Epoxy functionalized iron oxide magnetic nanoparticles for catalase enzyme covalent immobilization

Ali O. Ali1,4 · Korany A. Ali3,4 · Yasser E. Shahein1 · Abeer Shokeer1 · Hayat M. Sharada2 · Mohga S. Abdalla2

Received: 17 November 2021 / Accepted: 16 March 2022 / Published online: 4 April 2022 © The Author(s) 2022

Abstract
An aqueous solution of magnetite (Fe3O4) nanoparticles was synthesized using the method of co-precipitation. The nanoparticles were activated with epichlorohydrin for covalently immobilizing the catalase enzyme. The immobilization conditions were optimized as 0.07 mg/ml catalase for 1 h contact time. The properties of free and immobilized catalase were also investigated. The immobilized enzyme showed enhanced activity at alkaline pH and retained about 90% of its relative activity between pH (6–8) and resisted the high temperature and retained 90% of its relative activity at 50 °C. Kinetic parameters of free and immobilized catalase were investigated. While the Vmax value of the immobilized enzyme was reduced 2.4 fold compared to the free enzyme, the KM value of the immobilized catalase was higher by 2.2 fold than the free enzyme. The formulated matrix enhanced the velocity of the immobilized catalase more than the free one and was able to be used for about 18 cycles with retention of 80% of its activity. The immobilized catalase on epoxy functionalized iron oxide nanoparticles is promising as a nano-bio-catalyst carrying out in many industries and different fields.

Keywords Magnetite · Iron oxide nanoparticles · Epoxy · Immobilized catalase

Introduction
The oxidoreductase enzyme; catalase (EC 1.11.1.6) plays a crucial role in suppressing the action of hydrogen peroxide that is produced as a byproduct of several biological, medical, bioremediation, or industrial reactions. During the biological mechanisms, catalase participates in neutralizing the generated reactive species protecting the cell from oxidative stresses(Kaushal et al. 2018). The enzyme acts by decomposing the relatively long-lived hydrogen peroxide into molecular oxygen and water preventing it from oxidative modification of other proteins especially enzymes, DNA, and lipids as indicated by the following equation:

\[ 2\text{H}_2\text{O}_2 \overset{\text{Catalase}}{\longrightarrow} 2\text{H}_2\text{O} + \text{O}_2 \]

There are several industrial applications where catalase is used such as the textile industry (raw silk degumming, treatment of wool, softening of cotton, bio-polishing, fabric finishing, and bioremediation by treating bleaching materials) and dairy technologies (food wrappers, production of cheese, food preservation and determination of quality of milk)(Kaushal et al. 2018). This wide use of the enzyme requires the stabilization of the enzyme against the reaction medium including the pH and temperature, and recovery for reuse. To achieve this goal, enzyme immobilization has been developed to enhance efficiency of enzyme catalytic activity and its potential recycling compared to the free enzyme. In this context and to overcome the operational poor stability and short life of catalase on shelves, immobilization of the enzyme on different supports was conducted according to the structure of catalase and its functional interaction with the carrier.
Catalase was immobilized onto various supports ranging from natural (chitosan, dextran, agarose, wool fabrics, gelatin, and others) and synthetic (acrylamide, vinyl alcohol, silicone, and others) polymers as well as carbon allotropes and inorganic nanoparticles (Grigoras 2017). For example, Liu et al. had immobilized catalase on wool fabrics by applying the layer by layer deposition through an electrostatic assembly of one or more layers of the negatively charged catalase with one or more layers of positively charged poly(diallyl dimethylammonium chloride) carrier at pH7 (Liu et al. 2013). Also, the polymethacrylate synthetic polymer-based supports were used by Cerqueira et al. (Cerqueira et al. 2015). They prepared the poly (methylmethacrylate) which was functionalized by poly(ethyleneimine) to anchor catalase in presence of glutaraldehyde as a crosslinker in an enzymatic bioreactor. It was noticed that the efficiency of conversion of H2O2 increased with the decrease of solution flow rate through the bioreactor. This bioreactor retained the initial catalase activity for two weeks indicating good stability using this kind of carrier. A new matrix of carrageenan-alginate beads was designed and applied by Ali et al. (Ali et al. 2021), They showed that this carrier enhanced the catalytic properties at alkaline pHs compared to the free enzyme which maintained optimal activity at neutral pH.

