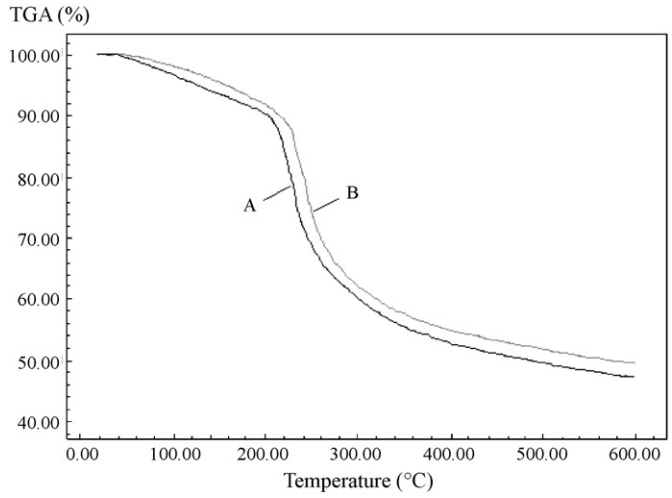

Fig. 3. TGA curves of Ch-bead (A) and CB-Ch-bead (B).

vibrations involving the C–O stretching) are characteristics of its saccharide structure. The bond at 1651 cm^{-1} is attributed the imine bond (N=C) formed from the interaction of glutaraldehyde and amine groups attached to the Ch for Schiff base formation.

In the FTIR spectrum of CB (Fig. 4.), the band at 1630 cm^{-1} shows a C=N stretching band in the structure of the triazine ring. The bands at 1080 and 1180 cm^{-1} represent symmetric stretching of S=O and asymmetric stretching of S=O, respectively. The band at 630 cm^{-1} is a C–Cl band.

The presence of CB onto Ch-beads was easily detected by the change of the Ch-bead's colour from white to blue form. It is shown in the spectrum of CB-Ch-beads that (Fig. 4.) after covalent attachment of CB, the C–Cl band at 600 cm^{-1} is lost.

3.2. Binding properties of catalase

3.2.1. Adsorption isotherms

In this stage, it was purposed that investigation of uptake of catalase via the chitosan beads and usability as a sorbent for catalase. The equilibrium adsorption of catalase on Ch-beads was determined in batch experiments. Immobilization capacity was calculated by using the following relationship:

$$Q = \frac{(C_0 - C_e)}{m} V \tag{3}$$

where Q is the amount of catalase immobilized onto unit mass of the chitosan beads (mg g^{-1}), C_0 and C_e the concentration of the catalase in the initial solution and in the aqueous phase after immobilization, respectively (mg mL^{-1}), V the volume of the solution (mL) and m is the mass of the chitosan beads (g).

The adsorption isotherm for catalase binding is shown in Fig. 5a. The adsorption of catalase onto chitosan beads, all corresponded to type L (Langmuir type) adsorption isotherms as listed in the Giles classification system for the adsorption of a solute from its solution [18]. The linear form of the Langmuir equation is

$$\frac{C}{Q} = \frac{1}{Q_{mon} K} + \frac{1}{Q_{mon}} C \tag{4}$$

where Q_{mon} is the mass of solute adsorbed per unit mass of adsorbent in forming a complete monolayer on the surface and K is a constant related to the energy. These values were determined from the straightline plot of C/Q against C by linear regression (Fig. 5b) and tabulated in Table 2.

In case of Ch-CAT, glutaraldehyde is bound to free amino groups of chitosan, a Schiff's base structure will be occurred. This structure should introduce free aldehyde groups of glutaraldeyhyde onto chitosan beads to facilitate the reaction with

