



Characterization of immobilized α -amylase on functionalized graphene oxide surface

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Carboxyl-functionalized graphene oxide (GO-COOH) and amino-functionalized graphene oxide (GO-NH₂) were prepared for use as carriers for α -amylase immobilization with 2-3% glutaraldehyde as a coupling agent. The α -amylase immobilized onto modified GO exhibited shifts in both working optimum pH and temperature with an increase from pH 6.0 to pH 7.0, and increased optimum temperature by 5-10°C compared with the free enzyme. The loading capacity of the carriers is 786.8 mg/g (GO-COOH) and 437 mg/g (GO-NH₂), respectively. The immobilized α -amylase exhibited a comparable stability activity in comparison with the free enzyme. The FT-IR spectra, UV-visible spectra as well as SEM analysis proved the presence of amine groups and carboxyl groups in the GO, and also covalent immobilization of α -amylase on the modified carrier. The constant values, the K_m was 3.541 mg·mL⁻¹, 4.072 mg·mL⁻¹ and 8.004 mg·mL⁻¹ for free enzymes, GO-COOH-E, and GO-NH₂-E, respectively, and their V_{max} were 7.341 mg·mL⁻¹·min⁻¹, 4.968 mg·mL⁻¹·min⁻¹ and 6.655 mg·mL⁻¹·min⁻¹, respectively. Furthermore, above 54% of the original activity of the immobilized enzyme was retained after 7 reaction cycles, indicating excellent reusability.

Keywords: Amination, Carboxylation, Graphene oxide, Immobilized enzyme, α -amylase

Enzymes not only play an important role in organisms but also are widely used in pharmaceutical, medical, food, and environmental fields. In order to ensure the stability and recyclability of the enzyme in industrial applications, immobilization of enzymes is one of the best methods^{1,2}.

In the past few years, nanocellulose, chitosan, dopamine, polymer microsphere, gelatin, and other materials have been widely used in the immobilization of enzymes. Graphene oxide (GO) is a single layer of carbon atoms in a honeycomb two-dimensional lattice, which has a high specific surface area and rich functional groups (mainly hydroxyl, carboxyl, carbonyl, epoxy, etc.). These functional groups make GO have good plasticity and biocompatibility, laying a good foundation for enzyme immobilization³⁻⁵. Li *et al.*⁶ immobilized *Yarrowia lipolytica* lipase with carboxyl-functionalized GO (GO-COOH) and confirmed that the immobilized lipase had high efficiency in the resolution of racemic compounds in organic solvents. On the basis of GO-COOH, Zhuang *et al.*⁷ prepared amino-functionalized GO (GO-NH₂) and utilized

glutaraldehyde to connect GO-NH₂ with *nuclease P1*. It was proved that the thermal stability of the cross-linked immobilized enzyme was higher than that of the free enzyme, and the storage stability and reusability were significantly enhanced.

Although GO has tremendous potential in the field of enzyme immobilization, studies have shown that the diversity of GO surface groups has certain effects on active biomolecules⁸⁻¹⁰. Therefore, understanding the effect of GO on protein function is helpful to optimize the implementation of protein stabilization strategy in the process of enzyme immobilization, to prolong the shelf life of immobilized enzyme¹¹⁻¹³. In this study, we selected α -amylase as a model enzyme, GO-COOH, and GO-NH₂ as immobilization carriers, investigated the effects of immobilization conditions on the catalytic performance of immobilized α -amylase, studied the catalytic effect of α -amylase on substrates, and characterized the FT-IR spectra, UV-visible spectra and SEM spectra of immobilized enzymes and carriers. The results showed that the enzymatic properties of carboxylated graphene oxide immobilized enzyme (GO-COOH-E) and aminated graphene oxide immobilized enzyme (GO-NH₂-E) were improved, but there were some differences in enzymatic properties between the two immobilized enzymes.

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Materials and Methods

Materials

Natural flake graphite (8000 mesh, 99.95%, Shanghai Maclean Biochemical Technology Co., Ltd.). α -amylase (Purified α -amylase from *Bacillus subtilis* with an activity of 4180 U/g was supplied by Beijing Suo Laibao Technology Co., Ltd.). Soluble starch (Tianjin Zhiyuan Chemical Reagent Co., Ltd.). Glutaraldehyde (50%, Shanghai Maclean Biochemical Technology Co., Ltd.). Ethylenediamine (Tianjin Komiou Chemical Reagent Co., Ltd.). Monochloroacetic acid, 3,5-dinitrosalicylic acid, concentrated sulfuric acid, potassium permanganate, potassium nitrate, disodium hydrogen phosphate, and sodium dihydrogen phosphate were analytical grade and used without further purification.