Among carriers that are used for enzyme immobilization, magnetic nanoparticles which mainly consist of a magnetic material such as iron and a chemical constituent exhibit many magnetic properties that differ from bulk counterparts. They have a superparamagnetic property in which the magnetic susceptibility is higher than paramagnets. They have also low Curie temperature and high coercivity which is related to thickness. Magnetic nanoparticles are of great interest for researchers due to their various applications including magnetic fluids, data storage, catalysis, and bio-applications (Gopal et al. 2015; Chouhan et al. 2007).

Due to the unique physical properties of size and shape, the low toxicity, simplicity of synthesis and efficient loading capacity with the enzymes and potential modification of its surface, the magnetic nanoparticles of iron oxide (Fe3O4) have been used for enzyme immobilization. Fe3O4 nanoparticles have potent magnetism which enables their retrieval from the reaction mixture (Atacan et al. 2016; Muley et al. 2018). However, several enzymes have been immobilized using the Iron oxide magnetic nanoparticles as cellulase (Jordan et al. 2011), lipase (Xie and Ma 2009) catalyzing the production of biodiesel.

This work aims to synthesize epoxy functionalized iron oxide nanoparticles for covalently immobilizing catalase enzyme, hence the role of the synthesized matrix upon enhancing the catalytic activity, kinetic parameters, and reusability of the immobilized enzyme were studied.

**Experimental**

**Materials**

Bovine liver catalase (EC 1.11.1.6) was purchased from Sigma Aldrich, ferrous chloride tetrahydrate (FeCl2.4H2O), ferric chloride hexahydrate (FeCl3.6H2O), epichlorohydrin and ammonia solution were purchased from ACROS Organics. All chemical reagents were of analytical grade and were used without further purification.

**Methods**

**Synthesis of iron oxide magnetic nanoparticles**

The co-precipitation technique was used to synthesize the magnetic nanoparticles following the methods described by (Dhavale et al. 2018; Jain et al. 2015; Çakmak et al. 2020). In brief, 15.9 g of FeCl2.4H2O and 25.95 g of FeCl3.6H2O were dissolved in 1600 ml of distilled water and stirred for about 1 h at 80 °C. Next, 200 ml of aqueous ammonia were added drop wisely to the previous solution, and kept at 80 °C. The prepared magnetic nanoparticles were left to cool at room temperature, magnetically separated, rinsed with distilled water till the pH of the filtrate become 7, and then dried to be ready for the functionalization step.

**Functionalization of iron oxide magnetic nanoparticles**

Fifteen grams of the synthesized magnetic nanoparticles were dispersed in 300 ml of 1 M NaOH, sonicated for half an hour at 28 Hz by using a sonicator, and then treated by slowly addition of 30 ml of epichlorohydrin. The prepared mixture was mixed by shaking at 60 °C in an orbital shaker at 165 rpm for 2 h. Following, the treated particles were magnetically separated, rinsed with distilled water/ethanol, and then left to dry at room temperature overnight (Çakmak et al. 2020).

**Immobilization of catalase enzyme**

To achieve the immobilization process, 0.1 g of the activated nanoparticles were soaked in 5 ml of the specific concentration of the catalase enzyme which was dissolved in 50 mM of phosphate buffer, pH 7.0 for about 1 h.
Activity assay for the immobilized catalase enzyme

The activity of the immobilized catalase was monitored (Aebi 1984) by dispersing 0.1 g of catalase immobilized particles in 3 ml of 30 mM H$_2$O$_2$. The reaction was left to proceed for 3 min. After that, the reaction was stopped by magnetically separating the catalase immobilized particles from the reaction mixture. The absorbance of H$_2$O$_2$ was recorded at 240 nm and activity was calculated according to the following equation:

\[
\text{Unit/g Nanoparticles} = \frac{(\Delta \text{OD/min}) \times 1000 \times \text{Volume of Reaction Mixture}}{\text{Molar Absorbity Coefficient} \times \text{Weight of Nanoparticles}}
\]

