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# Immobilization of catalase onto Eupergit C and its characterization

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ABSTRACT

Bovine liver catalase was covalently immobilized onto Eupergit C. Optimum conditions of immobilization: pH, buffer concentration, temperature, coupling time and initial catalase amount per gram of carrier were determined as 7.5, 1.0 M, 25 °C, 24 h and 4.0 mg/g, respectively. $V_{max}$ and $K_m$ were determined as $1.4(\pm0.2)\times10^5$ U/mg protein and $28.6\pm3.6$ mM, respectively, for free catalase, and as $3.7(\pm0.4)\times10^3$ U/mg protein and $95.9\pm0.6$ mM, respectively, for immobilized catalase. The thermal stability of the immobilized catalase in terms of half-life time (29.1 h) was comparably higher than that of the free catalase (9.0 h) at 40 °C. Comparison of storage stabilities showed that the free catalase completely lost its activity at the end of 11 days both at room temperature and 5 °C. However, immobilized catalase retained 68% of its initial activity when stored at room temperature and 79% of its initial activity when stored at 5 °C at the end of 28 days. The highest reuse number of immobilized catalase was 22 cycles of batch operation when 40 mg of immobilized catalase loaded into the reactor retaining about 50% of its original activity. In the plug flow type reactor, the longest operation time was found as 82 min at a substrate flow rate of 2.3 mL/min when the remaining activity of 40 mg immobilized catalase was about 50% of its original activity. The resulting immobilized catalase onto Eupergit C has good reusability, thermal stability and long-term storage stability.



## 1. Introduction

Catalase (EC 1.11.1.6) has many industrial applications, including the elimination of $H_2O_2$ after sterilization of milk [1] and textile bleaching [2], production of gluconic acid with glucose oxidase [3], synthesis of dihydroxyacetone phosphate with L-α-glycerophosphate oxidase [4], production of phenylpyruvic acid with D-aminoacid oxidase [5]. Moreover, catalase is also used in $H_2O_2$ biosensor [6], glucose biosensor with glucose oxidase [7], γ-aminobutyric acid biosensor with D-glutamate oxidase [8], glycolic acid biosensor with glycolate oxidase [9].

Catalase has been immobilized on numerous carriers by using adsorption, encapsulation, entrapment and covalent immobilization techniques [10–28]. Although there are many studies on catalase immobilization, further investigations are still necessary in order to improve the catalytic efficiency, stability, usability in reactors and to reduce the cost of the immobilization process. Eupergit C was used by many researchers as a carrier for immobilization of various enzymes and it was reported that this carrier was very stable and had good chemical and mechanical properties (simple immobilization procedure, high binding capacity, low water uptake, high flow rate in column procedures, excellent performance in stirred bath reactors, etc.) [29–41]. Eupergit C is a neutral, macroporous copolymer of methacrylamide, glycidyl methacrylate and allyl glycidyl ether, cross-linked with N,N′-methylene-bis(methacrylamide).

In the present study, immobilization parameters of catalase onto Eupergit C (pH, ionic strength, temperature, duration of immobilization, initial amount of catalase) were optimized for the first time. Kinetic constants ($K_m$, $V_{max}$, $k_{cat}/K_m$ and $E_a$), thermal and storage stabilities of immobilized catalase were determined and the results were compared with that of the free catalase. Operational stabilities of immobilized catalase in batch and plug flow type reactors were determined.

## 2. Materials and methods

### 2.1. Materials

Hydrogen peroxide was obtained from Merck AG (Darmstadt, Germany). Bovine liver catalase (44,500 U/mg protein), Eupergit C with an average pore diameter 25 nm (containing 0.8 mM epoxy groups per gram dry weight) and all the other chemicals were obtained from Sigma (St. Louis, MO).

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### 2.2. Immobilization procedure

A total of 1 g carrier were suspended in 9 mL of 1 mg/mL catalase solutions prepared in 1.0 M potassium phosphate buffer (pH 7.0) and the mixture was kept at 25 °C for 24 h and shaked gently during this period. The preparation was washed with buffer solution until no protein was detected in the filtrate. Protein value was determined by the method of Lowry et al. [42]. The amount of unbound enzyme protein was subtracted from the total amount of enzyme protein used for immobilization and the amount of bound enzyme protein was calculated as mg protein/g carrier. Immobilized catalase preparation were kept overnight in an incubator at 5 °C and then stored in closed glass tubes at the same conditions.

The Fourier transform infrared (FTIR) spectra were recorded by using PerkinElmer Spectrum RX/FTIR spectrophotometer, and the sample and KBr were pressed to form a tablet.

Images of the Eupergit C and immobilized catalase were obtained by using Leo 440 Computer-Controlled Digital Scanning Electron Microscope.

### 2.3. Optimization of immobilization conditions

#### 2.3.1. pH of immobilization

In order to determine pH of immobilization, catalase immobilization was carried out in 1.0 M acetate buffer (pH 5.0, 5.5), citrate buffer (pH 6.0), phosphate buffer (pH 6.5–8.0) and borate buffer (pH 9.0).

