Application of Fe_3O_4-chitosan nanoparticles for *Mucor racemosus* NRRL 3631 lipase immobilization

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Received 26 May 2013
Accepted 30 July 2013

Egyptian Pharmaceutical Journal
2013, 12:155–162

Introduction and purpose

Magnetic Fe_3O_4–chitosan (CS) nanoparticles are prepared by the coagulation of an aqueous solution of CS with Fe_3O_4 nanoparticles. Various factors such as time, elevated pH and temperatures affecting magnetic Fe_3O_4–CS nanoparticles were studied using the immobilization technology.

To improve the efficient use of lipase and reduce the cost of production, enzyme immobilization technology is applied.

Materials and methods

Fe_3O_4 nanoparticles were prepared by the coprecipitation method. The CS solution was prepared by dissolving 0.5 g of CS in 50 ml of 1% (v/v) acetic acid, followed by the addition of 12.5 ml of 1 mg/ml Tripolyphosphate (Tpp) solution as a cross-linker to enhance colloidal stability. The solution was used to resuspend the magnetic Fe_3O_4 nanoparticles. The resulting Fe_3O_4–CS was stored at 4°C until use. Uncoated and coated magnetite nanoparticles for immobilizing the lipase were characterized according to the particle size, as measured by atomic force microscopy or scanning force microscopy using contact mode by means of Agilent 5500. The infrared spectra were detected by a Fourier-transform infrared spectrophotometer.

Results and conclusion

To solve leaching problems of the adsorbed lipase and improve the conventional method of lipase immobilization, different concentrations of CS were used. A concentration of CS nanoparticles of 0.3 mg was proved to be more suitable, with an immobilization efficiency of 95.6%. On the basis of atomic force microscopy three-dimensional images, the diameters of the uncoated magnetite particles were determined to be 12 nm. The immobilized lipase shows better operational stability, including wider pH and thermal ranges. The pH optimum of the immobilized enzyme exhibited an acidic shift (pH 5–6). The immobilized lipase was stable in the pH range from 3 to 5 as compared with free lipase. Lipase immobilized by Fe_3O_4–CS nanoparticles remained fully active up to 40°C. At a temperature of 60°C, free lipase preserved about 40% of its residual activity for 1 h; however, the immobilized enzyme preserved about 72.9% of its residual activity at the same time. The kinetic constants V_{max} and K_m were determined to be 250 U/mg protein and 20 mmol/l, respectively, for immobilized lipase. The resulting immobilized lipase had better resistance to pH and temperature inactivation compared with free lipase and exhibited good reusability; after eight repeated uses, the immobilized lipase retained over 63.5% of its original activity.

Keywords:
Fe_3O_4–CS nanoparticles, *Mucor racemosus* NRRL 3631 lipase, lipase stability

Egypt Pharm J 12:155–162

1687-4315

Introduction

During the last decade, lipase has become of significant interest to the chemical and pharmaceutical industries owing to its usefulness in both hydrolytic and synthetic reactions. With recent advances in enzyme technology, many attempts have been made to use the enzyme on a laboratory scale. Some industrial processes have been elaborated [1–7]. Application of lipase can be achieved more economically and efficiently by immobilization to enhance its activity, selectivity, and operation stability. Therefore, a lot of effort has been made on the preparation of lipases in immobilized forms, which involves a variety of both support materials and immobilization methods. If lipase is immobilized onto nanoparticles that have a high specific surface area and low diffusion resistance, it would be very effective for catalysis. Nanophase materials have many advantages because of their unique size and physical properties. However, nanoparticles are difficult to separate from the solution, except through the use of high-speed centrifugation. Using the magnetic property is a good solution to this problem. With the rapid development of nanotechnology, magnetic nanoparticles are now being studied all over the world [8].

In recent years, magnetic nanoparticles have been synthesized through different approaches such as the chemical coprecipitation process [9], sol–gel

self-propagation [10], and in tiny pools of water-in-oil microemulsion [9]; they have been applied in the removal of heavy metals, in MRI contrast agents, and as biosensors [9,11]. Superparamagnetic iron oxide (Fe_3O_4) nanoparticles have attracted researchers' attention because of their multifunctional characteristics, including small size, superparamagnetism, low toxicity, and the ease with which they can be separated from the reaction system [8,12].