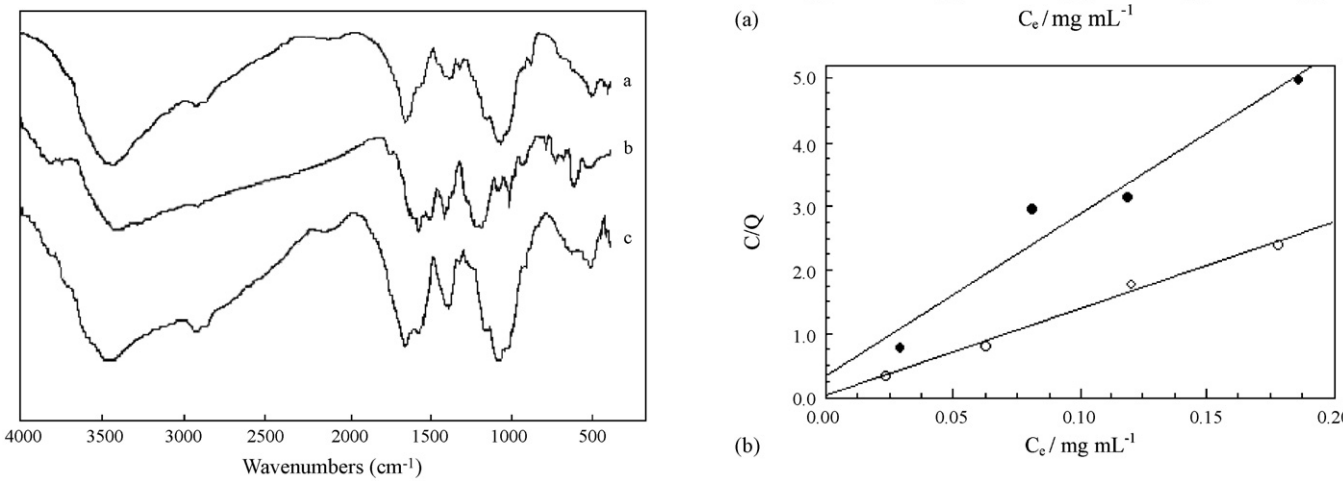

Fig. 4. FTIR spectra of Ch-bead (a), CB (b) and CB-Ch-bead (c).

Fig. 5. The isotherms of adsorption (a) and Langmuir graph (b): (●) CB-Ch-CAT and (○) Ch-CAT.

Table 2
Properties of free and immobilized catalase onto Ch-beads and CB-Ch-beads

Form of enzyme	Q_{mon} (μg g^{-1})	K (L mg^{-1})	V_{max} (μmol (mg protien)$^{-1}$ min^{-1})	K_m (mM)	Activity (U g^{-1})
Free CAT	–	–	32,000	35	–
Ch-CAT	74	696,610	4,800	18	3555
CB-Ch-CAT	39	72,546	14,250	41	5565

enzyme. After that, ε-amino group of Lys residues on protein surfaces can be bound to free aldehyde groups of glutaraldehyde [11].

In case of CB-Ch-CAT, it is possible that enzyme readily prefers interaction with dye molecules compared to free aldehyde groups on chitosan. Generally, whether specific or nonspecific protein–dye binding can be described in terms of electrostatic and/or hydrophobic interactions [19]. The isoelectric point of catalase is 5.6. Catalase is positively charged at pH 5.0. Because CB molecules on the CB-Ch-beads are negatively charged, the dominant force contributing to dye and protein interactions is electrostatic rather than hydrophobic. We found that maximum adsorption capacity of Ch-beads is much greater than the CB-Ch-beads. As amount of dye molecules increases on support, bound protein amount may increases. In this study, amount of immobilized protein may depend on amount of the dye molecules on chitosan. Thus, it is possible that the amounts of bound dye and so, the attached protein amount is lower than that of Ch-beads.

3.2.2. Thermodynamic behavior of immobilization

The study of the temperature effect on catalase immobilization by Ch-beads and CB-Ch-beads enabled us to determine the thermodynamic parameters (ΔH, ΔG and ΔS) of immobilization processes by using the following relationships:

$$\ln \frac{Q_2}{Q_1} = \frac{\Delta H}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right), \quad T_1 = 20^\circ\text{C and } T_2 = 30^\circ\text{C} \quad (5)$$

$$\Delta G = -RT \ln K \quad (6)$$

$$\Delta G = \Delta H - T\Delta S \quad (7)$$

These values are shown in Table 3. A negative ΔG value indicates that the immobilization process is spontaneous with both materials Ch-beads and CB-Ch-beads. A negative ΔH value shows also that immobilization of catalase is an exothermic process on both materials. The positive value of ΔS indicates an increasing disorder at the solid solution interface for Ch-CAT. The negative value of ΔS indicates a decreasing disorder at the solid solution interface for CB-Ch-CAT. The ΔH value of CB-Ch-CAT (>20 kJ mol^{-1}) indicates that the dominant force contributing to dye and CAT interactions is electrostatic.