#### 2.3.2. Concentration of immobilization buffer

In order to determine optimum concentration of immobilization buffer, catalase immobilization was carried out in 0.5, 0.75, 1.0 and 1.25 M buffer solutions at the optimal pH determined before.

#### 2.3.3. Temperature of immobilization

Catalase was immobilized at three different temperatures (5, 15 and 25 °C) at the predetermined optimal pH and buffer concentration.

#### 2.3.4. Time of immobilization

To determine time of immobilization, catalase immobilization was carried out for 1, 2, 3, 7, 12, 24, 48, 72 and 96 h at the predetermined optimal immobilization temperature.

#### 2.3.5. Initial amount of catalase

The effect of initial amount of catalase per gram of carrier was studied at six different catalase amounts (1.0, 2.0, 4.0, 6.0, 8.0 and 9.0 mg). The amount of bound enzyme and the activity were determined for each case.

### 2.4. Enzyme activity assay

The catalase activity was determined according to the Lartillot et al. [43] which is a modification of the method described by Bergmeyer [44]. In a typical experiment, reaction mixture was contained 2.5 mL of substrate made up of 10 mM hydrogen peroxide in a 50 mM phosphate buffer pH 7.0 and $3.16 \times 10^{-5}$ mg of free catalase or 5.0 mg of immobilized catalase. Reaction was carried out at 25 °C for 2 min and stopped by adding 0.5 mL of 1 M HCl. The amount of $H_2O_2$ was determined by measuring its absorbance at 240 nm using a specific absorption coefficient of 0.0392 $cm^2$ μmol $H_2O_2{}^{-1}$. One unit of activity is defined as the decomposition of 1 μmol hydrogen peroxide per min at 25 °C and pH 7.0. Activity of free catalase was given as U/mg protein and activity of immobilized catalase was given as U/mg protein or U/g immobilized catalase.

### 2.5. Characterization of free and immobilized catalases

#### 2.5.1. The effects of pH, buffer concentration and temperature on the activities of free and immobilized catalases

*2.5.1.1. Effect of pH.* Activities of free and immobilized catalases were determined by using $H_2O_2$ substrate prepared in 50 mM acetate buffer (pH 5.0, 5.5), citrate buffer (pH 6.0), phosphate buffer (pH 6.5, 7.0, 7.5, 8.0) and borate buffer (pH 9.0).

*2.5.1.2. The effect of buffer concentration.* Activities of free and immobilized catalases, depending on ionic strength, were determined by using 10 mM $H_2O_2$ solution prepared in 25, 50, 75 and 100 mM buffer solutions at predetermined optimal pH value to investigate effect of buffer concentration.

*2.5.1.3. The effect of temperature.* The effect of temperature on the activities of free and immobilized catalases was investigated at temperatures ranging of 10–60 °C at their optimal pH and buffer concentrations.

#### 2.5.2. Effect of immobilization on the kinetic constants

The activity assays were carried out in different $H_2O_2$ concentrations (5.0, 7.5, 10.0, 12.5 and 15.0 mM) to determine maximum reaction rates ($V_{max}$) and Michaelis–Menten constants ($K_m$) of free and immobilized catalases. $V_{max}$ and $K_m$ for free and immobilized catalases were determined from double reciprocal plots. Turnover numbers ($k_{cat}$) of free and immobilized catalases were calculated from the equation:

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

where $[E]_T$ is the total amount of enzyme in reaction medium. Catalytic efficiencies ($k_{cat}/K_m$) of free and immobilized catalase were also calculated.

The activation energies ($E_a$) were estimated by using the Arrhenius equation.

#### 2.5.3. Thermal and storage stabilities of free and immobilized catalases

*2.5.3.1. Thermal stability.* The thermal stabilities of free and immobilized catalases were determined by measuring the residual activity of the enzyme exposed to 40 and 50 °C. Samples were taken at different time intervals (1, 3, 7 and 15 h) during incubation. Free enzyme was kept as a solution of $1.6 \times 10^{-3}$ mg protein/mL in 50 mM pH 7.5 phosphate buffer, whereas immobilized catalase was kept as solid form. The first-order inactivation constant ($k_i$) and half-life ($t_{1/2}$) of enzyme was calculated from the equation:

$$\ln V = \ln V_0 - k_i t$$

where $V_0$ and $V$ are the initial activity and the activity after time $t$, respectively.

*2.5.3.2. Storage stability.* The storage stabilities of free and immobilized catalases were investigated at room temperature and 5 °C. Free enzyme stored as a solution of $1.6 \times 10^{-3}$ mg protein/mL in 50 mM pH 7.5 phosphate buffer, whereas immobilized catalase was stored as solid form in a closed glass tube. The residual activities were measured during 28 days of their storages.