Chitosan (CS), poly (β-(1-4))-linked-2-amino-2-deoxy-d-glucose), is the *N*-deacetylated product of chitin, which is a major component of arthropod and crustacean shells such as lobsters, crabs, shrimps, and cattle [13]. CS has been applied in many fields, such as metal adsorption, enzyme immobilization, protein adsorption, and in the controlled release of drugs because of its excellent properties such as nontoxicity, biocompatibility, mucus adhesion, and the controlled release of drugs and biodegradation. CS supports have been used for lipase immobilization, which allows at least 80% of the initial activity to be retained after 10 hydrolytic cycles [14–19].

To improve the efficient use of neutral lipase and reduce the cost of production, enzyme immobilization technology is applied. The literature reports mainly about the preparation of CS nanoparticles and their applications as carriers of drugs, but there are few reports regarding their application in enzyme immobilization. To the best of our knowledge, studies of *Mucor racemosus* lipase immobilized on magnetic Fe_3O_4–CS nanoparticles have not been reported. In this paper, magnetic Fe_3O_4–CS nanoparticles were prepared by the ionization gelation methodology, and characteristics of the enzyme immobilization were studied. Various factors such as time, elevated pH and temperatures affecting magnetic Fe_3O_4–CS nanoparticles were studied using the immobilization technology. Finally, the stability of magnetic Fe_3O_4–CS nanoparticle lipase was evaluated for repeated-batch enzyme reaction.

Materials and methods

Materials

Commercial lipase enzymes were prepared from *M. racemosus* NRRL 3631. All other materials were of analytical grade and used without further purification. These materials included tetraethyl orthosilicate (TEOS≥98%), ammonia solution (NH_3, 28 wt%), ferrous dichloride tetrahydrated ($FeCl_2O \times 4H_2O$), ferric trichloride hexahydrated ($FeCl_3O \times 6H_2O$), CS (medium molecular weight, 75–85% deacetylated, molecular weight ca. 400 000; Fluka, Switzerland), glucose ($C_6H_6O_6$), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), magnesium sulfate ($MgSO_4 \times 7H_2O$), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), peptone from animal protein, olive oil, gum Arabic, and acetone. All solutions were prepared with distilled, deionized water.

Microorganisms, medium, and growth conditions

M. racemosus NRRL 3631 was maintained on potato dextrose agar slants of PDA formula. The microorganism was grown in 250-ml Erlenmeyer flasks containing 100 ml of medium. The medium was inoculated with 4 ml of spore suspension, and the flasks were incubated for 72 h in an orbital shaker operating at 200 rpm at 30°C. For lipase production, the composition of the basal medium with an initial pH of 6.5 (9% w/v) was as follows: glucose, 1; olive oil, 1; peptone, 30; KH_2PO_4, 0.2; KCl, 0.05; and $MgSO_4 \times 7H_2O$, 0.05% [20]. The medium was heat sterilized (121°C for 15 min).

The standard method for enzyme activity assay

The lipase assay was performed with olive oil emulsion by the procedure described in the study of Starr [21]. The olive oil emulsion was prepared as follows: 10 ml olive oil and 90 ml of 10% Arabic gum were emulsified by a homogenizer for 6 min at 20 000 rpm. The reaction mixture composed of 3 ml olive oil emulsion, 1 ml 0.2 mol/l Tris-buffer (pH 7.5), 2.5 ml distilled water, and 1 ml enzyme solution incubated at 37°C for 2 h with shaking. The reaction was stopped by the addition of 10 ml acetone (95% v/v) immediately and the liberated free fatty acids (FFAs) were titrated against 0.05 N NaOH.

Analytical procedures

Protein analysis

Protein measurements were carried out by the method of Lowry *et al.* 1951. [22], using BSA as the standard. The amount of bond protein was determined indirectly from the difference between the amount of protein present in the filtrate and washing solutions after immobilization.

Partial purification of Mucor racemosus *lipase by ammonium sulfate*

To 900 ml of culture supernatant, ammonium sulfate was added (60% saturation) at 4°C. The precipitate was collected by centrifugation at 12 000*g* at 4°C for 20 min and dissolved in a constant amount of distilled water. The lipase activity and protein concentration were determined [23].

