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Enhancing the Stability of Immobilized Catalase on Activated Carbon with Gelatin Encapsulation

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ABSTRACT: Gelatin (Gel) encapsulation onto activated carbon (AC) with catalase (CAT) was developed as an alternative method for CAT immobilization in this study. The immobilized CAT with AC encapsulated by Gel as the supporter accounted for 65.69% of the native enzyme activity. Furthermore, the properties of the immobilized CATs were characterized by Fourier transform infrared spectroscopy and scanning electron microscopy. Among free CAT and the two immobilized CATs, the immobilized CAT with AC encapsulated by Gel as the supporter showed the highest relative enzymatic activity and a high stability in a broad range of pH values and temperatures, and its residual activity was 80% after 15 uses, whereas the immobilized CAT with AC as the supporter was retained at a level of only 50% under the same conditions. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 1498–1502, 2013

KEYWORDS: adsorption; biomaterials; catalysts; proteins

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INTRODUCTION

Catalase (CAT) is one of the most common enzymes in plants, animal tissues, and microorganisms and can decompose hydrogen peroxide (H_2O_2) into water and oxygen. It consists of four subunits, and each of them includes ferriporphyrin as a prosthetic group.[1] CAT is widely used in several large-scale processes, such as the removal of H_2O_2 after sterilization in the food industry and the control of the H_2O_2 concentration after textile bleaching to reduce difficulties in subsequent dyeing processes.[2,3] It can effectively reduce energy consumption and relieve environmental damage.

However, as a kind of multimeric protein, CAT can be inactivated via subunit dissociation under certain conditions.[4] At the same time, free CAT is always high in cost, nonreusable, and unstable in biological catalytic reactions; to a certain extent, these characteristics have restricted its application. Recently, with the aim of preventing its subunits from dissociation and improving the enzyme stability,[5–8] especially on detection with immobilized CAT, which serves as a component of biosensor systems, immobilization has been proposed as a method of easily recovering the enzyme by separation and then recycling it for another operation.[9] Furthermore, through the interactions between the enzyme and supporting matrix, the stability of the immobilized enzyme could be considerably improved because of changes in enzyme microenvironment.[10,11] Compared with free enzyme, immobilized CAT has the possibility of being used for diverse applications because of its unique potential and may thus result in more economic benefits.

Because the properties of an enzyme can be significantly affected by the matrix, it is crucial to select a proper supporting matrix in enzyme immobilization. In general, the matrix should be nontoxic to the enzyme and have a suitable bioaffinity and hydrophilic–hydrophobic properties that are beneficial for the immobilization of the enzyme.[12] Many inorganic supports (silicate, glass, alumina, etc.) and organic biopolymers (chitin, chitosan, gel, etc.) have been used for the immobilization of enzymes.[13–19]

In recent years, porous activated carbons (ACs) have attracted the most extensive attention for their properties, including an ideal pore structure, large surface area, good mechanical stability, and strong adsorption capacity.[20,21] However, porous ACs have always suffered from an inevitable leaching of enzyme in catalytic procedures. Therefore, modified methods have been developed. Wang et al.[22] coated silica onto multiwalled carbon

The first two authors contributed equally to this work and should be considered co first authors.