Preparation of GO, GO-COOH, and GO-NH₂

Preparation of GO

GO was prepared according to a modified Hummers method¹⁴. In brief, 1.5 g graphite particles, 1 g potassium nitrate and 44 mL sulfuric acid were stirred in an ice bath. 6 g potassium permanganate was slowly added and stirred for 20 min at 20°C. The reaction system was stirred at 40°C water bath 120 min. Subsequently, 100 mL deionized water was added and heated to 95°C, the reaction lasted 30 minutes at this temperature. After then, hydrogen peroxide (30%) was added until the reaction system turned bright yellow. The suspension was washed with an aqueous hydrochloric acid solution (0.5 mol/L) and deionized water until neutral pH. The prepared GO was then dispersed in water by sonication in a sonicating water bath, which was 250 W rated with a 40 kHz transducer.

Preparation of GO-COOH

0.5 g NaOH, 1.2 g chloroacetic acid (ClCH₂COONa) and 10 mL deionized water were added to 10 mL prepared GO suspension (1.5 mg/mL) for 4 h in an ice bath under sonication, followed by the addition of dilute hydrochloric acid to neutralize the resulting solution, which conjugated the acetic acid moieties to the -OH groups. The resulting GO-COOH solution was purified by repeated rinsing and filtration (at least 6 times).

Preparation of GO-NH₂

5% Glutaraldehyde was added slowly to the GO-COOH suspension and the mixture was sonicated in an ice water bath for 30 min followed by centrifugation by deionized water. Then, 5% ethylenediamine was

added and magnetically stirred overnight at room temperature, yielding the GO-NH₂ solution. The final product was purified by multiple centrifugations and ultrasonically dispersed in an ice water bath.

α -amylase covalently immobilized onto GO-COOH and GO-NH₂

10 mL GO-COOH solution (1 mg/mL) was activated by glutaraldehyde (1-7.5%) and the mixture was sonicated in an ice water bath for 30 min. The activated GO-COOH was then washed with 0.02 M PBS buffer (pH 6, Na₂HPO₄-NaH₂PO₄). 1 mL of the α -amylase solution (20 mg/mL, prepared from pH 6 and 0.02 M PBS buffer) was added to the activated support solution, and the mixture was magnetically stirred (300 rpm) for 12 h at room temperature. After the reaction was completed, the unbound α -amylase was removed by centrifugation in a 0.02 M PBS (pH 6) buffer to obtain a carboxylated GO immobilized α -amylase (GO-COOH-E).

The preparation procedure of GO-NH₂ immobilized enzyme (GO-NH₂-E) was similar to GO-COOH-E.

Determination of protein loading

The protein concentration was determined using the Bradford method¹⁵. Protein loading was defined as the amount of bound protein per gram dry carrier and calculated as follows:

$$(\text{Protein loading})/g = (A - B) / C$$

where A and B are the weights of total protein and unbound protein and C is the weight of the carrier.

Enzymatic activity assay

The activities of both free and immobilized α -amylase were determined in the presence of 1% (w/v) soluble starch in PBS buffer (0.02 M, pH=6.0) based on the Bernfeld method¹⁶. For free α -amylase, a sample of 1 mL was incubated for 5 min at room temperature with 1 mL of the substrate solution and the enzymatic reaction was interrupted by the addition of 2 mL of DNS reagent. The mixture was heated for 5 min in boiling water and then cooled at room temperature. Afterwards, the volume was set to 25 mL, the absorbance of the digested products was measured spectrophotometrically at wavelength 540 nm. A blank was prepared in the same manner without free α -amylase. For the immobilized α -amylase, after the enzyme and the substrate are incubated, the immobilized enzyme is separated by centrifugation and then 2 mL of DNS reagent was added to its supernatant. The subsequent procedures

were similar to those of free α -amylase. All activity measurement experiments were carried out at least three times.

Optimum conditions and reusability of immobilized α -amylase

The activity of free and immobilized α -amylase was measured at intervals of 5°C in the range of 55 to 85°C at pH 6. The pH dependence of α -amylase activity was evaluated at 7 different kinds of pH (5, 5.5, 6, 6.5, 7, 7.5, 8, and 8.5) at their respective optimal temperatures. The enzyme activity was determined using a 1% starch solution as a substrate under the respective optimum temperature and pH conditions, and the relative activity of the initial enzyme was 100%. After completion of each enzymatic hydrolysis experiment, the immobilized α -amylase was separated by centrifugation and washed 3 times with deionized water to remove the substrate and product solution adhering to the surface, followed by the next enzymatic hydrolysis experiment.

Determination of kinetic parameters

The kinetic parameters of free and immobilized α -amylase were determined by measuring the initial rates of enzymes with different substrates (starch concentration 10, 6.67, 5.0, 4.0, 3.33, 2.86, 2.5 mg/mL). The K_m and V_{max} values were calculated from GraphPad Prism 7.

