Synthesis of papain-polyacrylamide hydrogel microspheres and their catalytic application

Yuan Ma[1, 2], Yao Li[3], Xu Fei*[1], Jing Tian*[2], Longquan Xu [1], Yi Wang[2]

Supporting Information

1 Immobilization conditions of papain

The papain activity of PAHMs was studied when the mass ratio of enzyme to carrier was 1:2, 1:4, 1:6 and 1:8. As shown in Fig. S1a, it indicated that the mass ratio of 1:4, (papain/PAHMs) was the optimal enzyme loading ratio for papain-PAHM1, papain-PAHM2, and papain-PAHM3, which were 14.67±2.15 mg g^{-1}, 12.08±2.12 mg g^{-1}, 8.18±2.41 mg g^{-1}, respectively. Therefore, the mass ratio of 1:4 for papain/PAHMs was chosen as the best enzyme-loading ratio.

The influence of immobilization temperature on the loading of papain was further discussed. As shown in Fig. S1 b, the papain capacity of papain-PAHMs was higher between 30 and 40 ºC than other immobilization temperature. Combined with the physiological conditions of papain, 37 ºC was chosen as the optimal immobilization temperature. The effect of immobilization time on papain loading was investigated at 37 ºC under the conditions of enzyme to carrier mass ratio of 1:4. The papain capacities of papain-PAHMs immobilized for 3 h, 6 h, 9 h, 12 h and 15 h were determined. As shown in Fig. S1 c, the papain capacities of papain-PAHM1, papain-PAHM2 and papain-PAHM3 reached balance at 6 h, 9 h and 12 h, respectively, which were 8.17±1.34 mg g^{-1}, 12.23±0.88 mg g^{-1} and 14.67±0.04 mg g^{-1}.

In conclusion, the optimal immobilization condition was as follows: papain /PAHMs mass ratio of 1:4, immobilization temperature of 37 ºC , and immobilization time of 6-12 h. The results showed that PAHM3 had the best enzyme loading capacity.

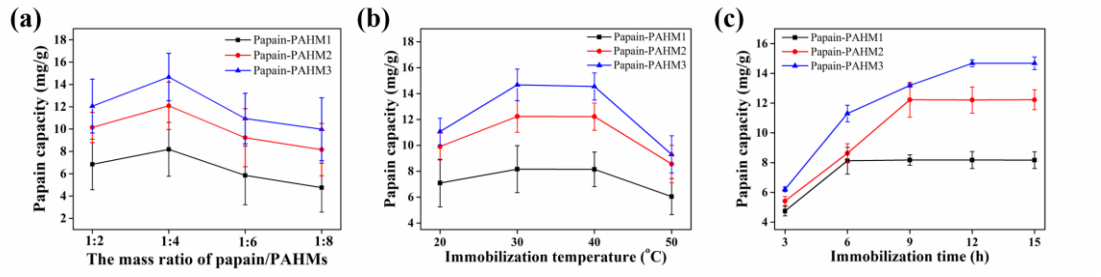

Fig. S1 The papain capacity of different mass ratio of papain/PAHMs (a), immobilization temperature (b) and immobilization time (c)

2 Standard curve

The standard curve of protein content was determined by Bradford protein assay. The assay was based on the observation that the absorbance maximum for Coomassie Brilliant Blue G-250 was appeared at 595 nm when binding to protein occurs. Within the linear range of the assay, the more protein present, the more Coomassie binds.

As shown in Fig. S2, the linear equation of the protein standard curve could be calculated as: $Y = 0.0087X + 0.0111$, where $R^2 = 0.99959$, Y was the absorbance in 595nm, and X was the protein content (μg). It indicated that the linear relationship of the standard curve was good when the protein content from 0 μg to 90 μg.