Preparation of Fe_3O_4 nanoparticles

The method of preparation was according to Massart [24], but without the use of hydrochloric acid. $FeCl_3 \times 6H_2O$

(4.05 g) and $FeCl_2 \times 4H_2O$ (1.98 g) were dissolved in 100 ml distilled water. The solution was purged with nitrogen to agitate the mixture and prevent the oxidation of Fe^{2+} ions. After 30 min of purging, 143 ml of 0.7 mol/l NH_4OH was added dropwise into the above Fe^{2+} ion solution and the basified solution was purged for an additional 10 min; during the addition of NH_4OH, it was noticed that the solution changed color from the original brown to dark brown and then to black. The precipitate was then magnetically separated by a permanent magnet and then washed with distilled water several times and allowed to dry in air. The resulting product was defined as M. The precipitate was then magnetically separated by a permanent magnet and then washed with distilled water several times and allowed to dry in air.

Preparation of Fe_3O_4–CS nanoparticles

The CS solution was prepared by dissolving 1 g of CS powder in 100 ml of 1% v/v hydrochloric acid (HCl, 38%), after which 25 ml of 10 mg/ml TPP solution was added to cross-link the CS [25]. After stirring for 10 min, 15 ml of 0.1 ml/mol $Fe(NH_4)_2(SO_4)_2$ solution was added into the mixture, under the protection of nitrogen and a controlled flow of oxygen (0.5% v/v). Then, 25 ml of 1 N NaOH were added slowly to the suspension to precipitate the coated nanoparticles. The resulting Fe_3O_4–CS nanoparticles were recovered from the suspension by applying a magnet. They were washed with deionized water several times until the pH reached 7.0, resuspended in 50 ml of deionized water, and stored at 4°C until use.

Characterization

Atomic force microscopy (AFM) or scanning force microscopy using contact mode by means of Agilent 5500 (USA) were performed. AFM is a very high-resolution type of scanning probe microscopy, with a demonstrated resolution on the order of fractions of a nanometer. AFM provides a three-dimensional (3D) surface profile. In addition, samples viewed by AFM do not require any special treatments that would irreversibly change or damage the sample, and does not typically suffer from charging artifacts in the final image. Most AFM modes can work perfectly well in ambient air or even a liquid environment. This makes it possible to study biological macromolecules and even living organisms. High-resolution AFM is comparable in resolution to scanning tunneling microscopy and transmission electron microscopy. The infrared (IR) spectra were recorded by a Fourier-transform infrared spectrophotometer (FT-IR), and the sample and potassium bromide (KBr) were pressed to form a tablet.

Immobilization of lipase onto magnetic Fe_3O_4–CS nanoparticles

PBS (pH 7) containing lipase was mixed with 0.01 g of magnetic Fe_3O_4–CS nanoparticles in a conical flask. The mixture was magnetically stirred at room temperature for 12 h. Then, 1 ml of 1.0 wt% glutaraldehyde was added to the mixture and stirred for another 20 min. Magnetic Fe_3O_4–CS nanoparticles with enzyme were dried under air condition at room temperature.

The activity of free and immobilized lipase was measured as per the method of Yamada and Machida [26], with some modifications. The measurement was based on the action of enzymes on olive oil. The substrate was prepared by thoroughly mixing 50 ml of olive oil with 150 ml of n-octane. Then a certain amount of Fe_3O_4–CS nanoparticles with immobilized lipase was dispersed into 10 ml of the oil mixture. After warming up for 10 min at 40°C, 10 ml of PBS (0.05 mol/1) was added. The reaction emulsion was allowed to be magnetically stirred for 30 min at the same temperature and was later centrifuged at 83.33 Hz (500 rpm) for 15 min. Both (1 ml) the aqueous phase and the oil phase were taken out and diluted to 10 ml by deionized water and ethanol–ether (volume ratio=1 : 1) solutions, respectively. Finally, 0.01 mol/1 of NaOH standard solution was titrated for FFA measurement. Enzyme activity is defined as the amount of FFA produced per milligram protein under the assay conditions.