Nanoparticles characterization

Transmission electron microscopy (TEM) was used to analyze the morphology and dimensions of the nanoparticles using the HR-TEM (JEOL-JEM-2100, Tokyo, JAPAN) device. The suspension of the magnetic nanoparticles was sonicated for 10 min using the Crest Ultrasonic, New Jersey (USA), then the grid loaded with the sample was examined. Dynamic light scattering (DLS) measurement was done for assessment of particle size. The particle size was measured by using a particle size analyzer (Nano-ZS, Malvern Instruments Ltd., UK). The sample was sonicated for 5 min. just before assessment. Fourier Transform Infrared Spectroscopy (FTIR) analyses of the particles (free magnetite, magnetite with epichlorohydrin, and catalase immobilized magnetite with epichlorohydrin) were performed to elucidate the chemical interaction for each step.

Optimization of immobilization conditions

Immobilization time To study the effect of time on loading capacity, 0.1 g of the functionalized magnetic nanoparticles was soaked in 5 ml of specific enzyme concentration for 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h. The particles were assayed for their immobilized catalase activity.

Effect of enzyme concentration 0.1 g of the activated particles was immersed in 5 ml of different catalase concentrations ranging from 0.01 to 0.1 mg/ml of catalase for 1 h. The particles were assayed for their immobilized catalase activity.

Catalytic parameters

Determination of optimum pH In order to follow the optimum pH of the free and immobilized catalase activity, 30 mM of H$_2$O$_2$ as a substrate was prepared using a wide range of universal buffer (citrate-borate-phosphate) with pHs ranging from pH 3.0 to 10 under assay conditions (Carmody 1961). The relative activity was measured according to the change in pH of reaction and was expressed as the ratio of the retained activity to the enzyme maximal activity.

Determination of optimum temperature The optimum temperature of free and immobilized catalase catalytic activity was tested using 30 mM of H$_2$O$_2$ substrate incubated at different temperatures ranging from 20 to 70 °C. The catalytic relative activity was measured according to the change in temperature of reaction and was expressed as the ratio of the retained activity to the enzyme maximal activity.

Kinetic parameters

Different H$_2$O$_2$ concentrations were used in a range from 10 to 80 mM to determine the Michaelis–Menten constant ($K_M$) and the maximum activity of the reaction ($V_{max}$) of both free and immobilized catalase under assay conditions.

Reusability study

The optimized particles were used to catalyze several cycles of reactions. After each cycle, the particles were rinsed with distilled water and used again to catalyze another cycle of reaction and the relative activity of each cycle was recorded.

Results and discussion

Synthesis and characterization of iron oxide magnetic nanoparticles

Both Figs. 1 and 2 illustrate the functionalization and catalase immobilization reactions on iron oxide magnetic nanoparticles as well as the magnetic separation of magnetic nanoparticles immobilized catalase from the reaction mixture, respectively. In Fig. 3, the TEM graphs show no morphological or size changes between the bare iron oxide nanoparticles and the treated ones. But the immobilized catalase increased the particle size distribution more than bare and treated one to reach 13 nm average diameter.

To assess the nanometric size of the particles, dynamic light scattering measurement was carried out. As shown in Fig. 4, the particle size was 53.70 nm which is larger than
the TEM measurement because of the hydrodynamic shell surrounding the particle.