Characterization

The samples used in the characterization were obtained by freeze-drying the corresponding solution for 6 h. The UV-visible spectrum scan range was 190-800 nm. The Fourier transform infrared spectrum scan range was 400 to 4000 cm^{-1} . The scanning electron microscope conditions were: the sample was analyzed after the platinum (Pt) coating at an accelerating voltage of 15 kV.

Results and Discussion

Characterization of carriers and immobilized α -amylase

As can be clearly seen from (Fig. 1A), graphite sheets were flat sheets. Graphite became into GO with fewer layers and a large number of folds on its surface by being oxidized and peeled. After carboxylation treatment (Fig. 1B), the surface of GO had hardly changed, which probably because carboxylation treatment only changed the content of surface functional groups, and the reaction conditions were relatively mild. After the GO-COOH loading enzyme, the sheet structure was consistent with GO-COOH,

but there were lots of white spots on GO-COOH-E, which may be the place where the enzymes were immobilized. As the enzyme bound to the carrier, causing changes on the surface of the GO-COOH. It can be seen from (Fig. 1D) that GO-NH₂ underwent severe agglomeration, and some small white spots appeared on the surface of GO-NH₂-E, which were similar to GO-COOH-E. This may also be the place where the enzymes were immobilized. However, due to the serious accumulation of GO-NH₂ and thicker layers, the morphology of GO-NH₂ and GO-COOH were different, so the effect of the immobilization on the morphology of the carrier was different.

Figure 2 illustrates that the solutions of GO, CO-COOH, and GO-COOH-E had basically the same appearance. There was no obvious change after standing at room temperature for 48 h, all showed

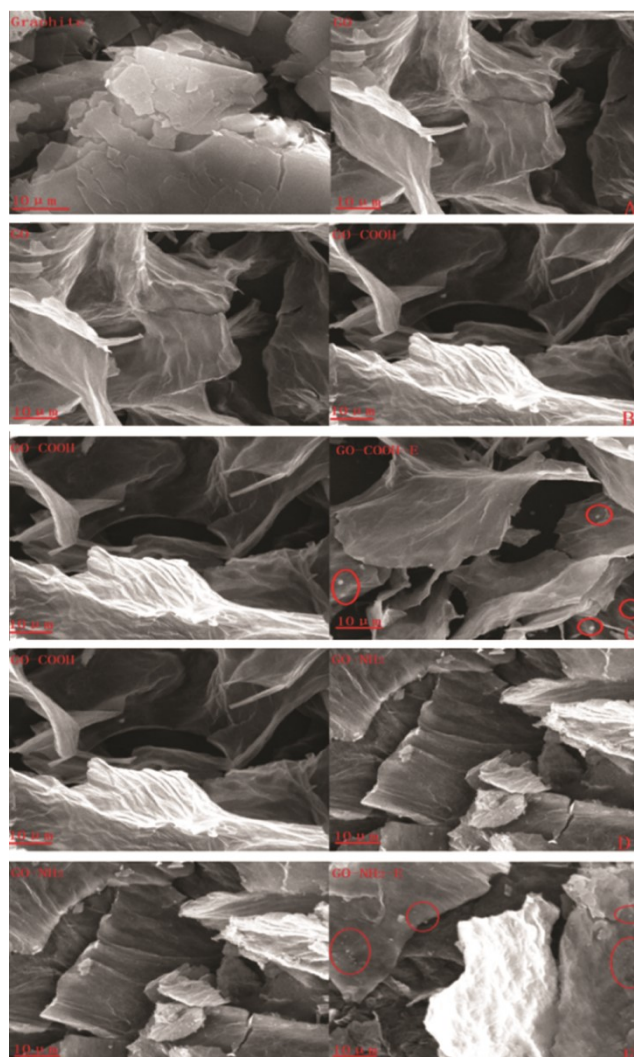


Fig. 1 —SEM images of Graphite (A) GO; (B) GO-COOH; (C) GO-NH₂; (D) GO-COOH-E; and (E) GO-NH₂-E