### 2.6. Application of immobilized catalase in batch and plug flow type reactors

Operational stability of immobilized catalase was investigated by using batch and plug flow type reactors. In batch type reactor, different amounts of immobilized catalase (10, 20 and 40 mg)

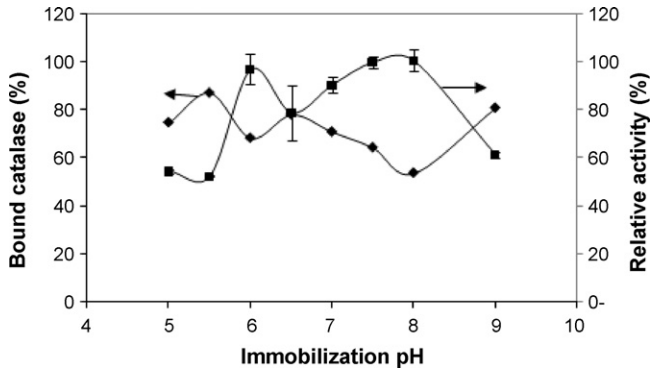


**Fig. 1.** The effect of immobilization pH on amount of bound catalase and activity of immobilized catalase [(♦) bound catalase (%) and (■) relative activity (%)].

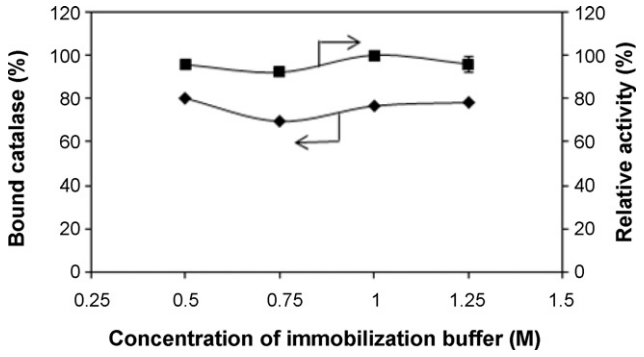


**Fig. 3.** The effect of the concentration of immobilization buffer on amount of bound catalase and activity of immobilized catalase [(♦) bound catalase (%) and (■) relative activity (%)].

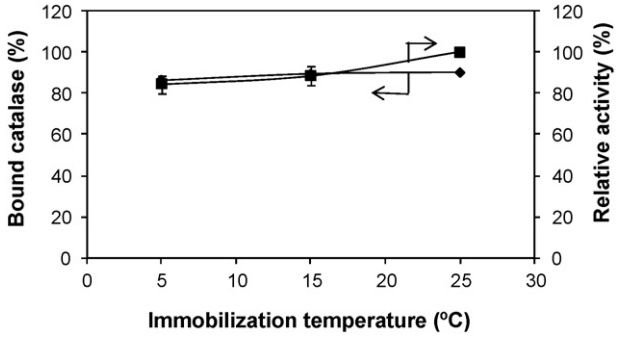


**Fig. 4.** The effect of immobilization temperature on amount of bound protein and activity of immobilized catalase [(♦) bound catalase (%) and (■) relative activity (%)].

were loaded into the reactor (7 cm length, 1 cm i.d.) and then 5 mL of 10 mM $H_2O_2$ solution in 50 mM phosphate buffer (pH 7.0) was added and the reaction was allowed to continue for 2 min at room temperature. After that the reaction mixture was removed immediately from the column and the activity was determined by measuring the absorbance of $H_2O_2$ at 240 nm.

In plug flow reactor experiments, immobilized catalase (10, 20 and 40 mg) was packed into small glass column with 15 cm length, 2 mm i.d. and 0.5 mm wall thickness and reactor was connected to a peristaltic pump which fed the reactor with 10 mM $H_2O_2$ in 50 mM potassium phosphate buffer (pH 7.0) with varying flow rates (2.3 and 5.6 mL/min) at room temperature. Absorbances were measured 30 s interval in a Hellma glass quartz flow cell in spectrophotometer until immobilized catalase completely lost its original activity.

## 3. Results and discussion

### 3.1. Optimization of immobilization conditions

To establish the optimum pH value for the immobilization of catalase onto Eupergit C, pH of the immobilization medium was changed between 5.0 and 9.0 and results were given in Fig. 1. Segura et al. [33] reported that proteins could be bound onto Eupergit C at slightly acidic pH with carboxyl groups, at neutral or slightly alkaline pH with the thiol groups and at pH > 9 with the amino groups. However, Novic et al. [41] reported that under neutral and alkaline conditions the amino groups on the enzyme are principally responsible for binding to Eupergit C (Fig. 2). Under acidic and neutral conditions sulfhydryl and carboxyl groups take part binding. It has been reported that epoxy groups show low reactivity at neutral pH values so that immobilization of enzyme on this support to be produced via a two-step mechanism: in the first step, a rapid and mild physical adsorption of the enzyme on the support is produced [45]. In the second step, a covalent reaction between the adsorbed enzyme and neighboring epoxide groups is occurred. The recommended immobilization conditions on this support include the use of high ionic strength (to force the hydrophobic adsorption of the proteins) because of a fairly hydrophobic nature of the support [46]. As shown in Fig. 1, the amount of bound catalase onto Eupergit C randomly changed depending on the pH of medium because Eupergit C binds proteins via their epoxide groups, which may react with different nucleophiles on the protein as a function of pH. The maximum catalase loading onto Eupergit C was obtained at pH 5.5 which is around the isoelectric point of bovine liver catalase (p*I* 5.4). However, the highest activity was determined for the preparation immobilized at pH 7.5. These results showed that catalase