To detect functional groups’ appearance or disappearance on nanoparticles’ surface, we used the FTIR spectroscopy. The functionalization step by epichlorohydrin and binding step of catalase was determined as shown in the spectra illustrated in Fig. 5. Two absorption bands at (3417 cm⁻¹) and (3144 cm⁻¹) indicate the presence of (–OH) stretching vibration, whereas the absorption band at (1398 cm⁻¹) corresponds to the (–OH) bending vibration. The absorption band at (572 cm⁻¹) corresponds to the (Fe–O) bond. These bands are characteristic for the magnetite and compatible with data obtained by (Bui et al. 2018). The appearance of two bands at (845 cm⁻¹) and (1050 cm⁻¹) are corresponding to the presence of oxirane group and (C–O) bond, respectively, which confirms the presence of epoxy groups on the surface of magnetite. After immobilization of catalase, the spectra show the disappearance of the characteristic band of the epoxy group at (845 cm⁻¹) and the appearance of (–NH) bending vibration band at (1644 cm⁻¹) ensuring the interaction of the catalase enzyme with the epoxy functionalized magnetic nanoparticles. However, the absorption band at (1398 cm⁻¹) corresponding to the (–OH) bending vibration at the surface of the magnetic nanoparticles was reduced after functionalization and immobilization steps ensuring the interaction of the hydroxyl group of the magnetic nanoparticles with epichlorohydrin and catalase enzyme.

**Optimization of immobilization conditions**

**Effect of immobilization time**

As indicated in Fig. 6, the activity of the loaded catalase enzyme increased gradually with time till it reaches its maximum activity of about 210 units/g nanoparticles after 1-h incubation of the enzyme with the nanoparticles, after which the activity gradually decreased to reach 40% drop in the activity after 8 h. This behavior could be interpreted as the appropriate incubation time for maximum activity of the immobilized catalase is one hour and upon increasing the incubation time more active sites of the enzyme are blocked.

Fig. 1 Schematic diagram illustrating the functionalization and catalase immobilization reactions on iron oxide nanoparticles

Fig. 2 Magnetic separation of magnetic nanoparticles immobilized catalase from the reaction mixture
Fig. 3  TEM images and particle size distribution of A Fe$_3$O$_4$ nanoparticles, B Epoxy functionalized Fe$_3$O$_4$ nanoparticles, and C Fe$_3$O$_4$ nanoparticles immobilized catalase. $d$ = mean diameter and $n$ = number of particles counted
Fig. 4 Dynamic light scattering measurement for iron oxide magnetic nanoparticles

Fig. 5 FTIR spectrum of A Magnetite, B Epichlorohydrin functionalized magnetite, C Catalase immobilized on the epichlorohydrin functionalized magnetite, and D Merged spectrum
It seems that the incubation time of one hour was enough to bind all epoxy groups (oxirane rings) of the epichlorohydrin with the amino groups of the catalase enzyme. Similar results were described by (Wu et al. 2013) who showed an increase in pectinase activity after only 15 min of incubation, almost constant activity then followed by a slight decrease after 6 h which was attributed to coverage of active centers of the enzymes by excess pectinase leading to less substrate reaching the active sites. In other words, increasing the immobilization time leads to the generation of multiple bonds between the enzyme and the carrier which may alter the active centers of the enzyme or cause shape modification and hence inactivate partially the enzyme (Wu et al. 2013; Wang et al. 2009). However, oligomerization processes would occur on the functionalized nanoparticles leading to a reduction in the enzymatic activity of the covalently bound enzyme after the specified immobilization period (Akhond et al. 2016). Consequently, we selected the one-hour time period for the following assays.

**Effect of enzyme concentration**

The immobilization capacity was gradually increased with increasing catalase concentration till it reached 187 U/g nanoparticles at an enzyme concentration of 0.07 mg/ml after an incubation time of one hour (Fig. 7). However, increasing the concentration of catalase enzyme did not affect immobilization capacity. Upon increasing the concentration of the enzyme during the immobilization process till the point of saturation, this will create steric hindrance between catalase molecules on the surface of the carrier leading to undesired interactions. This behavior may be attributed to the limited epoxy binding sites of the current carrier (Sohrabi et al. 2014). Accordingly, the catalytic reaction of catalase would not proceed effectively due to a shortage of the number of active sites as well as less accessibility to the hydrogen peroxide (Akhond et al. 2016).