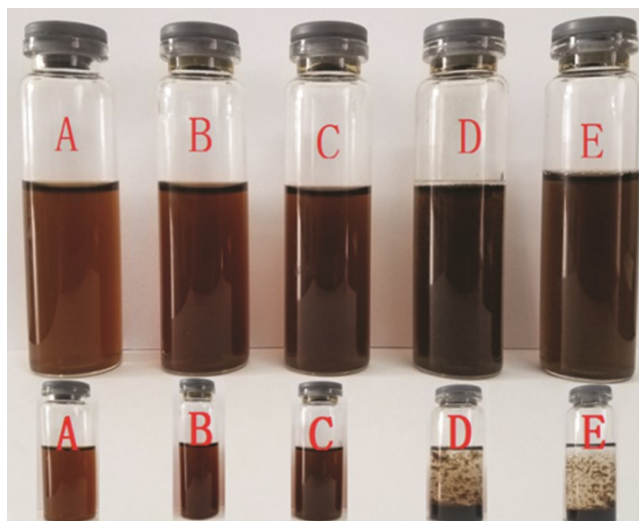


Fig. 2 —Photos of (A) GO; (B) GO-COOH; (C) GO-COOH-E; (D) GO-NH₂ and (E) GO-NH₂-E (The corresponding small picture below were the pictures after standing for 48 h)

good stability. This was consistent with the similar features of the structure shown in the SEM images. Notably, the color of the GO-NH₂ solution varied from yellowish to brown, implying that the GO was partly reduced. After 48 h of standing, severe delamination occurred. This may be due to the deletion of negative charges from the surface of GO-COOH upon amine groups addition which reduces repulsion of negative charges and probably increases ionic bonds and following aggregation. On the other hand, the addition of the cross-linking agent glutaraldehyde caused the carrier to self-polymerize in a way^{17,18}.

Figure 3 shows the UV-vis spectra of free α -amylase, GO-COOH, GO-COOH-E, GO-NH₂, and GO-NH₂-E. It can be seen from the figure that the free α -amylase had a strong absorption at 278 nm because the amino acid constituting the enzyme contained a benzene ring conjugated double bond¹⁹. The spectral change of GO-COOH was relatively flat, with weak absorption only around 300 nm, which was caused by the $n-\pi^*$ transition of the C=O double bond in GO²⁰. The trend of GO-NH₂ was steeper than that of GO-COOH, and the shoulder peak at 300 nm also disappears. GO-COOH-E and GO-NH₂-E had shoulder peaks around 278 nm, which was derived from protein absorption, indicating that the carriers had been loaded with α -amylase.

The FT-IR spectra of GO are presented in (Fig. 4), wherein the peaks at 1075 cm⁻¹, 1622 cm⁻¹, and 1730 cm⁻¹ were ascribed to the C-O-C, C-OH and C=O stretching vibrations, respectively. The evidence that GO had been successfully carboxylated was that

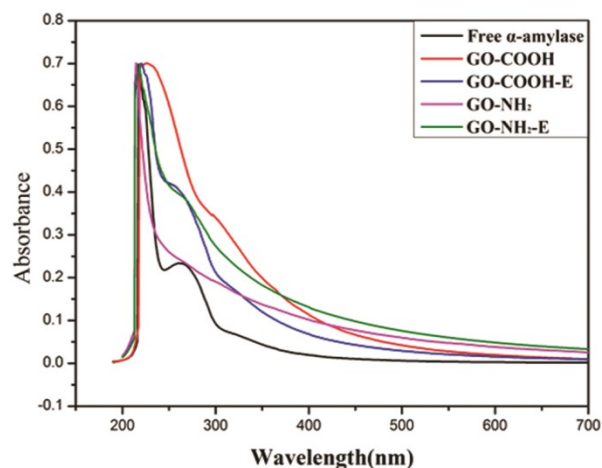


Fig. 3 — UV-vis spectra of free α -amylase; GO-COOH; GO-COOH-E; GO-NH₂ and GO-NH₂-E

the localized magnification of GO after carboxylation showed a significant increase in the absorption intensity at 1730 cm⁻¹ (Fig. 1B). From the GO-COOH-E spectrum, the C=O of the carboxyl group at 1730 cm⁻¹ had disappeared, and the newly added 1548 cm⁻¹ and 1457 cm⁻¹ correspond to the absorption peaks of N-H and C-N, respectively^{21,22}, indicating the α -amylase had been successfully immobilized onto GO-COOH. After the amination of GO-COOH, the carboxyl group disappeared at 1730 cm⁻¹. In addition, the three new characteristic peaks at 1643 cm⁻¹, 1582 cm⁻¹, and 1480 cm⁻¹ were -CONH amide band I, -NH amide band II and C=N stretching vibration, which indicated that GO-NH₂ was successfully prepared²³⁻²⁴. In the infrared scan of GO-NH₂-E, it was found that there were also absorption peaks at 1543 cm⁻¹ and 1448 cm⁻¹, which was similar to GO-COOH-E, which may be because both carriers were passed through one aldehyde group of the aldehyde was bonded to GO-COOH/GO-NH₂, and the other group was further linked to the enzyme by the amino group of the lysine residue^{25,26}. Therefore, the infrared absorption peaks of the two immobilized enzymes were similar, which showed that α -amylase effectively present in the samples confirming the binding of α -amylase to GO-NH₂.