After each batch, the particles with immobilized lipase were filtered and washed with water three times and dried in air at room temperature. Then the recycled enzyme was used repeatedly in subsequent reactions. The residual activity of the recycled enzyme was compared with the enzyme activity of the first time (100%).

$$\text{Recycling efficiency} = \frac{\text{Enzyme activity in the } n\text{th cycle}}{\text{Enzyme activity in the1st cycle}} \times 100$$

Biochemical characterization of *Mucor racemosus*-immobilized lipase

The thermostability of the immobilized enzyme was studied by incubating the biocatalyst at 30–80°C for 15, 30, and 60 min in a water bath. Likewise, to determine the stability at varying pH, the immobilized enzyme was separately preincubated in 0.2 mol/l of citrate phosphate buffer at pH 4 and 5, phosphate buffer at pH 6 and 7 and Tris-HCl buffer at pH 7.6, 8.0, 8.5, and 9.0 for 1 h and the residual activities were determined under standard assay conditions. Residual activity in samples without incubation was taken as 100%. The inactivation rate constants, K and the half-life time, $t_{½}$, were calculated with the following equation [27]:

Half-life = 0.693/slope of the straight line.

Deactivation rate constant (K) = slope of the straight line.

Result and discussion

Structure and shape of the support nanoparticles

Morphology and particle sizes of these samples were studied with the AFM technique at a resolution of 5 μm. It is worthwhile to note that the mean particle size is about 12 nm. The average roughness (R_a), the root mean square (R_{sq}), the surface skewness (S_{sk}), the coefficient of kurtosis (S_{ku}), and the roughness profile (R_{pr}) were calculated for each sample, and are presented in Table 1.

From Table 1, it can be seen that all these coefficients (R_{pr}, R_a, R_{sq}, S_{sk}, and S_{ku}) increased as a result of enzyme addition because of the lower porosity of the sample. We can say that the numbers of peaks on the surface of the enzyme sample are larger and higher than those observed on the surface topology of the normal sample. Figure 1a and b represent AFM 3D images of the Fe_3O_4–CS nanoparticle sample and Fe_3O_4–CS nanoparticle/enzyme sample. The R_a and the R_{sq} are two physical scales describing the roughness degree of the sample. S_{sk} is a statistical parameter describing the asymmetry of the average high distribution peaks in the histogram. For a symmetrical distribution (Gauss), S_{sk} is 0. The value S_{sk} less than 0 shows that the surface is porous, and when S_{sk} is greater than 0, the surface presents peaks. The S_{ku} is a statistical measure of the comparison of the measured profile and the Gaussian distribution characteristic of a perfectly random distribution of peak heights and valley depths. If S_{ku} is 3, there is a Gaussian distribution. When S_{ku} is less than 3, peaks prevail, and when Sku is greater than 3, pores prevail [28].

Table 1 Important physical scales describing the roughness degree of the samples

Physical scale	Magnetite + chitosan	Magnetite + chitosan/ enzyme
R_{pr} (nm)	243	269
R_a (nm)	22	98
R_{sq} (nm)	27	134
S_{sk}	−0.441	0.58
S_{ku}	3.35	4.17