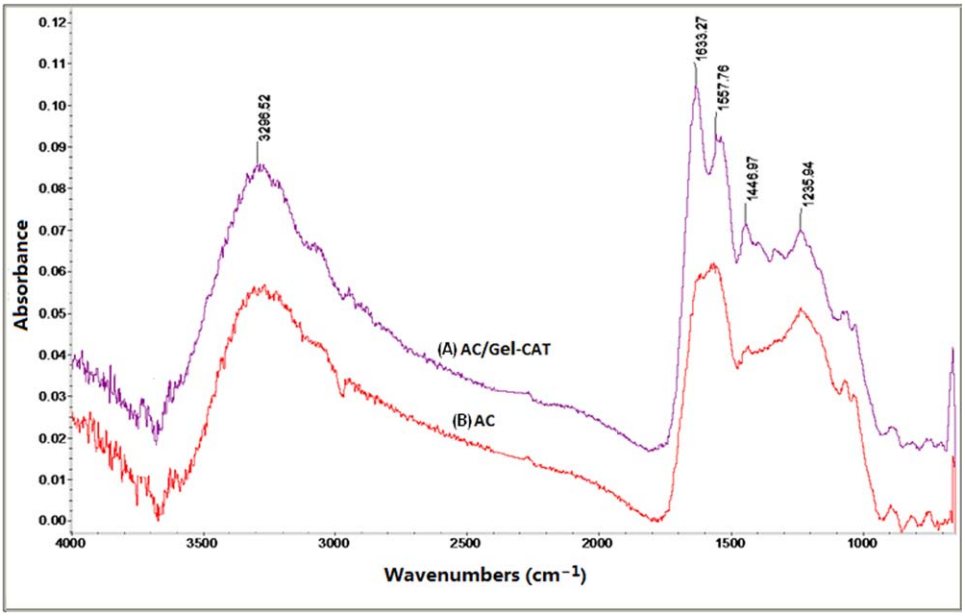

Figure 1. Fourier transform infrared spectra of (A) pristine AC and (B) AC/Gel–CAT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nanotube–papain bioconjugates by a biomimetic silicification process. The results of operational stability indicated that papain leakage was prevented. After seven cycles, the residual activity of this immobilized enzyme remained at 71%, which indicated that the operational stability was enhanced to a certain extent, although a further improvement is still required for its practical application.

Considering that gelatin (Gel) is a kind of nontoxic and biodegradable sol–gel material with hydroxyl, amino, and carboxyl functional groups,[23] there might be some advantages for employing it in for immobilizing CAT on AC; thus, we designed a novel method with Gel encapsulation[24–26] on the basis of the following reasons: (1) Gel is a protein that has the capability to form gels easily without inactivating the entrapped biocatalyst; (2) enriched with hydroxyl, amino, and carboxyl functional groups, Gel can effectively implement the crosslinking procedure with enzyme; and (3) Gel might inhibit the loss of enzyme by encapsulation to enhance the stability of the immobilized enzyme. Therefore, the use of Gel may become an efficient strategy for CAT immobilization.

EXPERIMENTAL

Materials

Porous ACs (type CGP 0334-7) were purchased from Norit, Inc. CAT (3500 units/mg) was purchased from Aladdin Reagent, Inc. Gel was obtained from animal bone (type B) in our laboratory. All of the other chemicals were analytical grade and were used as received.

Immobilization of CAT

The ACs were immersed in the CAT solution with continuous shaking until saturation was reached. After adsorption, the immobilized CAT with AC as a supporter (AC–CAT) was removed from the CAT solution and washed several times with 50 m*M* phosphate buffer solution (PBS; pH 7.0) until no CAT was detected in the washings.

AC–CAT was dispersed in a Gel solution (1 vol %) and reacted for 10 min at room temperature. Then glutaraldehyde (0.5 vol %), used as crosslinking agent, was added to the mixed solution, and after 10 min, it was freeze-dried for 4 h. The immobilized enzyme with AC encapsulated by Gel as the supporter (AC/Gel–CAT) was obtained and then incubated with PBS.

The total concentration of CAT in the solution was determined by the Bradford method with a UV specrophotometer.[27]

Activity Assay of CAT

The activity of CAT was determined spectrophotometrically by the direct measurement of the decrease in the absorbance of H_2O_2 at 240 nm due to its decomposition.[28] For free CAT, 10.0 mL of H_2O_2 (10 m*M*) was preincubated at 25°C for 10 min. An amount of 10 μL of free CAT (0.5 mg/mL) was added to start the reaction. After the enzymatic reaction proceeded at 30°C for 5 min, 5 mL of 10% sulfuric acid was added to immediately stop the reaction. The absorbance of the H_2O_2 was determined, and the activity was calculated. For the immobilized CATs, a sample of about 10 μL of immobilized CAT was mixed with incubated H_2O_2. Five minutes later, the immobilized CAT was removed by a vacuum filter. The absorbance of the reaction mixture was determined, and the activity was calculated as well. One unit of activity was defined as the decomposition of 1 μM H_2O_2 per minute at 25°C and at a pH of 7.0.

Determination of the Kinetic Parameters

The Michaelis–Menten kinetic constant (K_m) and maximum reaction rate (V_{max}) were determined by the study of the effect of the H_2O_2 concentration (10–50 m*M*) on the enzymatic reaction rate at 30°C and at a pH of 7.0. The kinetic parameters were estimated from the Michaelis–Menten equation.[29]