**Catalytic parameters**

**Optimum pH properties**

The effect of pH on the free and immobilized catalase enzyme was followed in separate cells containing hydrogen peroxide as substrate in the range of pH 3.0–10.0 and the results were depicted in Fig. 8. As shown, the gradual increase in pH value has enhanced the relative activity for both free and immobilized enzymes till it reaches pH 6.0. After that, there was a sharp decrease in the free catalase relative activity with increasing pH value, whereas the treated magnetic nanoparticle matrix has enhanced the catalase relative activity to form a broadband between pH 6.0 and 8.0.
The immobilized catalase shows the highest optimum pH with almost 80% relative activity at pH 9.0. Almost similar results were obtained by (El-Shishtawy et al. 2021) using similar bovine catalase immobilized on chitosan/ZnO or chitosan/ZnO/Fe₂O₃ nanomaterials. They showed pH optimal profiles at 7.5 for both nanocomposites used for immobilization and in our study, the optimal pH was at 7.0.

Although the epoxy immobilized catalase retained around 80% and 30% of its relative activity till pH 9.0 and 10, respectively, the alkaline pH is still unfavorable for most enzymes due to the conformational changes caused in the structure of the active sites (Akhond et al. 2016). At pH 9.0 the catalase bound to chitosan/ZnO and chitosan/ZnO/Fe₂O₃ showed relative activities around 65% and 80%, respectively, indicating that the type of support and/or the linker may play a role. Moreover, the bonding potentials between the support and the enzyme may increase resistance against both environmental changes and the structural changes may arise from pH (Jun et al. 2019).

**Optimum temperature properties**

The reaction is generally increased when the collisions between molecules are increased by increasing the temperature of the reaction. Unfortunately, free enzymes lose their activities in high temperatures due to their nature as being proteins (Tümtürk et al. 2007; Doğaç and Teke 2013). Therefore, immobilization is an approach to confer stability for enzymes to be used in specified applications that apply temperature ranges above the optimal degrees of free enzymes (Li et al. 2017). The activity of both free and immobilized catalase enzyme was assayed at different temperatures between (20–70 °C). In Fig. 9, as temperature increases, the relative activities for both free and immobilized catalase also increase till they reach their optimum value at 35 °C. After that, the relative activity for free catalase sharply decreases whereas the immobilized one resists the high-temperature value and forms a broadband between 35 and 50 °C. The epoxy immobilized catalase has retained about 55 and 30% of its relative activity at 60 and 70 °C, respectively. In the case of chitosan/ZnO and chitosan/ZnO/Fe₂O₃ mixtures, the catalytic activity was reported to be 41 and 53%, respectively (El-Shishtawy et al. 2021). The intermolecular covalent bonds generated between catalase and the epoxy functionalized nanoparticles improved the thermal behavior of immobilized enzymes and restricted protein denaturation (Sun et al. 2017). Hence, these results show significant thermal improvement of using this support to immobilize the catalase enzyme.

**Kinetic parameters**

The effect of substrate concentrations on kinetic parameters ($K_M$ and $V_{max}$) of both free and immobilized catalase was studied as indicated in Fig. 9 and Table 1. The reaction rate was plotted against the concentration of H₂O₂ as substrate to represent the Michaelis–Menten equation ($K_M$) and $V_{max}$ give the maximum reaction rate (Fig. 10). The $K_M$ value of the immobilized catalase was about two times higher than that of the free one and the specific activity of immobilized catalase is much smaller than free catalase (Table 1). It is

![Fig. 9](image1.png) The optimum temperature of free and immobilized catalase. Assay conditions: 3 ml of 30 mM H₂O₂ in citrate-borate-phosphate buffer, pH 7.0, for 3 min

![Fig. 10](image2.png) Kinetic parameters of free and immobilized catalase by using Michaelis–Menten plot. Assay conditions: 3 ml of specific H₂O₂ concentration in phosphate buffer, pH 7.0, for 3 min, $T=25$ °C

| Enzyme               | $K_M$ (mM) | $V_{max}$ (µmole/min/mg) |
|----------------------|------------|--------------------------|
| Free catalase        | 30.5       | 2124                     |
| Immobilized catalase | 69.7       | 872                      |