Table 3
Thermodynamic parameters of chitosan beads

Beads	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
Ch-CAT	−22.79	−32.774	+34.00
CB-Ch-CAT	−63.26	−27.26	−122.26

3.3. Kinetic parameters, optimum temperature and optimum pH of free and immobilized catalase

The kinetic parameters, K_m (Michaelis constant) and V_{max} (maximum reaction rate) for free and immobilized catalase were determined by varying the concentration of hydrogen peroxide in the reaction medium. These parameters are presented in Table 2.

As expected, the K_m and V_{max} values were significantly affected after immobilization into chitosan beads. The change in the affinity of the enzyme to its substrate is probably caused by structural changes in the enzyme introduced by the immobilization procedure or by lower accessibility of the substrate to the active site of the immobilized enzyme. This indicated that the structure of enzyme probably occurs to a certain degree of change, or large deformation of enzymatic conformation due to the interaction of enzyme molecules or enzyme and support. The change in the affinity might be attributed to the structural change of the enzyme introduced by the immobilization procedure, which can lower accessibility of the substrate to the active side [20]. The V_{max} value of immobilized catalases was found lower than that of free catalase. The loss in activity might be attributed to the interaction of enzyme and the functional groups on the surface of beads or large areas of contact between enzyme and support causing large deformations of enzymatic conformation. In this study, we found that the V_{max} value of CB-Ch-CAT was bigger than that of the Ch-CAT. Similar results involving change in K_m and V_{max} values of enzyme after immobilization have been reported in literature [9,10,21–24].

The temperature dependence of the activities of the free and immobilized catalase were studied in 50 mM phosphate buffer (pH 7.0) in temperature range 25–55 °C and temperature profiles of free and immobilized catalase are shown in Fig. 6. This results show that CB-Ch-CAT activity was higher than that of the Ch-CAT for higher temperature. Optimum temperature was found at about 35 °C for free catalase and immobilized catalase.

The effect of pH on the activity of free and immobilized catalase preparations for hydrogen peroxide degradation was studied at various pH values at 35 °C. The reactions were carried out in acetate and phosphate buffers and the results are presented in Fig. 7. Optimum pH for free and immobilized enzymes is the same as pH 7.0. Catalase activity decreases by half either by decreasing pH from 7.0 to 3.0 or increasing pH from 7.0 to 8.0.

3.4. Thermal stability of catalase

Thermal stability was investigated by incubating immobilized catalases and free catalase at temperatures ranging from 25 to 65 °C in 50 mM phosphate buffer (pH 7.0) for 5 h and then determining the activity at 35 °C. The effect of tempera-

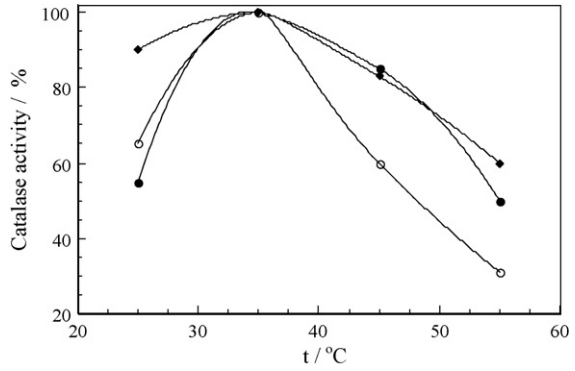

Fig. 6. Temperature profiles of catalase: (●) CB-Ch-CAT, (◯) Ch-CAT and (♦) CAT (activities were assayed at indicated temperatures by using 10 mM H_2O_2 prepared in 50 mM phosphate buffer at pH 7.0).

