Supplementary material

Immobilization of lipase on zwitterionic polymer-modified magnetic chitosan microspheres for enhancing enzyme performance

Experimental

Synthesis of zwitterionic *polymers*

Zwitterionic polymers were synthesized according to the previous method [1], as shown in Fig. S1. In brief, 1 g of poly (isobutene-*alt*-maleic anhydride) (about 6.5 mmol anhydride group) was dissolved in 5 mL of *N, N*-dimethylformamide. Then 0.44 mL of *N, N*-dimethylethylenediamine (DMEA) was added to the polymer solution under magnet stirring for 12 h at room temperature. The resultant polymer was precipitated by adding excess acetone, and then recovered by centrifugation and washed with acetone. Subsequently, the final polymer was dried in vacuum. The ring-opening degree of the anhydride groups in PIMA was determined by ^{1}H NMR spectroscopy (Fig. S2) with a 500 MHz NMR spectrometer (Varian, California, USA), in which the ratio of the peaks of the methyl proton (δ~ 2.86 ppm) held by the nitrogen atom to the methyl proton of polymer backbone (δ~ 0.99 ppm) was close to 0.7.

Determination of enzyme activity

0.1 mL of enzyme sample was added into the substrate solution comprised of 2.4 mL of 50 mM phosphate buffer (pH 7.0) and 0.5 mL of 10 mM *p*-nitrophenyl acetate (*p*-NPA) in acetonitrile at 37 ℃ and 170 rpm. After 10 min, 3.0 mL of ethanol was added to terminate the reaction, and then the absorbance of the solution was measured immediately at 400 nm [2]. One unit (U) of CRL activity is defined as the amount of enzyme required to generate 1 μmol of *p*-nitrophenol per minute under the test conditions.

Reference

1. Zhang, C.Y., Sun, Y. and Dong, X.Y., (2022), Conjugation of a zwitterionic polymer with dimethyl chains to lipase significantly increases the enzyme activity and stability. *Chinese Journal of Chemical Engineering*, **47** , 48-53. https://doi.org/10.1016/j.cjche.2021.04.023
2. Ren, L., Jia, H., Yu, M., Shen, W., Zhou, H. and Wei, P., (2013), Enhanced catalytic ability of *candida rugosa* lipase immobilized on pore-enlarged hollow silica microspheres and cross-linked by modified dextran in both aqueous and non-aqueous phases. *Biotechnology & Bioprocess Engineering*, **18**(5), 888-896. https://doi.org/10.1007/s12257-013-0044-7