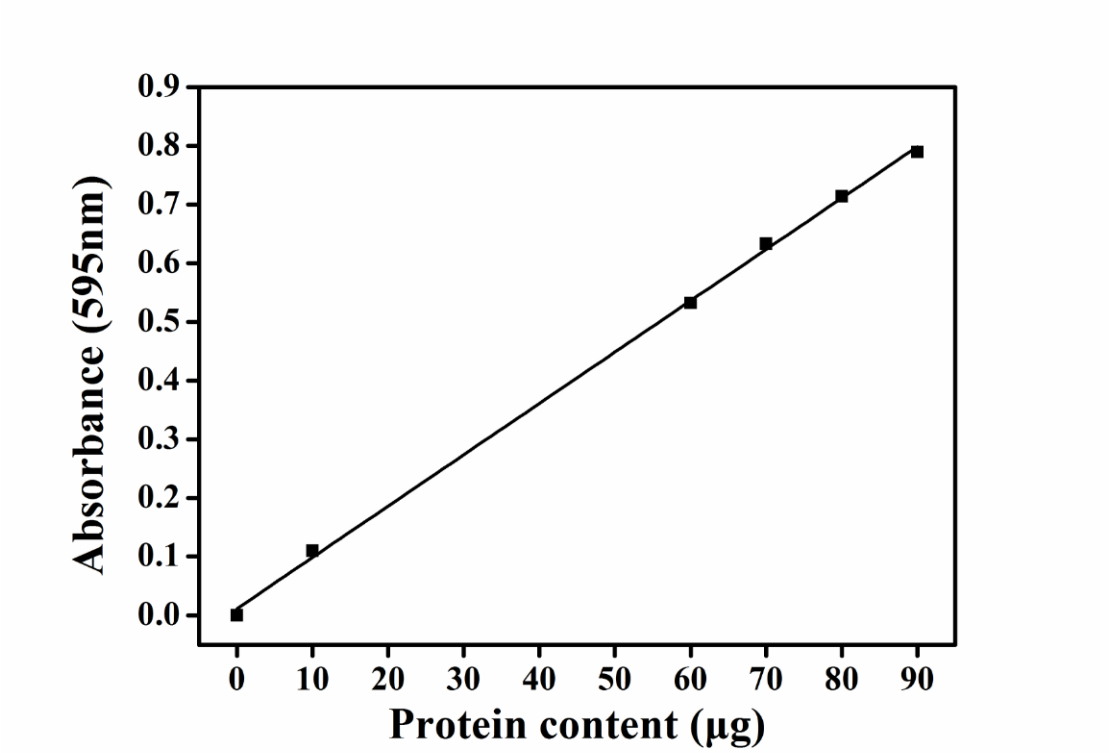

Fig. S2 The standard curve of protein content by Bradford protein assay

3 The N_2 adsorption/desorption isotherm on PAHMs

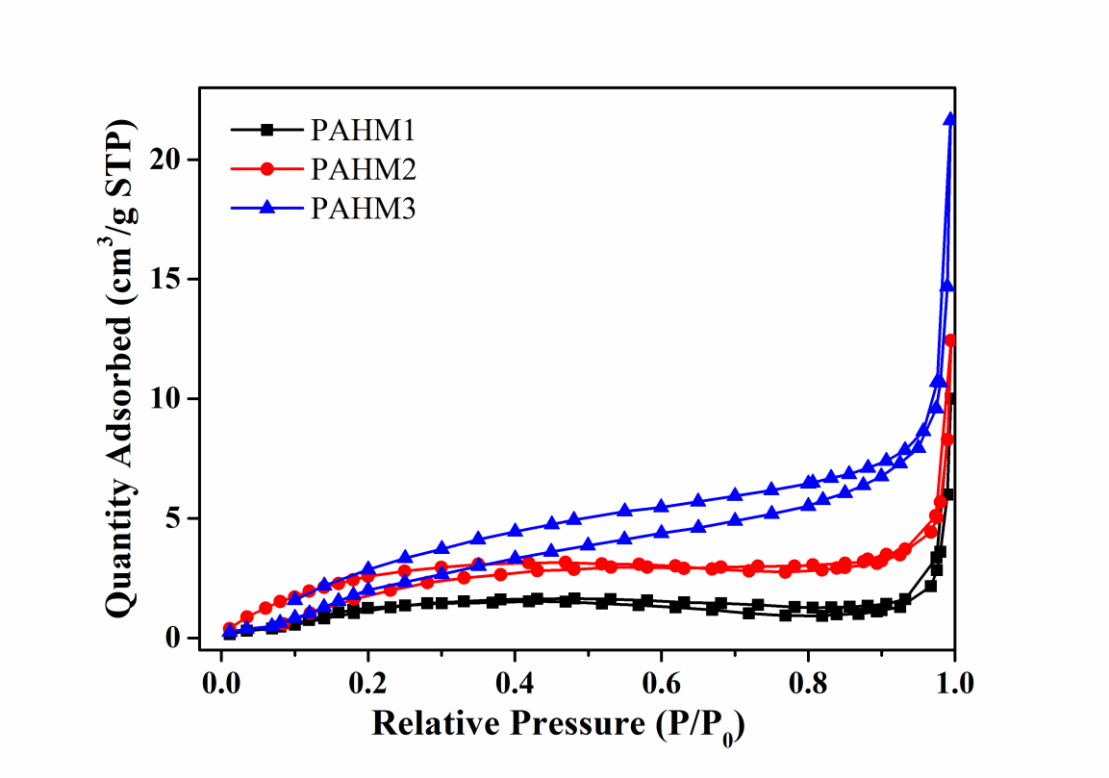

Fig. S3 N_2 adsorption/desorption isotherm on PAHMs

Table S1 The specific surface area and pore size of the PAHMs

Samples	Surface area (m^2/g)	Average pore diameter (nm)
PAHM1	2.22	24.01
PAHM2	3.602	14.96
PAHM3	8.12	11.21

4 The SEM and EDS of papain-PAHMs