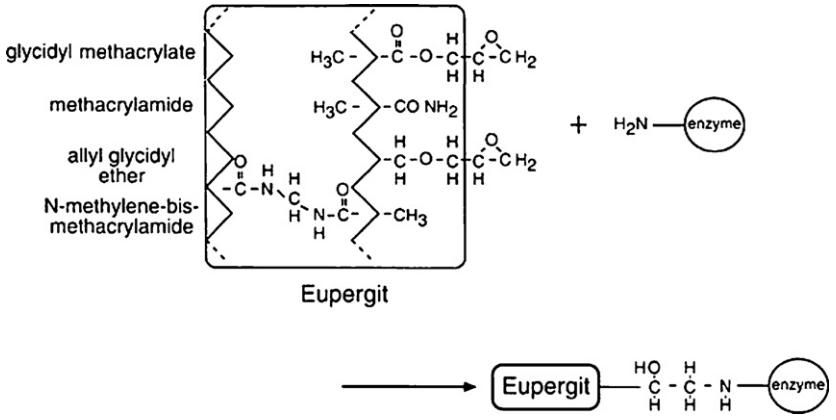


**Fig. 2.** Structure of Eupergit C and covalent immobilization of catalase (adapted from Novick et al. [41], Katzir and Kraemer [50]).

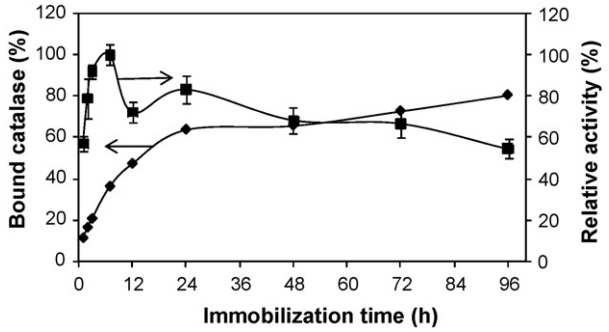


**Fig. 5.** The effect of immobilization time on amount of bound catalase and activity of immobilized catalase [(♦) bound catalase (%) and (■) relative activity (%)].

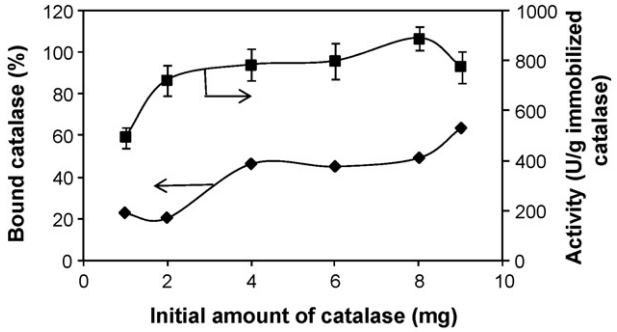


**Fig. 6.** The effect of initial amount of catalase on amount of bound catalase and activity of immobilized catalase [(♦) bound catalase (%) and (■) activity (U/g immobilized catalase)].

retained its active conformation at pH 7.5 after bound onto Eupergit C. In another words, it is best to bind catalase to Eupergit C at the pH at which activity is optimum for catalase. The amount of bound catalase was slightly affected from the medium buffer concentration in the range of 0.5–1.25 M (Fig. 3). However, maximum immobilized catalase activity was observed for the immobilized catalase prepared in 1.0 M pH 7.5 phosphate buffer. Therefore, considering the highest activity of immobilized catalase, we selected immobilization buffer concentration as 1.0 M. As shown in Fig. 4, when immobilization temperature was increased from 5 to 25 °C the amount of bound catalase did not change significantly and the activity of immobilized catalase slightly increased, for that reason, the immobilization temperature was chosen as 25 °C.

The binding yield of the immobilized catalase notably depended on the immobilization time (Fig. 5). The percentage of bound catalase increased from 11 to 80% when the immobilization time increased from 1 to 96 h. At the end of 7th h, as immobilization time, immobilized catalase showed its highest activity. However, the percentage of bound catalase was 36%. When immobilization time was 24 h, the relative activity of immobilized catalase was 83% and the percentage of bound catalase was 64%. Therefore, optimum immobilization time was chosen as 24 h by considering the amount of bound catalase onto carrier and also the activity of immobilized catalase together. A long immobilization time might result in a much higher operational stability of the immobilized enzyme due to increased multipoint attachment [39]. The amount of bound catalase onto per unit weight of Eupergit C was almost directly proportional with initial amount of catalase (Fig. 6). The percentage of bound catalase increased from 23 to 64% when initial amount of catalase was increased from 1.0 to 9.0 mg. Activity of immobilized catalase was increased from $493 \pm 43$ to $890 \pm 46$ U/g immobilized catalase by increasing initial amount of catalase from 1.0 to 8.0 mg. When initial amount of catalase was above 8.0 mg, the activity of immobilized catalase slightly decreased. When 4.0 mg catalase was used in immobilization, the percentage of bound catalase and activity of immobilized catalase were 46.5% and $784.2 \pm 63.6$ U/g immobilized catalase. Therefore, optimum initial amount of catalase was chosen as 4.0 mg by considering binding yield of catalase and also the activity of immobilized catalase together.