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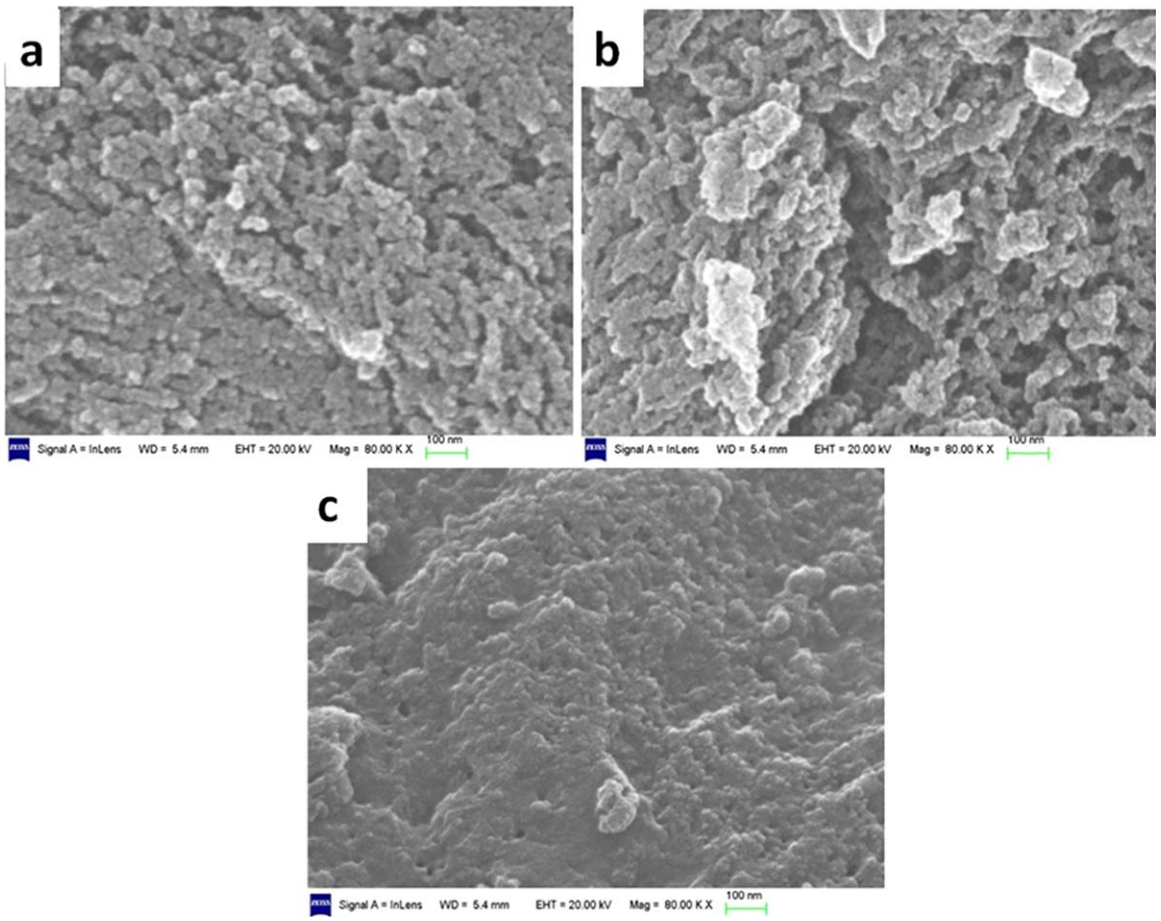

Figure 2. Scanning electron microscopy of (a) AC, (b) AC–CAT, and (c) AC/Gel–CAT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Optimization of the pH and Temperature

The optimum pH and temperature for the free CAT and immobilized CAT were studied by the evaluation of the enzyme activity at different pH values from 3 to 9 (0.05*M* acetate buffer with a range of pH values from 3 to 5 and 0.05*M* phosphate buffer with a range of pH values from 6 to 9 were used) at 35°C. The temperatures of the enzymatic reaction covered a range from 15 to 60°C. The highest value was defined as a value of 100%.

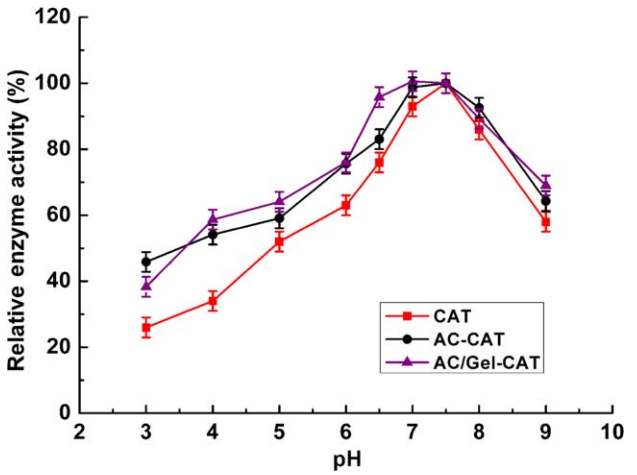

Figure 3. Effect of the pH on the activities of CAT, AC–CAT, and AC/Gel–CAT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Thermal Stability

The thermal stability of the free and immobilized CAT was investigated by the measurement of the residual activity of the enzyme. The free CAT solution and immobilized CAT were preincubated in a water bath in PBS (pH 7.0) for 2 h at different temperatures in a range from 15 to 65°C.