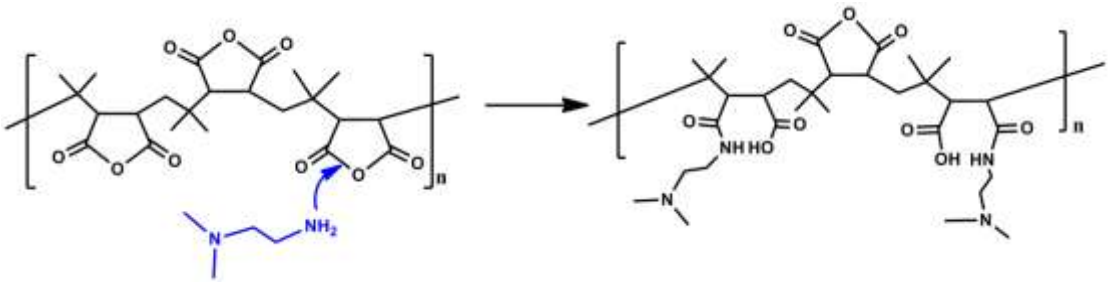

Fig. S1. Synthesis of polymer

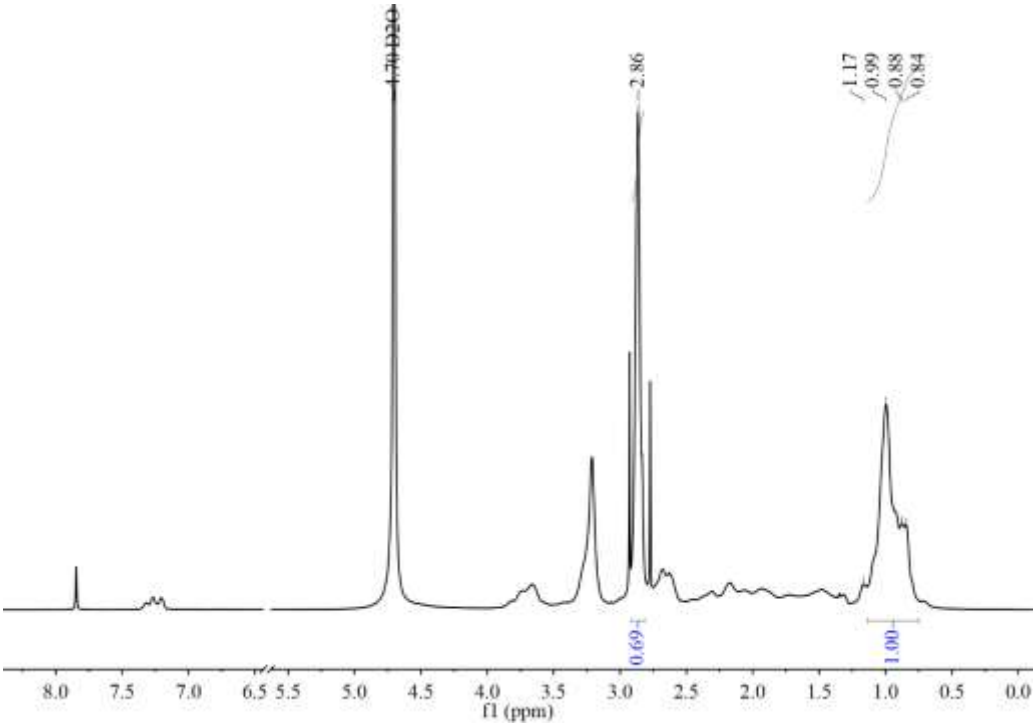

Fig. S2. ^{1}H NMR spectrum of pID0.7.

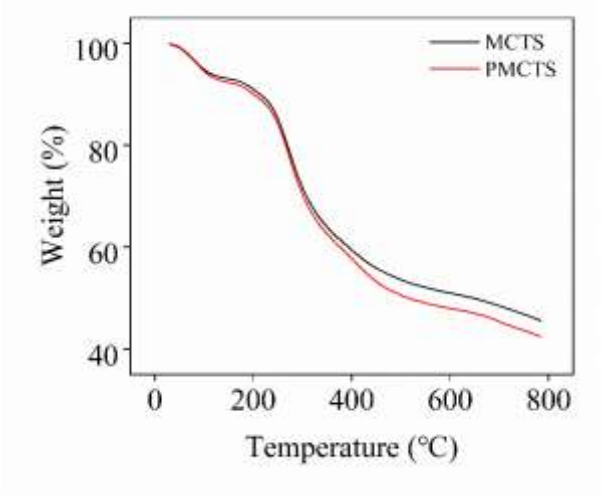

Fig. S3. TGA curves of MCTS and PMCTS.