Properties of free α -amylase, GO-COOH-E, and GO-NH₂-E Effects of glutaraldehyde dosage on the Activity and loading rate

Since glutaraldehyde is both a crosslinking agent and a deactivator, it is necessary to strictly control the use of glutaraldehyde and to immobilize the enzyme on the carrier as much as possible while maintaining the enzyme activity to the greatest extent^{27,28}. Figure 5

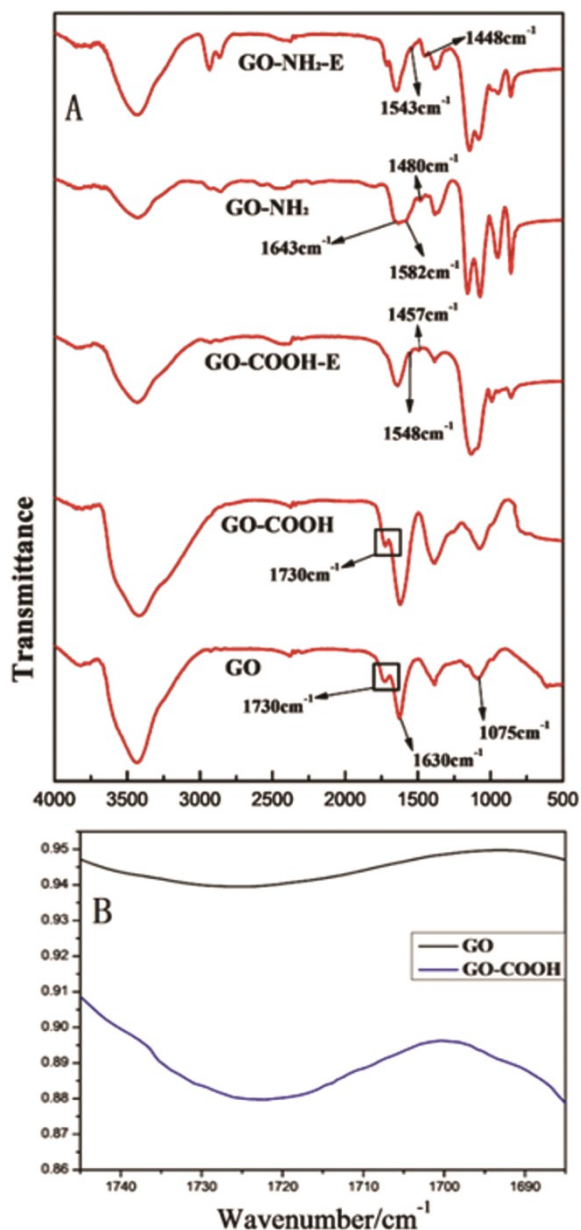


Fig. 4 — (A) FT-IR spectra of GO; GO-COOH; GO-COOH-E; GO-NH₂; GO-NH₂-E; and (B) GO and GO-COOH partial enlargement at 1730 cm⁻¹

reveals that the relative activities of GO-COOH-E, and GO-NH₂-E were lacking when the concentration of glutaraldehyde was insufficient (< 2%). Considering the relative loading rate, the incomplete activation of GO-COOH and GO-NH₂ led to this phenomenon. With the increase in the amount of glutaraldehyde, the enzyme loading of both carriers showed an increasing trend. When the amount of glutaraldehyde were 2.0% and 3.0%, the relative activities of GO-NH₂-E and GO-COOH-E reached a maximum, their enzyme

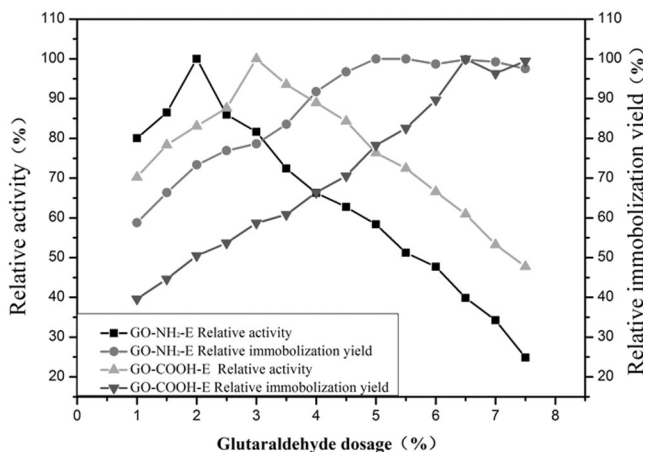


Fig. 5 —Effect of the amount of glutaraldehyde on α -amylase activity and loading rate