RESULTS AND DISCUSSION

Structure and Morphology

Bovine CAT is mainly in β-sheet form and has several histidine and tryptophan residues.[30] Previous literature has shown that the β-sheet structure was observed at 1625 cm^{-1}.[31] As for the AC/Gel–CAT [Figure 1(b)], there was an obvious absorption band at 1633 cm^{-1}, which indicated that the CAT was immobilized successfully. The shift of the β-sheet absorption bands around 1625 cm^{-1} may have been caused by the binding effect of CAT on the carbon matrix.

Similarly, the spectral bands of Gel were demonstrated. The representative characteristics of them are were N—H stretching at 3300–3500 cm^{-1} for amide I, N—H deformation at 1560 cm^{-1} for the amide II, and other bands associated with C—N

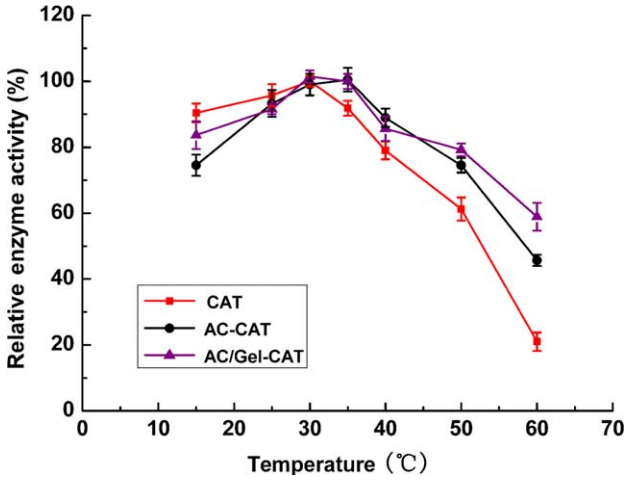

Figure 4. Effect of the temperature on the activities of CAT, AC–CAT, and AC/Gel–CAT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

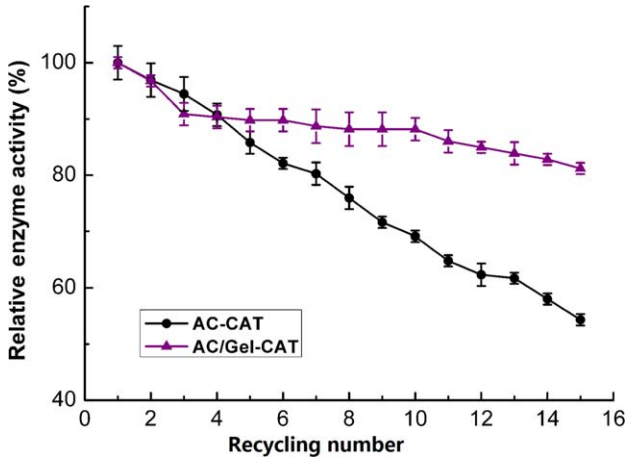

Figure 6. Operational stability of the AC–CAT and AC/Gel–CAT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

stretching and N—H deformation for amide III groups.[32,33] As shown in Figure 1(b), the absorption peaks were located at 3296 and 1558 cm^{-1}, respectively; this demonstrated that Gel was successfully encapsulated on AC as the supporter.

In Figure 2, the surface morphologies of AC and the two immobilized CATs are shown. In a comparison of the pristine AC [Figure 2(a)] and AC–CAT [Figure 2(b)], after the encapsulation step [Figure 2(c)], the morphology showed obvious changes. The Gel film was obvious on the surface of the AC–CAT, and this was consistent with our designed immobilized CAT structure.

Activity

Compared with AC–CAT (127.63 U/mg), AC/Gel–CAT exhibited a slightly decreased activity (114.96 U/mg), which was about 65.69% of the native enzyme activity. Previous research indicated that covalent attachment is often maintained at about 50% or less because of the changes in enzyme 3D structures.[34] The encapsulation method was reported to retain only 30% of the glucose oxidase native activity on mesocellular carbon foam.[35] Compared with the previously proposed immobilized enzymes, AC/Gel–CAT was able to effectively maintain the activity at a high level.