FT-IR spectra of the Fe_3O_4 and Fe_3O_4–CS nanoparticles

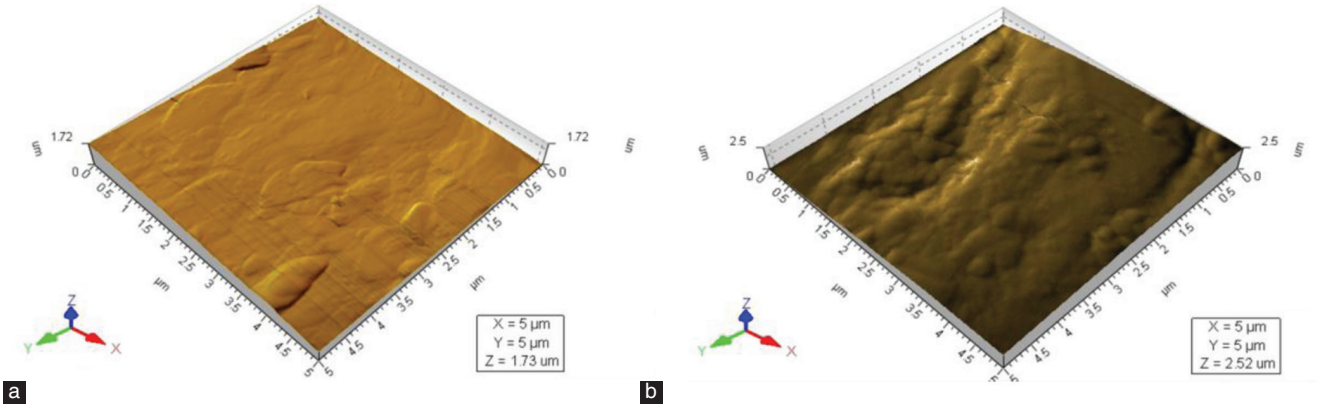
The FT-IR spectrum of magnetite is shown in Figure 2a. A factor group analysis, reported in a classic IR work on spinels, suggested that there were four IR active bands. However, in most cases, including magnetite, only two of them are observed between 400 and 800 cm^{-1}. Here, Fe_3O_4 showed a broad band that consisted of two slightly splitting peaks identified at 585 and 477 cm^{-1}; these peaks were attributed to the stretching vibration of the Fe–O bond and confirmed the occupancy of Fe^{3+} ions at tetrahedral sites in a consistent manner with that reported in the literature [23,29,30]. The characteristic bands of CS shown in Figure 2b were at 3450, 2950 (N–H stretching and O–H stretching vibrations), and 1075 cm^{-1} (C–O–C stretching vibration), consistent with a previous report [31], respectively, as shown in Figure 2b. Characteristic bands of a protein (i.e. lipase) at 1645 (amide) and 1570 (amine) cm^{-1} (C–O–C stretching vibration) are shown in Figure 2c; this revealed that the lipase was immobilized on the CS-coated magnetite nanoparticles [32,33].

Effect of different concentrations of CS on lipase adsorption

To solve the leaching problems of the adsorbed lipase and improve the conventional method of lipase immobilization, CS, which is an abundant long-chain polymer with enough NH_2 groups, was added at different concentrations to adsorb on and into the pore of magnetite nanoparticles.

The Fe_3O_4–CS nanoparticles prepared in different concentrations of CS (0.2, 0.3, and 0.5 mg) in contact

Figure 1



a b

Atomic force microscopic three-dimensional images (a and b) for Fe_3O_4–CS nanoparticles and Fe_3O_4–CS immobilized lipase nanoparticles.

with 1.5 ml neutral lipase solution are shown in Figure 3. The highest immobilization efficiency was 95.6% when the concentration of CS nanoparticles was 0.3 mg; this shows that CS could improve the stabilization of lipase and could be used as a protector of the immobilized enzyme; beside, lipase molecules would also be cross-linked in the CS through their own NH_2 groups. Therefore, a large and stable network covering both the external and the internal surfaces of the support among the lipase and the CS molecules was formed [34,35].

Effect of the amount of lipase added on the immobilization efficiency

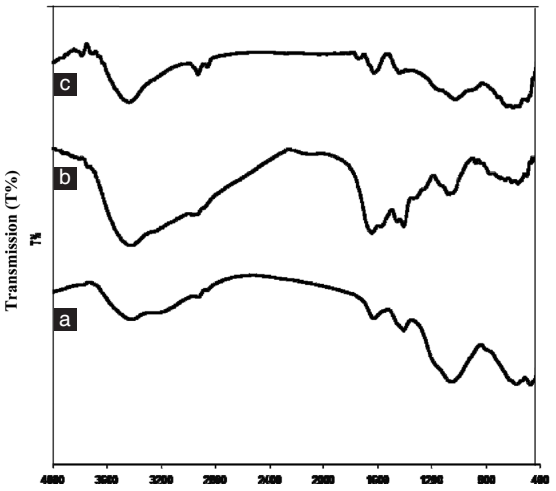
The effect of different concentrations of lipase solution on the immobilization efficiency is illustrated in Figure 4. The higher the amount of lipase (22 and 3 ml), the lower the immobilization efficiency (78 and 67%, respectively). The immobilization efficiency gradually increased when the amount of enzyme added was more than 0.5 mg/ml. The maximal immobilization efficiency was 92.3% when the lipase amount added was 1.5 mg/ml. This means that Fe_3O_4–CS nanoparticles could adsorb lipase quickly, and exhibited a high sorption capacity. The activity of the immobilized enzyme decreased with an increase in the amount of enzyme. This could be explained by the fact that when the amount of enzyme bounded increased, too many layers of enzymes filled the pores of the Fe_3O_4–CS nanoparticles, and thus the surface layer prevented contact between the substrate and enzymes in lower layers [31,36].