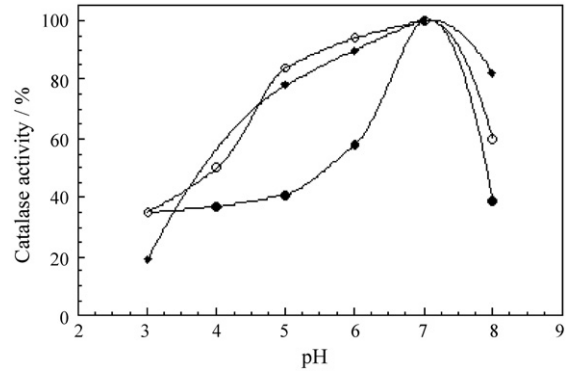

Fig. 7. pH profiles of catalase: (●) CB-Ch-CAT, (◯) Ch-CAT and (♦) CAT (activities were assayed at 35 °C by using 10 mM H_2O_2 prepared in appropriate buffer solution).

ture on stability of catalase is illustrated in Fig. 8. The activity of free enzyme decreased with increase in temperature. Ch-CAT and CB-Ch-CAT have significant activity about 25–45 °C. Immobilized catalases have shown high thermal stability at 35–45 °C. According to these results, we can say that immobilization of catalase in chitosan beads preserve tertiary structure of the enzyme and it protects the enzyme from conformational changes caused from environmental effect. It is often observed that immobilized enzyme has a higher thermal stability than the

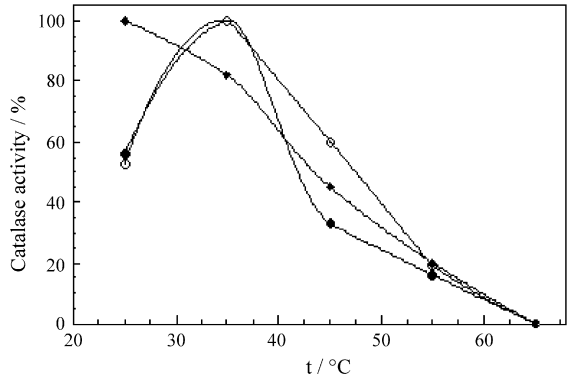

Fig. 8. Thermal stability of catalase: (●) CB-Ch-CAT, (◯) Ch-CAT and (♦) CAT (after incubation at indicated temperature, activities were assayed at 35 °C by using 10 mM H_2O_2 prepared in 50 mM phosphate buffer at pH 7.0).

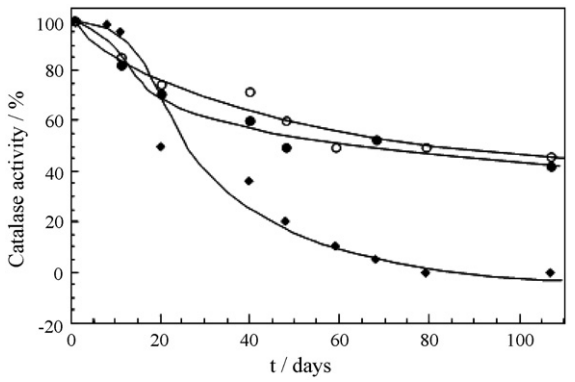

Fig. 9. Storage stability of catalase: (●) CB-Ch-CAT, (◯) Ch-CAT and (♦) CAT (immobilized enzymes were stored at 4 °C in 50 mM phosphate buffer at pH 7.0. Activities were assayed at 35 °C by using 10 mM H_2O_2 prepared in 50 mM phosphate buffer at pH 7.0).

corresponding free enzyme because of the reduction of conformational flexibility in the immobilized enzyme [15].