Table 1 Kinetic parameters of free and immobilized catalase

© Springer
known that the $K_M$ value indicates the affinity of an enzyme to its substrate and as the value is low, this refers to high affinity of the enzyme to the substrate (Akhond et al. 2016). In our case, the free catalase has a higher affinity to $H_2O_2$ as compared to the magnetic nanoparticles immobilized catalase where the $K_M$ of free enzyme was 30.5 mM and the $K_M$ of immobilized was 69.7 mM. This low affinity of the immobilized enzyme is probably because the effect of covalent bonding that occurred during immobilization may cause some distortion (not destructive) effect on the catalase tertiary structure without probable effect on the active sites (Mandal 2019). However, the values of $K_M$, $V_{max}$, and the activity of the immobilized catalase are proper confirmations that the epoxy functionalized magnetic nanoparticles do not bind the active sites of catalase.

**Reusability study**

The reusability of an enzyme is considered a crucial character in enzyme-based applications. The number of reuses of immobilized catalase under standard assay conditions was studied and the results are shown in Fig. 11. The immobilized catalase was able to retain almost 80% of its initial relative activity after 18 cycles of its use. The relative activity was reduced to 60% after 21 cycles of use. Previous studies showed the reusability of the magnetic nanoparticles immobilized catalase (using glutaraldehyde as a cross-linker) 11 times with retention of 49% of its initial relative activity (Doğaç and Teke 2013). Other studies using chitosan as an immobilization carrier for catalase showed only 33.3% of retained relative activity after 6 cycles (Ran et al. 2010). The thermos-responsive poly($N$-isopropyl acrylamide) bovine liver catalase exposed to 20 cycles of reusability presented only 15% of its relative activity by the end of the last cycle (Shakya et al. 2010). The high reusability of the formulated composite was due to the stable covalent bonds formed between catalase and the epichlorohydrin-treated magnetic nanoparticles. The covalent bond is so strong that prevents enzyme leakage during reusability and hence, more reaction cycles could be achieved (Wang et al. 2015). This promising result gives the current synthesized matrix a good opportunity for usage in the industry.

**Conclusion**

A simple co-precipitation method has been successfully used to synthesize magnetite nanoparticles that were activated by epichlorohydrin for covalent immobilization of catalase. The method and subsequent results may pave the way to use this catalase in industrial applications. The selection of the magnetite nanoparticles and epichlorohydrin to form the carrier has improved the immobilization efficiency of catalase. The data presented herein indicated that following the immobilization process, the magnetic nanoparticles showed no phase change. The immobilization reaction time was one hour for the optimized catalase concentration of 0.07 mg/ml. The chemical and thermal studies showed that the immobilized catalase was stable more than the free catalase at higher pH and temperature values. Interestingly, the immobilized catalase was able to perform its catalytic function up to 18 constitutive cycles with 80% retention of its initial activity. It is worth mentioning that the reaction using the immobilized catalase followed the standard behavior of Michaelis–Menten kinetics. In conclusion, the immobilized catalase on epoxy functionalized iron oxide nanoparticles is a promising nano-bio-catalyst carrying out in many industries and other different fields.

**Acknowledgements** The authors would like to thank the financial support offered by the National Research Centre (NRC) to the doctoral thesis of Ali O. Ali through the NRC funding program for theses.
**Funding** Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

**Declarations**

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. You have to obtain permission for the following third party content: Images or other material in this article are the property of the copyright holder(s), unless otherwise stated in a credit line. If you wish to use such material you will need to obtain permission from the copyright holder(s). Information on how to obtain permission is available from the Elsevier Rights Department by e-mail to rights@elsevier.com or by telephone to the rights department in your country. Any third party material in this article is included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

**References**

Aebi H (1984) [13] Catalase in vitro. in: methods enzymol, Vol 105. Academic Press

Akhound M, Pashanghe K, Karbalaei-Heidari HR, Absalan G (2016) Efficient immobilization of porcine pancreatic α-amylase on amino-functionalized magnetite nanoparticles: characterization and stability evaluation of the immobilized enzyme. Appl Biochem Biotechnol 180(5):954–968. https://doi.org/10.1007/s12010-016-2145-1