Biochemical properties of the free and the immobilized lipase

Effect of pH on the specific enzyme activity of lipase immobilization by the Fe_3O_4–CS nanoparticles was studied by varying the pH of the reaction medium from 3 to 9 using 0.1 mol/l citrate phosphate buffer (pH 3–7) and 0.1 mol/l Tris (hydroxymethyl) amino methane buffer (pH 7.5–9), and the pH profile is shown in Figure 5a. After Fe_3O_4–CS immobilization, the pH optimum of the immobilized enzyme exhibited an acidic shift (pH 5–6). Generally, binding of enzymes to polycationic supports would result in an acidic shift in the optimum pH [37,38]. The variation of the residual activity of the free and immobilized lipase with pH is shown in Figure 5b. The immobilized lipase was more stable in the pH range from 3 to 5 as compared with free lipase. This indicated that immobilization appreciably improved the stability of lipase in the acidic region.

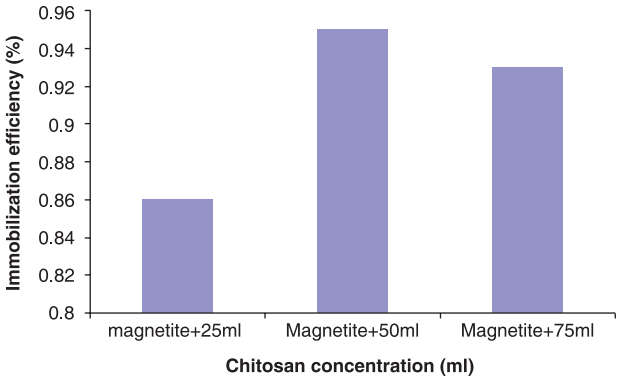
Thermal stabilities of the free and the immobilized lipase in terms of the residual activities were compared

Figure 2



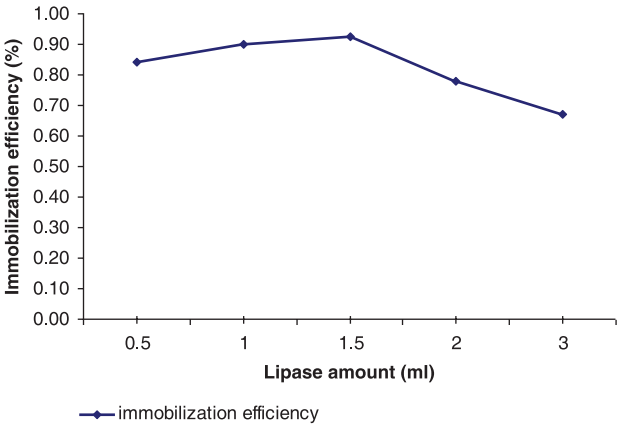
(a) Fourier-transform infrared spectrophotometric spectra of Fe_3O_4, (b) Fe_3O_4–CS without immobilized lipase, and (c) Fe_3O_4–CS with immobilized lipase.

Figure 3



Effect of different concentrations of chitosan coated on the magnetic nanoparticles on the immobilization efficiency.

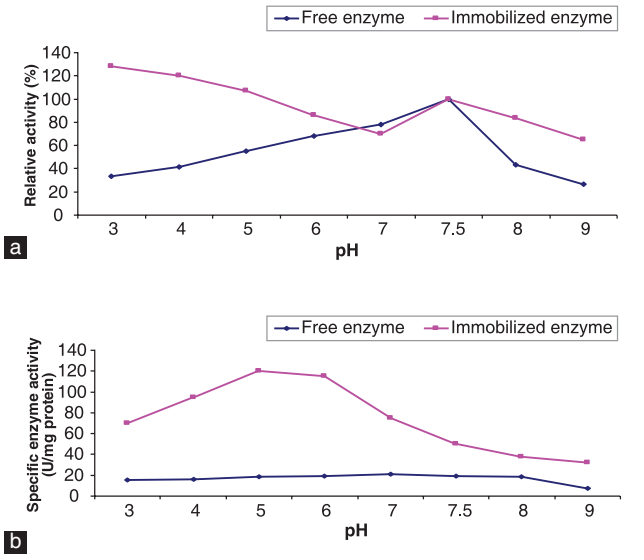
Figure 4



Effect of lipase loading on the immobilization efficiency.