loadings were 437 mg/g and 786.8 mg/g, respectively. When GO-COOH-E and GO-NH₂-E reached the maximum relative activity, the contents of glutaraldehyde were different, and the enzyme loading of GO-COOH was 1.8 times than that of GO-NH₂. One reason might be that the preparation of GO-NH₂ was based on GO-COOH. After the part surface of GO-COOH was combined with ethylenediamine, GO-NH₂ was obtained, so there were fewer active groups on the surface of GO-NH₂ compared with GO-COOH. Another reason was that GO-NH₂ was agglomerated seriously, which made it possible to bind to enzymes with fewer groups. When the amount of glutaraldehyde reached 5.0% and 6.5%, the enzyme loadings of GO-NH₂ and GO-COOH no longer changed, reaching the maximum enzyme loadings of 595.9 mg/g and 1340 mg/g, respectively. As the amount of glutaraldehyde continued to increase, the relative activity of the immobilized enzyme decreased and the enzyme load no longer changed. On one hand, due to the excess of glutaraldehyde, the enzyme conformation may change, thereby reducing the enzyme activity. On the other hand, an excess of glutaraldehyde caused the groups on the surface of the two supports had been fully utilized and the loading is saturated^{29,30}.

Effects of Temperature on the Activity

The effect of temperature on the activity of free and immobilized α -amylase for starch hydrolysis at pH 6.0 in the temperature range of 50-85°C was shown in (Fig. 6). It was found that the optimum temperature of the free enzyme was 60°C, while the optimum temperature of the immobilized enzyme was increased: GO-COOH-E was 70°C, GO-NH₂-E was 65°C. The relative activity of free enzymes varied by

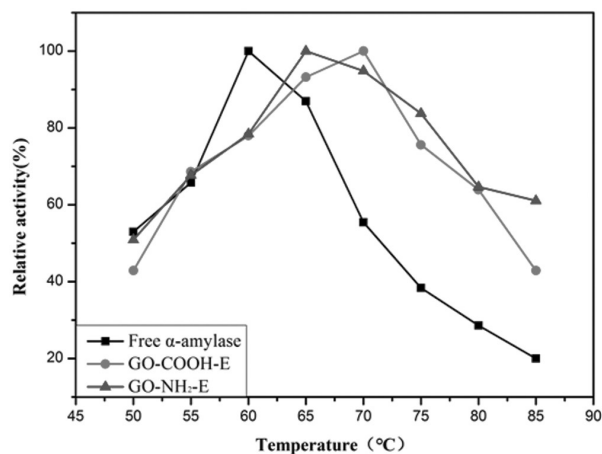


Fig. 6 —Optimum Temperature of the free and immobilized enzymes

80% in the whole test range (50–85°C), while GO-COOH-E and GO-NH₂-E were 49.11% and 57.14%, respectively, which revealed that the heat resistance and temperature resistance of the immobilized α -amylase were improved. The activity of the immobilized enzymes was limited when the temperature was within 55–65°C. This may be due to the change of the conformation of the enzyme after the immobilization of α -amylase, which required higher energy to make the enzyme more active^{31,32}. At higher temperatures (65–85°C), the activity of free enzymes almost showed a straight line downward trend, while the activity of immobilized enzymes began to show a slower downward trend after reaching their optimal temperatures. It can be seen from the figure that although the optimum temperature of the two immobilized enzymes was higher than that of the free enzyme, there were some differences between the two immobilized enzymes: the optimum temperature of GO-COOH-E was 70°C, while GO-NH₂-E was 65°C. The reason may be that the space arm of GO-COOH-E was much shorter than that of GO-NH₂-E. The shorter space arm made the enzyme more closer to GO, GO gave more protection to the enzyme, while the longer space arm of GO-NH₂-E made the activity of the enzyme wider and the protection of the enzyme by graphene oxide smaller. Therefore, the optimum temperature of GO-COOH-E was 5°C higher than that of GO-NH₂-E.

Effects of pH on the Activity

One of the most important factors affecting the catalytic activity of α -amylase is the pH value of the buffer solution. Because the change of pH value will directly affect the dissociation state of α -amylase, especially the dissociation state of amino acid