3.5. Storage stability of catalase

Free and immobilized catalases were stored in a phosphate buffer (50 mM, pH 7.0) at 4 °C and the activity measurements were carried out for a period of 100 days (Fig. 9). Free catalase lost about 50% of its activity within 20–25 days and lost its remaining activity within 70 days, whereas Ch-CAT and CB-Ch-CAT lost about 50% of their activity within 60 and 70 days, respectively. These two immobilized catalase protected 50% of their activity after 70 days. The half-life of Ch-CAT and CB-Ch-CAT were found higher than that of free catalase. The decrease in activity was explained as a time-dependent natural loss in enzyme activity and this was prevented to a significant degree by immobilization.

3.6. Operational stability of catalase

Free enzymes can be used only one time but immobilized enzymes can be used several times [15]. Operational stability curve was shown in Fig. 10. Operational stability of the

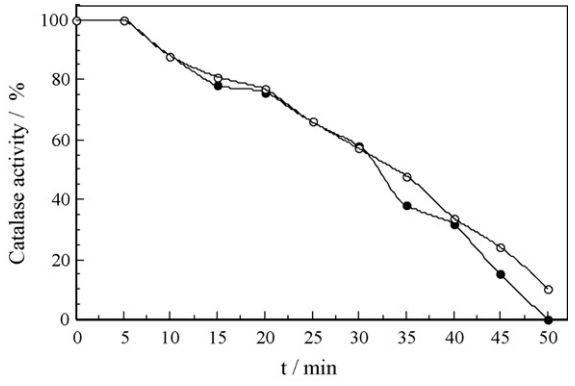

Fig. 10. Reusability of catalase: (●) CB-Ch-CAT and (◯) Ch-CAT (activities were assayed at 35 °C by using 10 mM H_2O_2 prepared in 50 mM phosphate buffer at pH 7.0).

immobilized catalase was determined for eight successive batch reactions at 35 °C for 5 min. It was observed that immobilized catalase retained about 50% of its activity after 7–8 cycles. It was found that Ch-CAT and CB-Ch-CAT have higher operational stability.

3.7. Desorption and regeneration of Ch-CAT and CB-Ch-CAT

Desorption of catalase from chitosan beads were carried out in batch system. Ch-CAT and CB-Ch-CAT were placed within the desorption medium containing 0.1 M NaSCN (pH 8.0) at room temperature for 2 h. After the beads were washed, they were reused for immobilization of catalase. To investigate the reusability of chitosan beads support, the adsorption–regeneration cycle was repeated four times using the same chitosan beads. There is no significant adsorption capacity loss observed (data not shown).

4. Conclusion

Chitosan beads and cibacron blue F3GA attached chitosan beads were prepared. Some properties of these beads were investigated. The immobilization of catalase onto these matrixes was investigated. The following conclusions can be drawn from the results of the present study:

- Since chitosan beads contain chemically reactive groups (−OH and $-NH_2$ groups), their surfaces can be easily coupled with various affinity ligands. So, CB can be coupled to the Ch-beads easily.
- The adsorption isotherms have a Langmuirian shape for Ch-beads and CB-Ch-beads. With increasing the equilibrium catalase concentration, the amount of adsorbed catalase increases almost linearly low concentration, then increases less rapidly and approaches saturation.
- The CAT adsorption capacity of Ch-beads is higher than that of CB-Ch-beads. But activity of the CB-Ch-CAT is higher than that of Ch-CAT. The CB-Ch-beads swell much greater than the Ch-beads in distilled water and various pH values. Changes in swelling of beads affect bead porosity and diffusion of substrate. So the substrate in solution is diffused into the CB-Ch-beads easily and the CB-Ch-CAT showed better activity towards the Ch-CAT.
- The isoelectric point of catalase is 5.6. Catalase is positively charged at pH 5.0. Because CB molecules on the CB-Ch-beads are negatively charged, the dominant force contributing to dye and protein interactions is electrostatic rather than hydrophobic.
- Optimum temperature values were found as 35 °C and optimum pH values were determined as 7.0 for CAT, Ch-CAT and CB-Ch-CAT.
- Immobilized catalases showed better thermal and storage stabilities.
- The enzyme could retain about 50% of its activity after 7–8 repetetive cycles.
- The present work demonstrated potential use of cross-linked chitosan beads in dye-affinity systems and enzyme immobilization.

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