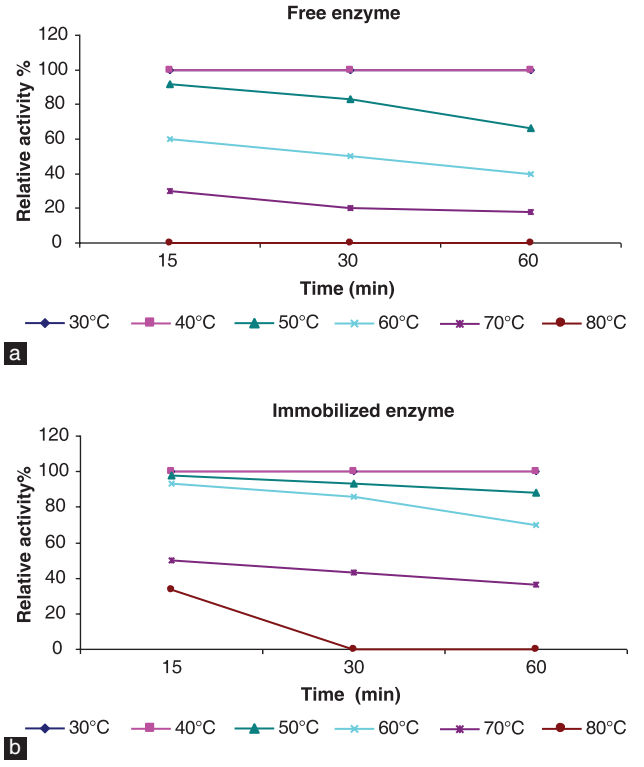
in Figure 6. Lipase immobilized by Fe_3O_4–CS nanoparticles remained fully active up to 40°C. These results are similar to those obtained by Huang *et al.* [38], who found that *Candida rugosa* binary immobilized lipase was fully active at 40°C. However, inactivation of the enzyme occurred on treatment at higher temperatures. Free lipase preserved about 40% of its activity at a temperature of 60°C for 1 h; however, the immobilized enzyme preserved about 72.9% of its residual activity at 60°C for 1 h. At 80°C, the free enzyme was fully activated, whereas the immobilized form preserved about 37.8% of its residual activity after 15 min. Hiol *et al.* [39] studied the thermostability of the free enzyme of *Rhizopus oryzae* and found that the enzyme was highly inactivated at 45°C and almost all activity was lost at 50°C after 40 min' incubation. This thermal stabilization could be explained by the location of lipase inside the micropores of the support, where the enzyme is protected against alterations of the microenvironment. The Michaelis–Menten kinetics of the hydrolytic activity of the free and the immobilized lipases is investigated in Table 2 using varying initial concentrations of olive oil as the substrate. Michaelis constant (K_m) and maximum reaction velocity (V_{max}) were evaluated from the double-reciprocal plot. The V_{max} value of 250 U/mg protein exhibited by lipase immobilized to silica was found to be higher than that of (50 U/mg protein) free lipase. The K_m value (20 mmol/l) determined for the immobilized lipase was about three-fold higher than that (6.66 mmol/l) of the free lipase, which indicated a lower affinity towards the substrate. This increase in K_m might be either due to the structural changes in the enzymes induced by the immobilization or the lower accessibility of the substrate to the active sites [39,40]. The rate of heat of inactivation of the soluble and immobilized lipase was investigated between 50 and 70°C. In general, the immobilization processes protected the enzymes against heat inactivation. For example, the calculated half-life values at 50, 60, and 70°C for the immobilized enzyme were 630, 533, 391.5 min, respectively, which are higher than those (231, 198, 187.3 min) of the free enzyme as shown in Table 3; that is, our free enzyme showed the half-life at 50°C to be 10.5 h, at 60°C to be 8.88 h, and at 70°C to be 6.5 h. Our results are nearly similar to those of Kumar *et al.* [41], who reported the half-life of *Bacillus coagulans* BTS_3 lipase at 55 and 60°C to be 2 and 30 min, respectively. Also, he reported the half-life of another mesophilic bacteria (*Bacillus* spp.*)* to be 2 h at 60°C. The calculated deactivation rate constants (1.1×10^{-3}, 1.3×10^{-3}, and 1.7×10^{-3}) of the experimentally immobilized enzyme at temperatures of 50, 60 and 70°C, respectively, were lower than those (3×10^{-3}, 3.77×10^{-3}, and 4.8×10^{-3}) of the free

Figure 5



(a, b) Effect of pH values on the stability and activity of free and immobilized *Mucor racemosus* lipase.