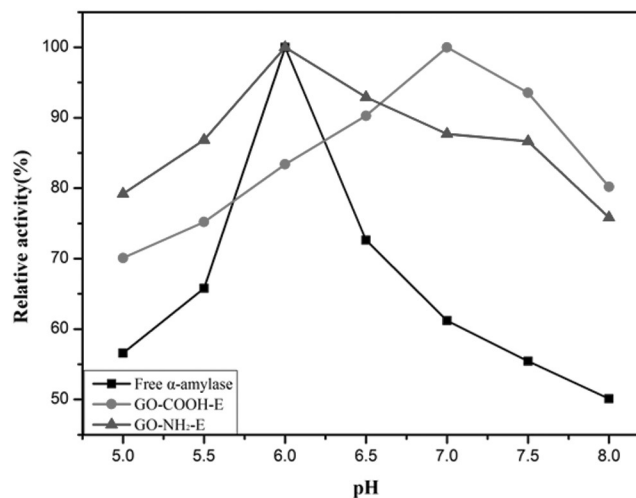


Fig. 7 —Optimum pH of the free and immobilized enzymes

residues near the enzyme activity center. Under the condition of peracid or alkali, the activity of the enzyme will be limited or even inactivated^{33–35}. Therefore, the optimum pH of the immobilized enzyme was explored in this paper. Results were shown in (Fig. 7), the optimum pH values of free enzymes, GO-NH₂-E and GO-COOH-E were 6.0, 6.0, and 7.0, respectively. The sensitivity of immobilized enzymes to pH decreased significantly, and the relative activity of GO-NH₂-E changed only 24.18%, while the change of GO-COOH-E and free enzymes was 29.9% and 49.9%, respectively. The optimum pH of GO-NH₂-E had no change, which was different from another study. Zhuang *et al.*⁷ indicated that the amino or imino on the surface of the carrier might decrease the concentration of H⁺ and the optimum pH of the immobilized enzyme could be increased to a certain extent. This might contribute to the fact that the content of amino or imine on the surface of the carrier was not enough to reduce the concentration of H⁺ in this experiment, so the optimum of GO-NH₂-E was consistent with the free enzyme. The optimal pH of GO-COOH-E varies obviously, which may be owing to the influence of space arm. Although GO-COOH-E had a shorter space arm, which limited the activity of enzymes to a certain extent, the shorter space arm made the enzymes well protected by carriers. This may be related to the nature of the GO carrier itself, the carboxyl group at the edge of the GO was protonated and the hydrophobic properties of the GO sheet were increased. Therefore, the proton concentration in the microenvironment of the immobilized enzyme was lower than the proton concentration in the bulk solution³⁶.

Operational stability

Reusability of immobilized enzymes demonstrates the imperative parameter insight of great range industrial applications since the processing cost is considerably decreased due to their use in repeated batch operations³⁷. As can be seen from (Fig. 8), the covalent immobilization procedure has a significant stabilizing effect on α -amylase for both supports. The activity of GO-COOH-E decreased significantly to 82.7% after the first use of GO-COOH-E, which seemed to correlate to the incomplete washing after the immobilization of carrier and enzyme. Some enzyme was deposited on the surface of the carrier or the enzyme molecule with weak binding was shedding. After the second reaction, the decrease in enzyme activity tended to be gentle. A possible explanation was that the exfoliation or inactivation of the susceptible enzymes immobilized on the surface of the carrier during reaction or centrifugation. Another reason was that some enzymes were immobilized in the interlayer or groove of GO-COOH, the space arm of GO-COOH-E was short and the range of activity was limited, which hindered the transfer of products and reduced the chance of contact between enzymes and substrates, thus affecting the activity of immobilized α -amylase^{38,39}.

The activity of the GO-NH₂-E decreased with a number of times of reuse compared to its initial value and the GO-NH₂-E kept nearly 70% after 7 times reuse. The longer space arm of GO-NH₂-E provided a larger range of activity for the enzyme, which was conducive to the contact between the enzyme and the substrate. Notably, GO-NH₂-E exhibited higher residual activity than that of GO-COOH-E after 14 cycles (the relative activity of GO-COOH-E was 54.6% and GO-NH₂-E was 68.36%). However, GO-COOH-E

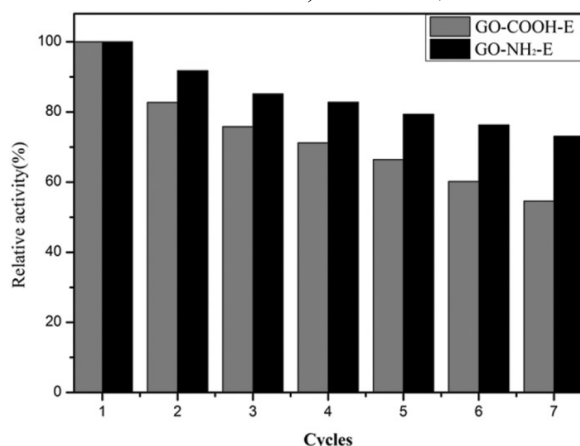


Fig. 8 — Reusability of GO-COOH-E and GO-NH₂-E

retained more activity of free enzymes (the initial activity of GO-COOH-E was 4180 U/g; the initial activity of GO-NH₂-E was 3340 U/g), the performance of the two immobilized enzymes were similar in repeatability. Both showed good operational stability.

Kinetic parameters

Michaelis-Menten kinetic parameters K_m and maximum reaction rate V_{max} of free and immobilized enzymes were obtained using GraphPad Prism 7 in this report. The simulation results were shown in (Fig. 9).

