Supporting Information

α-Glucosidase immobilization on metal-organic frameworks composite membrane for enzyme inhibitors screening

Ning Wen[a #1], Guang-Zhen Wan[a #1], Ke Sun[b], Fang-Di Hu [a*], Juan Chen [a*]

[a] School of Pharmacy, Lanzhou University, Lanzhou 730000, China

[b] LanZhou Foci Pharmaceutical Co.,Ltd., Lanzhou 730000, China

The supporting information includes the influences of reaction temperature and pH on enzyme activity (S1 and Fig. S1), performance study of immobilized α-glucosidase (S2 and Fig.S2) and the study of enzyme kinetics (Fig. S3).

[#1] These authors contributed equally to this work and should be considered co-first authors.

[*]Correspondence authors: Juan Chen; Tel: 86-931-8915686; Fax: 86-931-8915686; E-mail: chenjuan@lzu.edu.cn (J. Chen).

S1: Influences of reaction temperature and pH on enzyme activity

The reaction pH affects the dissociation degree of the enzyme molecule from the substrate and subsequently their binding to each other. Under the optimal pH condition, the dissociation of enzyme, substrate and co-enzyme is most suitable for their mutual binding and maximizes the rate of enzymatic reaction. Therefore, the pH tolerance of immobilized and free α-glucosidase was evaluated in the range of 5.0-9.0. As demonstrated in Fig. S1a, the catalytic activity of both enzymes increased and then decreased as the reaction pH gradually increased, and the optimal enzymatic pH of immobilized enzyme was 8.0, while that of free enzyme was 7.0. Immobilized α-glucosidase had higher alkaline tolerance compared to free one. This might be due to the fact that the carrier affects structural properties or surface charge of the enzyme after immobilization, and a large number of anions accumulates on the enzyme surface, resulting in a change in its optimum pH. In general, the immobilized enzyme has higher acid-base stability relative to the free one. In the subsequent experiments, 7.0 and 8.0 were used as the optimal enzymatic reaction pH value for the free and immobilized α-glucosidases, respectively.

Enzyme catalysis is also affected by reaction temperature, and a suitable temperature increases the collision between enzyme molecules and substrates and improves the catalytic activity of the enzyme. The effect of reaction temperature on the catalytic activity of free and immobilized α-glucosidase in the range of 40-80°C was examined. As shown in Fig. S1b, free and immobilized enzymes showed the maximum catalytic activity at 60 °C and 70 °C, respectively. The optimal enzymatic reaction temperature of α-glucosidase increased after immobilization, which indicated that the immobilized enzyme was more tolerant to temperature compared to the free one. This can be explained that the enzyme was encapsulated in situ in crystal lattice of ZIF-8 making the conformation of enzyme molecule more stable. Consequently, 60°C and 70°C were used as the optimum reaction temperature for the free and immobilized enzyme, respectively.

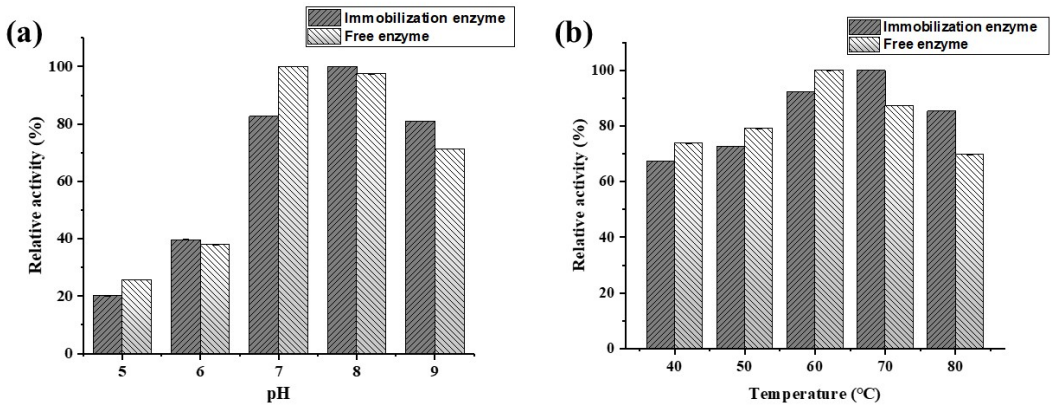

Figure S1. Influence of (a) enzymatic reaction pH and (b) enzymatic reaction temperature on the relative activity of immobilized and free α-glucosidase.

S2: Performance study of immobilized α-glucosidase

Five different batches of α-Glu@ZIF-8@PDA@PVDF were prepared by the same method for batch-to-batch reproducibility evaluation, and the RSD value was measured to be 5.9%, indicating that the immobilization method was stable and reliable and can be used for subsequent experiments.