Ali AO, Abdalla MS, Shahein YE, Shokeer A, Sharada HM, Ali KA (2021) Grafted carrageenan: alginate gel beads for catalase enzyme covalent immobilization. J Biotech 11(7):341. https://doi.org/10.1017/s12305-021-02875-9

Atacan K, Çakıroğlu B, Özacar M (2016) Improvement of the stability and activity of immobilized trypsin on modified Fe3O4 magnetic nanoparticles for hydrolysis of bovine serum albumin and its application in the bovine milk. Food Chem 212:460–468. https://doi.org/10.1016/j.foodchem.2016.06.011

Bui TQ, Ton SN-C, Duong AT, Tran HT (2018) Size-dependent magnetic responsiveness of magnetite nanoparticles synthesised by co-precipitation and solvothermal methods. J Sci: Adv Mater Dev 3(10):107–112. https://doi.org/10.1016/j.jsandm.2017.11.002

Çakmak R, Topal G, Çinar E (2020) Covalent immobilization of candida rugosa lipase on epichlorohydrin-coated magnetite nanoparticles: enantioselective hydrolysis studies of some racemic esters and HPLC analysis. Appl Biochem Biotechnol 191(4):1411–1431. https://doi.org/10.1007/s12010-020-03274-1

Carmedy WR (1961) Easily prepared wide range buffer series. J Chem Educ 38(11):559. https://doi.org/10.1021/ed038p559

Cerqueira MRF, Santos MSF, Matos RC, Gutz IGR, Anges L (2015) Use of poly(methyl methacrylate)/polyethyleneimine flow micro-reactors for enzyme immobilization. Microchem J 118:231–237. https://doi.org/10.1016/j.microm.2014.09.009

Chouhan G, Wang D, Alper H (2007) Magnetic nanoparticle-supported proline as a recyclable and recoverable ligand for the Cu catalyzed arylation of nitrogen nucleophiles. Chem Commun 45:4809–4811. https://doi.org/10.1039/B711298J

Dhavale RP, Parit SB, Sahoo SC, Kollu P, Patil PS, Patil PB, Chougule AD (2018) α-amylase immobilized on magnetic nanoparticles: reusable robust nano-biocatalyst for starch hydrolysis. Mater Res Expr 5(7):075403. https://doi.org/10.1088/2053-1591/aaeef1

Doğan YL, Teke M (2013) Immobilization of bovine catalase onto magnetic nanoparticles. Prep Biochem Biotechnol 43(8):750–765. https://doi.org/10.1080/10826068.2013.773340

El-Shishaty RM, Ahmed NSE, Almulaiky YQ (2021) Immobilization of catalase on chitosan/ZnO and chitosan/ZnO/Fe2O3 nanoparticles: a comparative study. Catalysts 11(7):820

Gopal SV, Mini R, Jothy VB, Joe IH (2015) Synthesis and characterization of iron oxide nanoparticles using DMSO as a stabilizer. Mater Today: Proc 2(3):1051–1055. https://doi.org/10.1016/j.mtpr.2015.06.036

Grigoras AG (2017) Catalase immobilization—a review. Biochem Eng J 117:1–20. https://doi.org/10.1016/j.bej.2016.10.021

Jain M, Sebatini M, Sharmila G, Muthukumaran C, Baskar G, Tamilarasan K (2015) Fabrication of a chitosan-coated magnetic nano-biocatalyst for starch hydrolysis. Chem Eng Technol 38(8):1444–1451. https://doi.org/10.1002/ceat.201400493

Jordan J, Kumar CSSR, Theegala C (2011) Preparation and characterization of cellulose-bound magnetite nanoparticles. J Mol Catal B Enzym 68(2):139–146. https://doi.org/10.1016/j.molcatb.2010.09.010