The FTIR spectra of the Eupergit C and immobilized catalase were presented in Fig. 7. The broad peak between 3450 and 3400 $cm^{-1}$ was due to N–H stretch, and a methylene vibration was observed between 2950 and 2990 $cm^{-1}$. The absorption

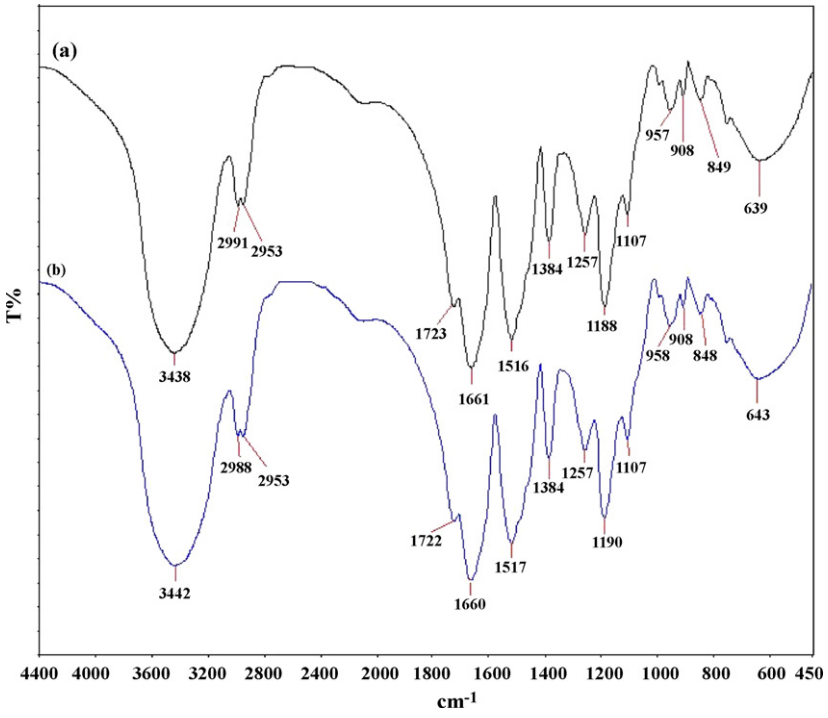


**Fig. 7.** FTIR spectra of Eupergit C (a) and its immobilized catalase (b).

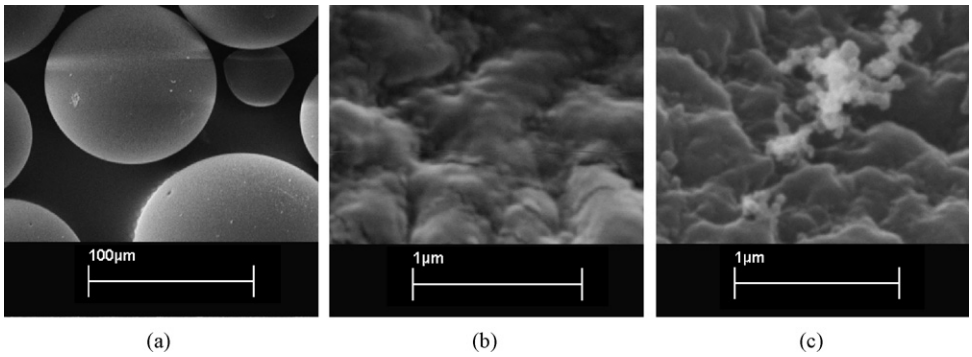


**Fig. 8.** Electron micrographs of Eupergit C (a and b) and catalase immobilized onto Eupergit C (c).

peaks at 1723 and 1661 $cm^{-1}$ were representing amide bond. Antisymmetric and symmetric stretching band of carbonyl groups was observed at 1384 and 1257 $cm^{-1}$, respectively. The broad band about at 630 $cm^{-1}$ indicated primary amides. The absorption peaks of the stretching vibration of the epoxy groups on the Eupergit C should be at 908 and 849 $cm^{-1}$ [47]. The area of epoxide peaks were calculated both of Eupergit C and immobilized catalase onto Eupergit C. As determined from Fig. 7, the area of the epoxide peak of immobilized catalase onto Eupergit C (b) was about 6% smaller than that of Eupergit C alone (a). This corresponds about 48 μmol epoxide groups per gram of carrier opened upon immobilization. This may indicate the covalent bond occurred between catalase and Eupergit C. The small parts of epoxide groups were applied to couple with catalase so there is still the absorption representing epoxy group in the IR spectrum (b).