To evaluate the reusability of α-Glu@ZIF-8@PDA@PVDF, the relative activity of immobilized enzyme was tested after 10 consecutive reactions. The results were displayed in Fig. S2, the immobilized enzyme activity retained 97.77% of its original value after three consecutive catalytic reactions. Overall, the relative activity of the immobilized α-glucosidase gradually decreased with the number of the assay, but remained at 79.32% after 10 cycles. The decrease in activity might be due to the denaturation and shedding of the enzyme in each catalytic experiment. Compared with free enzyme which can only be used once and cannot be recovered, α-Glu@ZIF-8@PDA@PVDF exhibited better reusability.

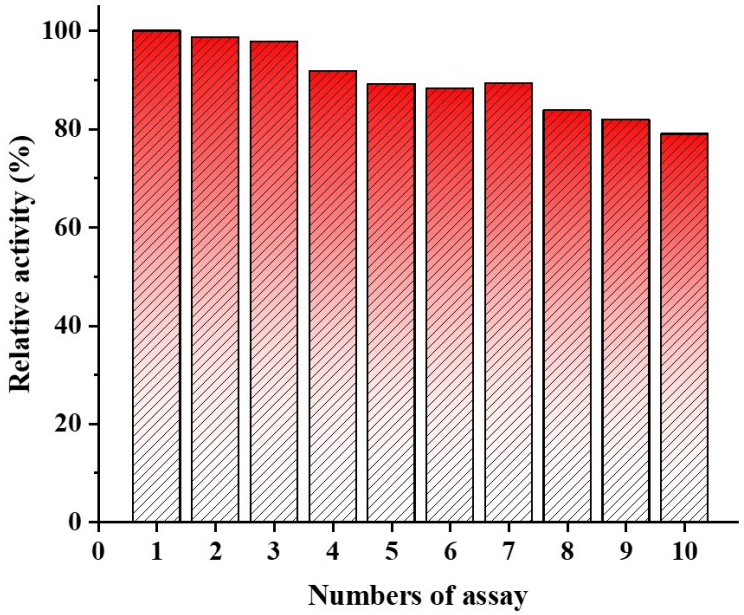

Figure S2. Reusability of α-Glu@ZIF-8@PDA@PVDF.

S3: Enzyme kinetics and inhibition kinetics study

K_m is the most important constant in kinetic studies of enzymatic reaction. As illustrated in Fig. S3, the Lineweaver-Burk plots of free and immobilized enzyme with substrate concentrations in the range of 0.25-4.0 mM were plotted based on $1/v$ versus $1/[S]$. According to Eq. (1), the corresponding linear regression equations were calculated to be $1/v = (1.026 \pm 0.011) \times (1/[S]) + (0.3233 \pm 0.0034)$, $R^2 = 0.9995$ and $1/v = (0.9929 \pm 0.017) \times (1/[S]) + (0.3486 \pm 0.0074)$, $R^2 = 0.9984$. From the above equation, the K_m values of free and immobilized enzyme were calculated to be 3.17 mM and 2.85 mM, respectively. The immobilized enzyme has a lower K_m value compared with the free one, showing its higher affinity towards the substrate. This may be due to the increase of optimum enzyme reaction temperature after immobilization, which accelerates the molecular movement of enzyme and makes it easier for the active site to come into contact with substrate [1].

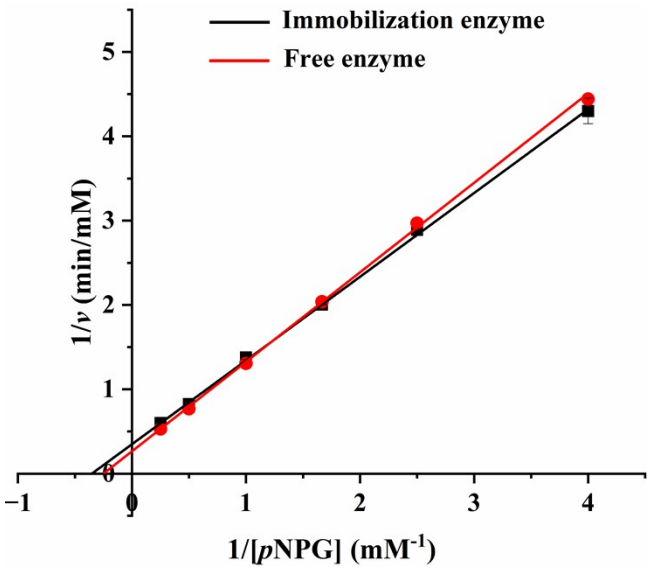

Figure S3. Lineweaver-Burk plots of the free and immobilized α-glucosidase.

Reference:

[1] Hu, Y.; Dai, L.; Liu, D.; Du, W.; Wang, Y., Progress & prospect of metal-organic frameworks (MOFs) for enzyme immobilization (enzyme/MOFs). Renewable and Sustainable Energy Reviews. 2018, 91, 793-801.