Jun LY, Mubarak NM, Yon LS, Bing CH, Khalid M, Jagadish P, Abdullah EC (2019) Immobilization of peroxidase on functionalized MWCNTs-buckypaper/polyvinyl alcohol nanocomposite membrane. Sci Rep 9(1):2215. https://doi.org/10.1038/s41598-019-39621-4

Kaushal J, Mehandia S, Singh G, Raina A, Arya SK (2018) Catalase enzyme: application in bioremediation and food industry. Bio-Catal Agric Biotechnol 16:192–199. https://doi.org/10.1016/j.bioct.2018.07.035

Li ZL, Cheng L, Zhang LW, Liu W, Ma WQ, Liu L (2017) Preparation of a novel multi-walled-carbon-nanotube/cordierite composite support and its immobilization effect on horseradish peroxidase. Process Saf Environ Prot 107:463–467. https://doi.org/10.1016/j.psep.2017.02.021

Liu J, Wang Q, Fan XR, Sun XJ, Huang PH (2013) Layer-by-Layer Self-Assembly Immobilization of Catalases on Wool Fabrics. Appl Biochem Biotechnol 169(7):2212–2222. https://doi.org/10.1007/s12010-013-0093-6

Mandal M Immobilization of fungal cellulase on chitosan beads and its optimization implementing response surface methodology. In, 2019.

Muley AB, Thorat AS, Singhal RS, Harinath Babu K (2018) A tri-enzyme co-immobilized magnetic complex: process details, kinetics, thermodynamics and applications. Int J Biol Macromol 118:1781–1795. https://doi.org/10.1016/j.ijbiomac.2018.07.022

Ran J, Jia S, Liu Y, Zhang W, Wu S, Pan X (2010) A facile method for improving the covalent crosslinking adsorption process of catalase immobilization. Biores Technol 101(16):6285–6290. https://doi.org/10.1016/j.biortech.2010.03.043

Shakya AK, Sharma P, Kumar A (2010) Synthesis and characterization of thermo-responsive poly(N-isopropylacrylamide)-bovine liver catalase bioconjugate. Enzyme Microb Technol 47(6):277–282. https://doi.org/10.1016/j.enzmictec.2010.07.018

Sohrabi N, Rasouli N, Torkzadeh M (2014) Enhanced stability and catalytic activity of immobilized α-amylase on modified Fe3O4 nanoparticles. Chem Eng J 240:426–433. https://doi.org/10.1016/j.cej.2013.11.059

Sun H, Jin X, Long N, Zhang R (2017) Improved biodegradation of synthetic azo dye by horseradish peroxidase cross-linked on nanocomposite support. Int J Biol Macromol 95:1049–1055. https://doi.org/10.1016/j.ijbiomac.2016.10.093
Tümtürk H, Karaca N, Demirel G, Şahin F (2007) Preparation and application of poly(N, N-dimethylacrylamide-co-acrylamide) and poly(N-isopropylacrylamide-co-acrylamide)/κ-carrageenan hydrogels for immobilization of lipase. Int J Biol Macromol 40(3):281–285. https://doi.org/10.1016/j.ijbiomac.2006.07.004

Wang G, Xin Y, Han W, Uyama H (2015) Immobilization of catalase onto hydrophilic mesoporous poly(ethylene-co-vinyl alcohol) monoliths. J Appl Polym Sci 132(38):42556. https://doi.org/10.1002/app.42556

Wang Q, Fan X, Hu Y, Yuan J, Cui L, Wang P (2009) Antibacterial functionalization of wool fabric via immobilizing lysozymes. Bioprocess Biosyst Eng 32(5):633–639. https://doi.org/10.1007/s00449-008-0286-5

Wu R, He B-H, Zhao G-L, Qian L-Y, Li X-F (2013) Immobilization of pectinase on oxidized pulp fiber and its application in whitewater treatment. Carbohyd Polym 97(2):523–529. https://doi.org/10.1016/j.carbpol.2013.05.019

Xie W, Ma N (2009) Immobilized lipase on Fe3O4 nanoparticles as biocatalyst for biodiesel production. Energy Fuels 23(3):1347–1353. https://doi.org/10.1021/ef800648y

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.