The surface morphologies of Eupergit C and immobilized catalase were exemplified by the electron micrographs in Fig. 8. As clearly seen at Fig. 8a and b, Eupergit C has a spherical form and a rough surface. It was observed small white particles on the surface of Eupergit C after catalase immobilization, they might be catalases bound onto surface of Eupergit C (Fig. 8c).

### 3.2. Characterization of free and immobilized catalases

#### 3.2.1. The effects of pH, buffer concentration and temperature on the activities of free and immobilized catalases

A comparative study between free catalase and immobilized catalase was performed at eight different pHs. As shown in Fig. 9, free and immobilized catalases showed their maximum activity at pH 7.5 and 7.0, respectively. The activity of immobilized catalase remained almost constant for pHs 6.0–7.5 whereas this was not case for the free catalase. This was probably due to the covalent bonding of catalase with oxirane group of Eupergit C resulting in physico-chemically stable enzyme. This would stabilize the pH

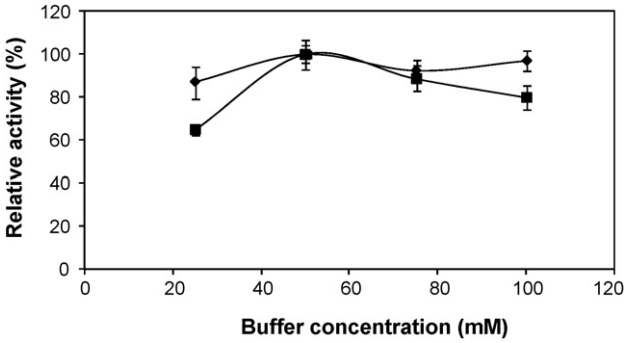


**Fig. 10.** The effect of the buffer concentration on the activity of free catalase (♦) and immobilized catalase (■).

of the microenvironment surrounding the enzyme which could play an important role on the state of protonation of the enzyme molecules. Another factor may be hydrophobic nature of Eupergit C which may effectively reduce the dielectric constant of the microenvironment with consequent modification of the acidity constants of acidic and basic groups on the immobilized enzyme.

Free and immobilized catalases showed their maximum activities at 50 mM buffer concentration (Fig. 10). When the buffer concentration was increased from 25 to 100 mM, the activity of immobilized catalase was more affected than the activity of free catalase. At 100 mM buffer concentration, free catalase retained 96.9% of its maximum activity although immobilized catalase retained 80.0% of its maximum activity.

The effect of temperature on the activities of free and immobilized catalases was shown in Fig. 11. The optimum temperature for free catalase was 25 °C. However; it was 40 °C for the immobilized catalase. In our previous studies, the maximum activities of catalase immobilized via glutaraldehyde onto florisil, via glu-

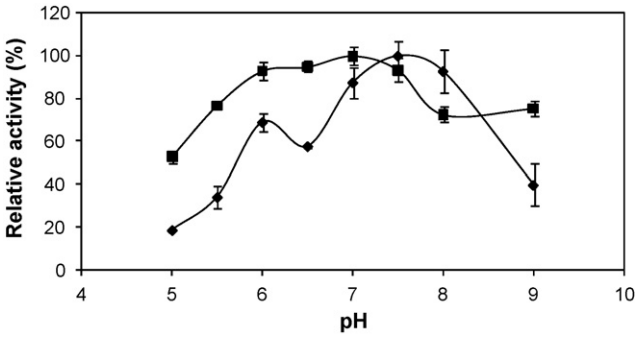


**Fig. 9.** The effect of the pH on the activity of free catalase (♦) and immobilized catalase (■).

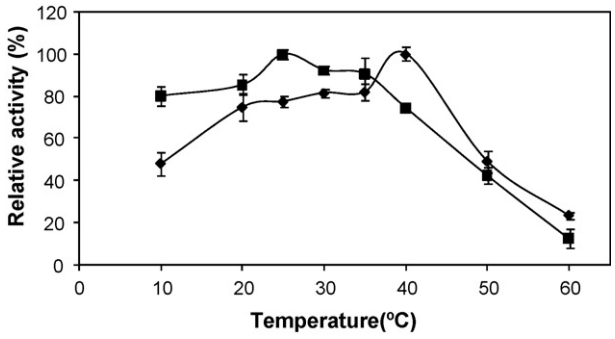


**Fig. 11.** Effect of the temperature on the activity of free catalase (♦) and immobilized catalase (■).

**Table 1**
Kinetic constants ($K_m$, $V_{max}$), catalytic efficiency ($k_{cat}/K_m$) and activation energy ($E_a$) of free and immobilized catalases.

| Enzyme form | $K_m$ (mM) | $V_{max}$ (U/mg protein) | $k_{cat}/K_m$ ($M^{-1}\,s^{-1}$) | $E_a$ (kJ/mol) |
|---|---|---|---|---|
| Free catalase | 28.6 ± 3.6 | $1.4(\pm0.2)\times10^5$ | $2.1\times10^7$ | 14.4 ± 3.5 |
| Immobilized catalase | 95.9 ± 0.6 | $3.7(\pm0.4)\times10^3$ | $1.6\times10^5$ | 15.4 ± 2.9 |

taraldehyde + 3-aminopropionic acid (spacer) onto florisil and eggshell were 35, 25 and 30 °C, respectively [25,28]. In these studies, our results showed that the optimum temperatures of immobilized catalases were similar or not with free catalase depending on the nature of the carrier and immobilization technique.