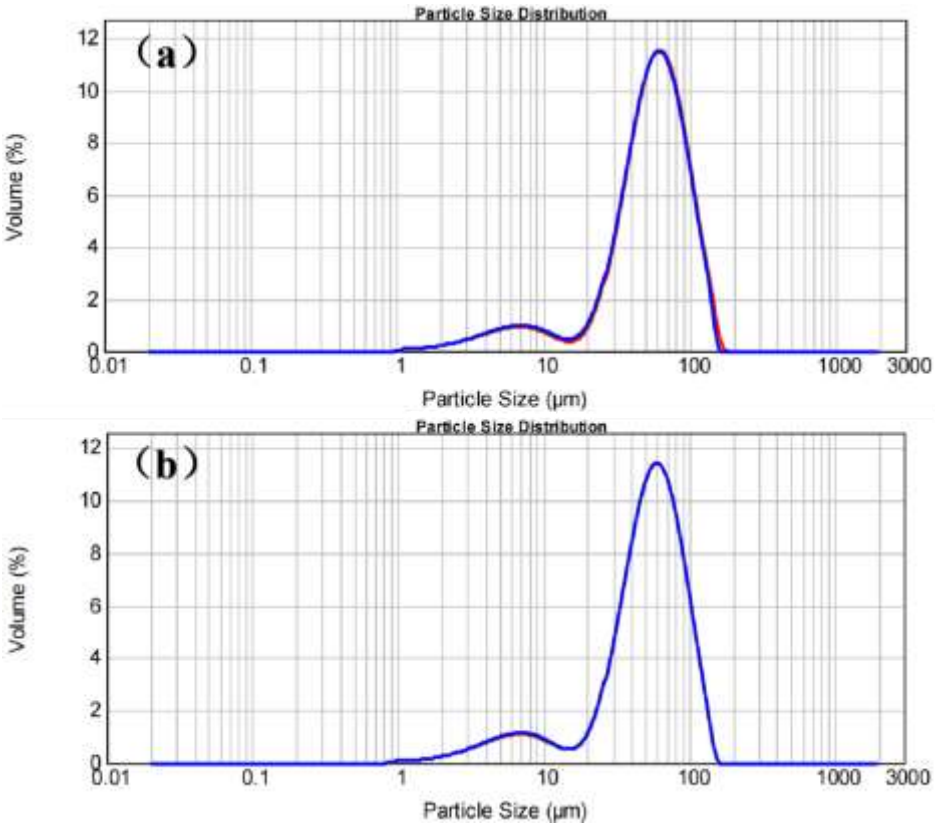

Fig. S4. The particle size distribution of (a) MCTS and (b) PMCTS

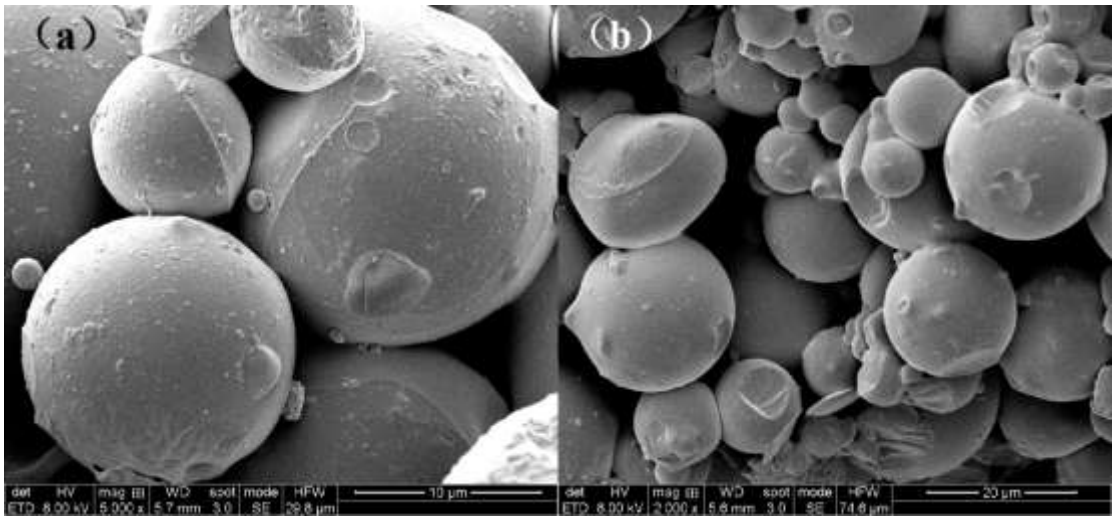

Fig. S5. SEM images of (a) MCTS and (b) PMCTS

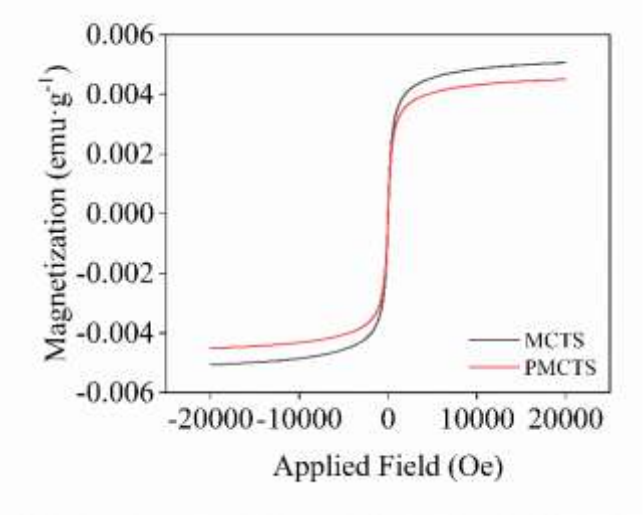

Fig. S6. Hysteresis loops of MCTS and PMCTS

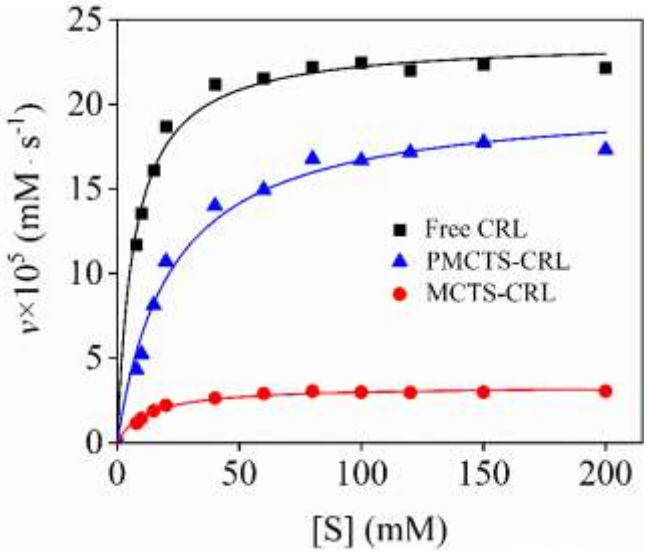

Fig. S7. The kinetic curves of the enzymatic reactions of free and immobilized CRLs. The solid lines are calculated from Michaelis-Menten equation