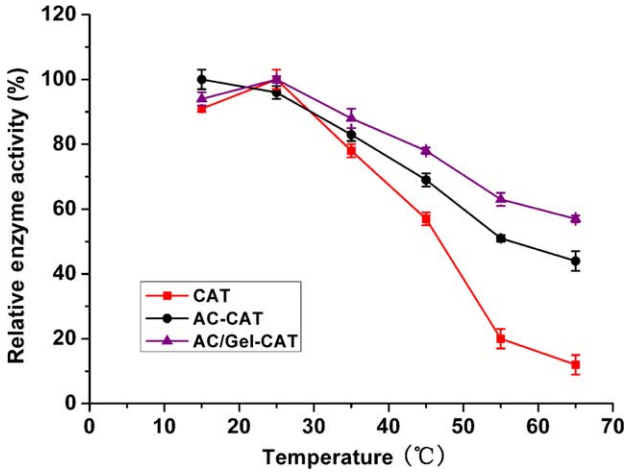

Figure 5. Thermal stability of CAT, AC–CAT, and AC/Gel–CAT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Determination of K_m and V_{max}

The kinetic parameters of CAT and AC/Gel–CAT were determined by the Lineweaver–Burk method to evaluate the kinetic performance of the enzymatic reaction. Variations in K_m and V_{max} indicated that the active site structure of CAT was perturbed by the attachment procedure.[29] The K_m values of free CAT and AC/Gel–CAT were 32.33 and 45.91 m*M*, respectively. In contrast to free CAT, AC/Gel–CAT showed a higher K_m value. This may have been because the substrate had a poor bond to the active site of the immobilized enzyme and a lower capability of transporting the substrate and the products into and out of the supporting matrix.[36,37] The V_{max} of free CAT was 33,000 U/mg, which was a little higher than that of the AC/Gel–CAT (25,000 U/mg). The decrease in V_{max} was ascribed to the low diffusion of the substrate into the Gel matrix.

Optimization of the pH and Temperature

The effect of the pH on the activity of catalase was investigated at 35°C (Figure 3). The maximum activity of the CAT was taken to be 100%, and the optimum pH value for the free CAT and AC–CAT was 7.5; this was mainly attributed to the comparatively low charge density on the carbon surface, which may have contributed to the modification of the microenvironment for CAT.[38–40] For AC/Gel–CAT, the relatively higher charge density induced the optimum pH value shift from 7.5 to 7.0. Additionally, AC–CAT and AC/Gel–CAT also showed higher stability than the free CAT under the same pH.

The temperature dependence of the activity for the CAT, AC–CAT, and AC/Gel–CAT are illustrated in Figure 4. The optimum temperature for free CAT was 30°C, whereas it shifted to 35°C for the AC–CAT and AC/Gel–CAT. Moreover, at higher temperatures, the immobilized enzymes revealed obviously higher activities. Thus, we observed that the activity of immobilized enzyme remained at a considerably high level in a comparatively broad range of temperatures in terms of the enzyme's application.

Thermal Stability and Operational Stability

As shown in Figure 5, the curve exhibited a similar trend in which the relative activity decreased as the temperature increased. After 2 h of incubation at 60°C, only about 10% of the activity was maintained for the free CAT, whereas approximately 44 and 57% of the original activity were maintained for AC–CAT and AC/Gel–CAT, respectively. Clearly, AC/Gel–CAT was more stable than both free CAT and AC–CAT at relatively high temperatures and had a better thermal stability. The increase in the thermal stability of the AC/Gel–CAT was caused by the stabilization through the multipoint attachment of the enzyme to the support; this decreased the protein–protein interactions.[41] Additionally, the Gel encapsulation imposed movement constraints and protected the function of CAT.

The operational stability of an immobilized enzyme is an important feature for its applications. In this research, the operational stability of immobilized enzymes was examined at 30°C and pH 7.0 (Figure 6) with the initial activity taken as 100%. After 15 replications, the residual activities of AC–CAT and AC/Gel–CAT were 50 and 80%, respectively. The results indicate that AC/Gel–CAT had a higher operational stability than AC–CAT. Therefore, we concluded that Gel encapsulation is a promising method to prevent leakage during the recycling process.

CONCLUSIONS

In this research, CAT immobilized on an AC by Gel encapsulation was prepared successfully. Compared with free CAT and AC–CAT, AC/Gel–CAT had a stronger pH and temperature resistance in the catalytic decomposition of H_2O_2 and also excellent operational and thermal stability. After 15 replications, AC/Gel–CAT still retained about 80% of its primary activity; this indicated that the stability of the AC–CAT was improved with Gel encapsulation, and it might have great potential for the continuous degradation of H_2O_2.

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