### 3.2.2. The effect of immobilization on kinetic constants

Table 1 gives a comparative data on the kinetic constants and activation energies of both free and immobilized catalases. It may be seen from this table that there occurred an alteration in the kinetics of the bound catalase as expected for enzymes upon immobilization. It is well known that $V_{max}$ reflects the intrinsic characteristics of the immobilized enzyme and can be affected by diffusion constrains, while $K_m$ reflects the effective characteristics of the enzyme and depends upon both partition and diffusion effects. The higher $K_m$ value of the immobilized catalase was due to either the conformational changes of the enzyme, which resulted in a lower possibility of forming a substrate–enzyme complex, or a less accessibility of the substrate to the active sites of the immobilized enzyme. In this study, catalytic efficiency of immobilized catalase ($1.6\times10^5\,M^{-1}\,s^{-1}$) was about 1% of catalytic efficiency of free catalase ($2.1\times10^7\,M^{-1}\,s^{-1}$). Catalase produces oxygen, which is subjected to limited solubility in aqueous media. Oxygen solubility might be exceeded in the microenvironment. The porous support might serve as a nucleation site for oxygen bubbles that could clog the pores, resulting in a transport barrier and hence the low observed catalytic activity [48]. In our previous studies, the catalytic efficiencies of catalase immobilized via glutaraldehyde onto florisil, via glutaraldehyde + spacer onto florisil and eggshell were about 0.1, 0.2 and 0.2% of catalytic efficiency of free catalase, respectively [25,28]. Jürgen-Lohmann and Legge immobilized bovine liver catalase in tetraethoxyorthosilicate based sol–gels [48]. They reported that catalytic efficiency of immobilized catalase was about 0.05% of catalytic efficiency of free catalase. Catalytic efficiency of catalase immobilized onto Eupergit C was better than those of above-mentioned immobilized catalase preparations.

The activation energies of free and immobilized catalase were determined as $14.4\pm3.5$ and $15.4\pm2.9\,kJ\,mol^{-1}$ by using the Arrhenius equation, respectively. The activation energy of catalase was slightly increased upon immobilization. This result may be explained with internal diffusion restrictions or reduced conformational flexibility of the catalase molecule to reorganize to the appropriate conformation for catalysis to occur at 10–40 °C. The activation energy of an enzyme reaction may or may not change as a consequence of the immobilization process. For example, the activation energies of immobilized catalase via glutaraldehyde and immobilized catalase via glutaraldehyde + spacer onto florisil were almost similar with that of the free catalase [25]. On the other hand, the activation energy of catalase covalently immobilized onto controlled pore glass increased in comparison to that of the free catalase [49].

### 3.2.3. Thermal and storage stabilities of free and immobilized catalases

The half lives of free catalase at 40 and 50 °C were 9.0 and 6.7 h, respectively, and; these correspondingly were 29.1 and 4.8 h for immobilized catalase. The thermal inactivation rate constants ($k_i$) at 40 and 50 °C were calculated as 0.0771 and $0.103\,h^{-1}$, respectively, for free catalase, and as 0.0238 and $0.143\,h^{-1}$, respectively, for immobilized catalase. Immobilized catalase was thermally more stable than free catalase at 40 °C. However, their thermal stabilities were in the same rank at 50 °C. The high thermal stability of immobilized catalase onto Eupergit C may suggest the formation of multipoint covalent attachments between a high proportion of the enzyme molecule and Eupergit C and this may be the cause of low catalytic efficiency obtained for the immobilized catalase.

Fig. 12 shows the residual activities of the free and immobilized catalases depending on storage time at 5 °C and room temperature. The experimental results indicated that the immobilization holds the enzyme in a stable position in comparison to the free counterpart. The activity of the immobilized catalase decreased more slowly than that of the free catalase. The activities of immobilized catalase samples were 68 and 79% of the initial activity when immobilized catalase stored for 28 days at 5 °C and room temperature, respectively. However, free catalase completely lost its activity at the end of 11 days both at room temperature and also at 5 °C. Eupergit C and the immobilization method provide higher shelf-life compared to that of free enzyme since the covalent bonds formed between enzyme and support enhanced the conformational stability of the immobilized enzyme.