Figure 6



Thermal stability of (a) free and (b) immobilized *Mucor racemosus* lipase.

Table 2 Kinetic parameters (V_{max} and K_m) for free and immobilized enzymes

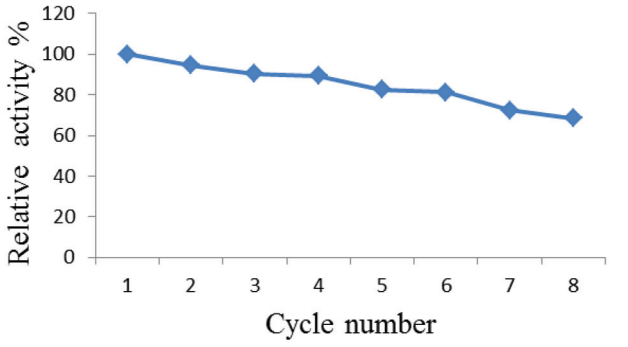
Types	V_{max} (U/mg protein)	K_m (mmol/l)
Immobilized lipase	222.22	13.3
Free lipase	50	6.66

K_m, michaelis constant; V_{max}, maximum reaction velocity.

Table 3 Kinetic parameters (half-life time and deactivation rate constant) for free and immobilized enzymes

Types	Half-life time (min)			Deactivation rate constant		
	50°C	60°C	70°C	50°C	60°C	70°C
Immobilized lipase	866	266	247.5	1.8×10^{-3}	2.6×10^{-3}	2.8×10^{-3}
Free lipase	231	198	187.3	3×10^{-3}	3.7×10^{-3}	4.8×10^{-3}

Figure 7



Operational stability of immobilized *Mucor racemosus* lipase on the hydrolysis process.

enzyme, respectively, at the same temperatures. These results could be related to hydrophilic or hydrophobic environments. A hydrophilic microenvironment allowed the immobilized derivatives to follow a double experimental decay in their activities, whereas a hydrophobic microenvironment makes the enzymatic activity suffer a single experimental decay under storage conditions [42].

Hydrolytic stability of immobilized lipase

Operational stability is commonly the most important issue associated with the application of enzyme immobilization because inactivation is inevitable when free enzyme is exposed to inadequate ambient conditions. To investigate the reusability, the immobilized lipase was washed with phosphate buffer (0.1 mol/l, pH 7.0) after one catalysis run and reintroduced into a fresh olive oil solution for another hydrolysis at 37°C. Figure 7 shows the variation of activity of the immobilized lipase after reuses. It was observed that the immobilized lipase still retained 63.5% of their original activities after the eighth reuse. It was concluded that immobilized lipase with cross-linking CS of a concentration of 0.3 mg has a good durability and reusability [19,36].

Conclusion

Taking the above results into consideration, Fe_3O_4–CS nanoparticles have been proven to be an efficient support for lipase immobilization. On the basis of AFM 3D images, diameters of the uncoated magnetite particles were determined to be 12 nm. The pores in the Fe_3O_4 sample decreased as a result of enzyme adsorption on the surface. Thermal and pH stabilities of the lipase increased on immobilization. The optimal pH and temperature of the immobilized lipase were 5, 6, and 40°C, respectively. There was a slight decrease in the residual activity of the immobilized lipase. An industrially important hydrolase (lipase) immobilized on the magnetic CS carriers developed showed long-term stability without leaching of enzyme from the support; the enzymes could be used repeatedly without significant loss of their activity.

Acknowledgements

The authors express their sincere gratitude to Dr A.H. Ashour, Professor in Physics Department, National Center for Radiation Research and Technology, Cairo, Egypt, for the AFM analysis performed. They also thank Dr Mousa Allam, Assistant Professor in Physics Department, National Research Centre, Dokki, Giza 12311, Egypt, for the FT-IR analysis performed.

Conflicts of interest

There are no conflicts of interest.

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