The results revealed that the K_m values of free enzymes, GO-COOH-E and GO-NH₂-E were 3.541 mg·mL⁻¹, 4.072 mg·mL⁻¹, and 8.004 mg·mL⁻¹, respectively. The V_{max} values were 7.341 mg·mL⁻¹·min⁻¹, 4.968 mg·mL⁻¹·min⁻¹ and 6.655 mg·mL⁻¹·min⁻¹, orderly. The K_m value was known as the affinity of the enzymes to substrates and the lower values of K_m emphasize the

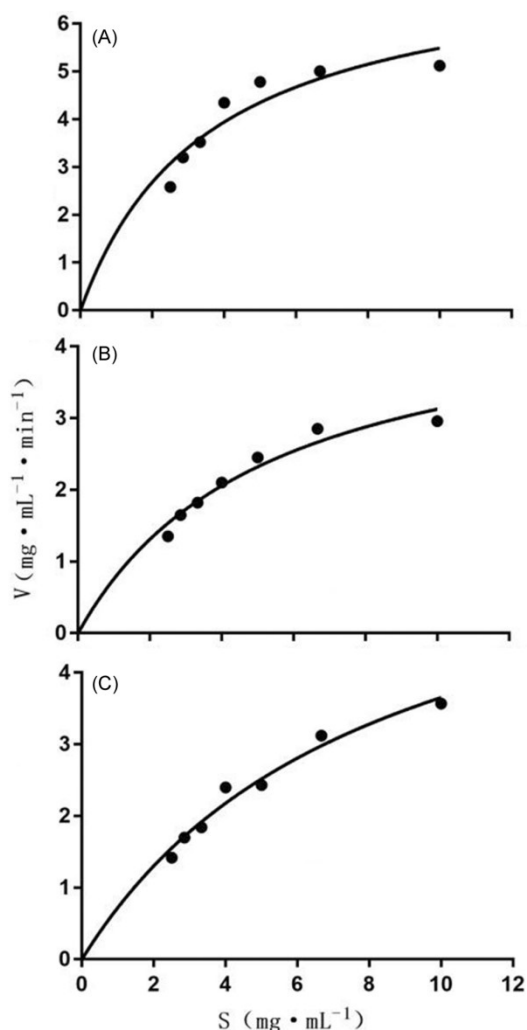


Fig. 9 — Michaelis-Menten plots for (A) free α -enzyme; (B) GO-COOH-E; and (C) GO-NH₂-E

higher affinity between enzymes and substrates. The increase of K_m values reflected the weakening of the binding ability between the substrate and the enzyme, which may be due to the influence of glutaraldehyde on the tertiary configuration of the enzyme, thus changing the binding ability between the enzyme and the substrate⁴⁰. Generally speaking, K_m values of immobilized enzyme were different from that of the free enzyme due to diffusion limitation, space effect and ionic strength. The change of substrate affinity was also caused by the change of enzyme structure introduced by the immobilization process and the decrease of the approachability of the substrate to the site of immobilized enzyme activity. According to the analysis of SEM and optical pictures mentioned above, the structure of GO-COOH was close to GO, making their K_m values similar; while the agglomeration of GO-NH₂ was serious, so the K_m value of GO-NH₂-E increases more obviously than that of free enzymes. Therefore, the increase in the K_m value of GO-NH₂-E after immobilization may be due to the impediment of substrate or product transport caused by transitional deposition. In addition, GO-NH₂-E obtained V_{max} larger than GO-COOH-E, which may be due to the long space arm providing enough free space for the enzyme to perform its function^{41,42}.

Conclusions

In this study, α -amylase was immobilized on GO-COOH and GO-NH₂ by chemical crosslinking with glutaraldehyde as a crosslinking agent. Compared with free enzymes, the pH stability, thermal stability, and reusability of immobilized enzymes were improved. At the same time, GO-COOH-E and GO-NH₂-E showed their characteristics: GO-COOH-E had higher enzyme load, stronger resistance to temperature, and easier to bind to the substrate, but GO-COOH-E owned relatively poor reusability; GO-NH₂-E had better reusability, higher V_{max} value, but lower initial enzyme activity retained.

Michaelis-Menten kinetic data reflected that K_m of the two immobilized enzymes increased, especially GO-NH₂-E, which indicated that the affinity between the immobilized enzymes and the substrate was not improved, but the binding force was weakened. Although further work was needed to study and observe the effects to improve the catalytic performance, our work showed that the effects of support materials on the activity of biological macromolecules can be regulated by surface modification, and GO and its derivatives also show great potential for application in high-efficiency biocatalyst systems.

Conflict of interest

All authors declare no conflict of interest.

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