### 3.3. Application of immobilized catalase in batch and plug flow type reactors

Operational stabilities of immobilized catalase were determined in batch and plug flow type reactors. The results were compared in terms of reuse numbers, operation times and total amounts of $H_2O_2$ decomposed when the remaining activity of immobilized catalase was about 50% of its original activity. The reuse numbers were 11, 15 and 22 cycles of batch operation in the reactors loaded with 10, 20 and 40 mg of immobilized catalase, respectively (Fig. 13). The total amounts of $H_2O_2$ decomposed were determined as 127, 241 and 722 μmol $H_2O_2$ in batch type reactor loaded with 10, 20 and 40 mg of immobilized catalase, respectively. In the case of plug flow reactor loaded with 10, 20 and 40 mg of immobilized catalase the operation times were determined as 32, 41.5 and 82 min, respectively, for 2.3 mL/min flow rate of substrate, however, when the substrate flow rate was increased to 5.6 mL/min corresponding operation times were determined as 13.5, 24.5 and 45 min (Fig. 14). The total amounts of $H_2O_2$ decomposed were calculated as 361, 601

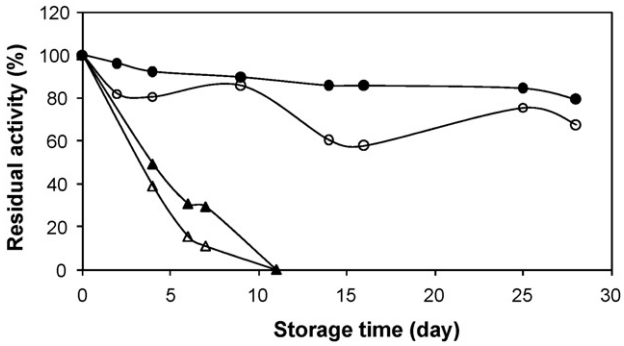


**Fig. 12.** Storage stability of free and immobilized catalases at 5 °C and room temperature (RT). [(▲) Free catalase (5 °C), (△) free catalase (RT), (●) immobilized catalase (5 °C) and (○) immobilized catalase (RT)].

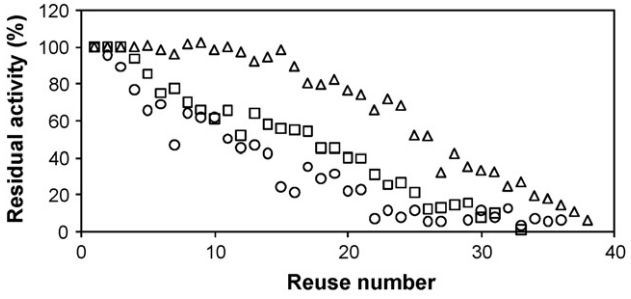


**Fig. 13.** Operational stabilities of immobilized catalase in batch type reactor (○) 10 mg immobilized catalase, (□) 20 mg immobilized catalase and (△) 40 mg immobilized catalase.

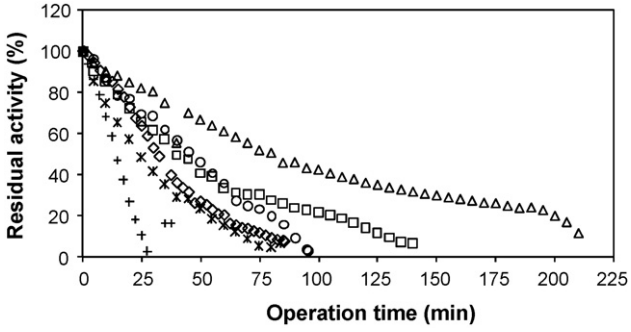


**Fig. 14.** Operational stabilities of immobilized catalase in plug flow type reactor. Flow rate 2.3 mL/min [(◊) 10 mg, (□) 20 mg, (△) 40 mg], flow rate 5.6 mL/min [(+) 10 mg, (✱) 20 mg and (○) 40 mg].

and 1344 μmol $H_2O_2$, respectively, when the reactor loaded with 10, 20 and 40 mg of immobilized catalase and the substrate flow rate was 2.3 mL/min. The total amounts of $H_2O_2$ decomposed were 283, 591 and 1485 μmol $H_2O_2$, respectively, for the same conditions except substrate flow rate as 5.6 mL/min. It was clearly seen that, increasing the flow rate of substrate from 2.3 to 5.6 mL/min did not significantly affect the total amount of $H_2O_2$ decomposed in plug flow type reactor. Plug flow type reactor was better than batch type reactor in terms of the total amount of $H_2O_2$ decomposed when the same amount of immobilized catalase.

## 4. Conclusion

- Optimization studies on catalase immobilization onto Eupergit C clearly showed that a high catalase binding yield did not correspond necessarily with a high activity. Therefore a successful immobilization should combine a maximal load and also a maximal activity.
- Catalytic efficiency of immobilized catalase was lower than that of free catalase but thermal stability at 40 °C and storage stability at room temperature and 5 °C were higher than that of free catalase.
- The use of immobilized catalase in plug flow type reactor may prefer to the use of it in a batch type reactor.

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