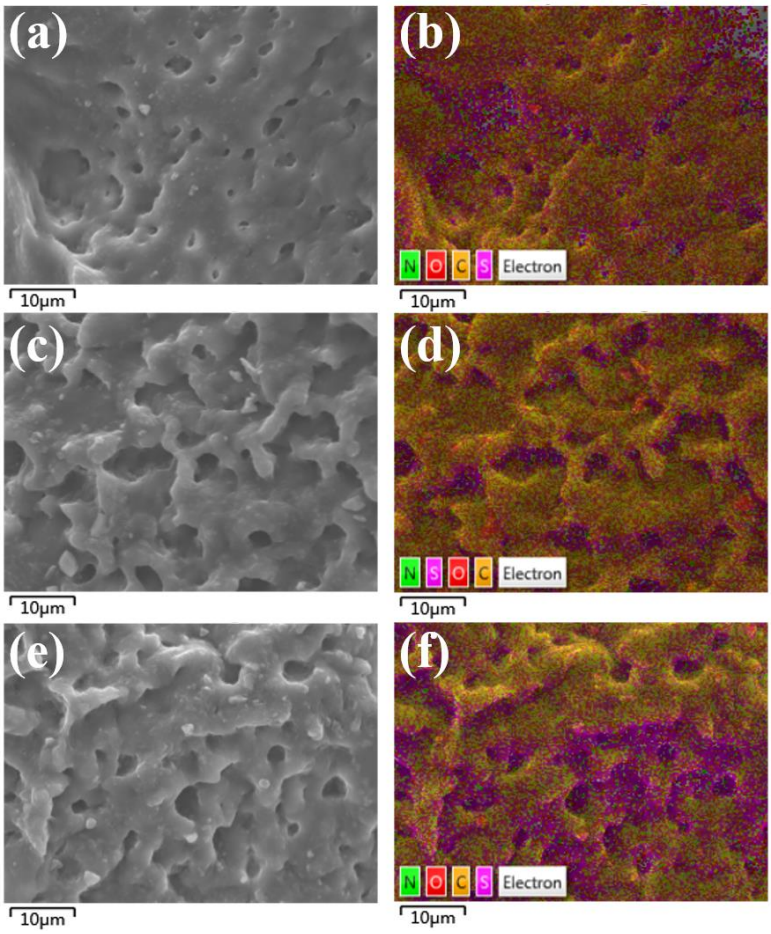

Fig. S4 The surface image of papain-PAHM1 (a), papain-PAHM2 (c), papain-PAHM3 (e) by SEM, (b, d, f) element analysis of papain-PAHM1, papain-PAHM2 and papain-PAHM3 by EDS

Table S2 The S element content of papain-PAHMs by EDS

Samples	Apparent Concentration	Wt %
Papain-PAHM1	0.42	0.51±0.02
Papain-PAHM2	0.47	0.57±0.02
Papain-PAHM3	0.52	0.81±0.02

4 The particle size distribution of PAHMs before and after swelling

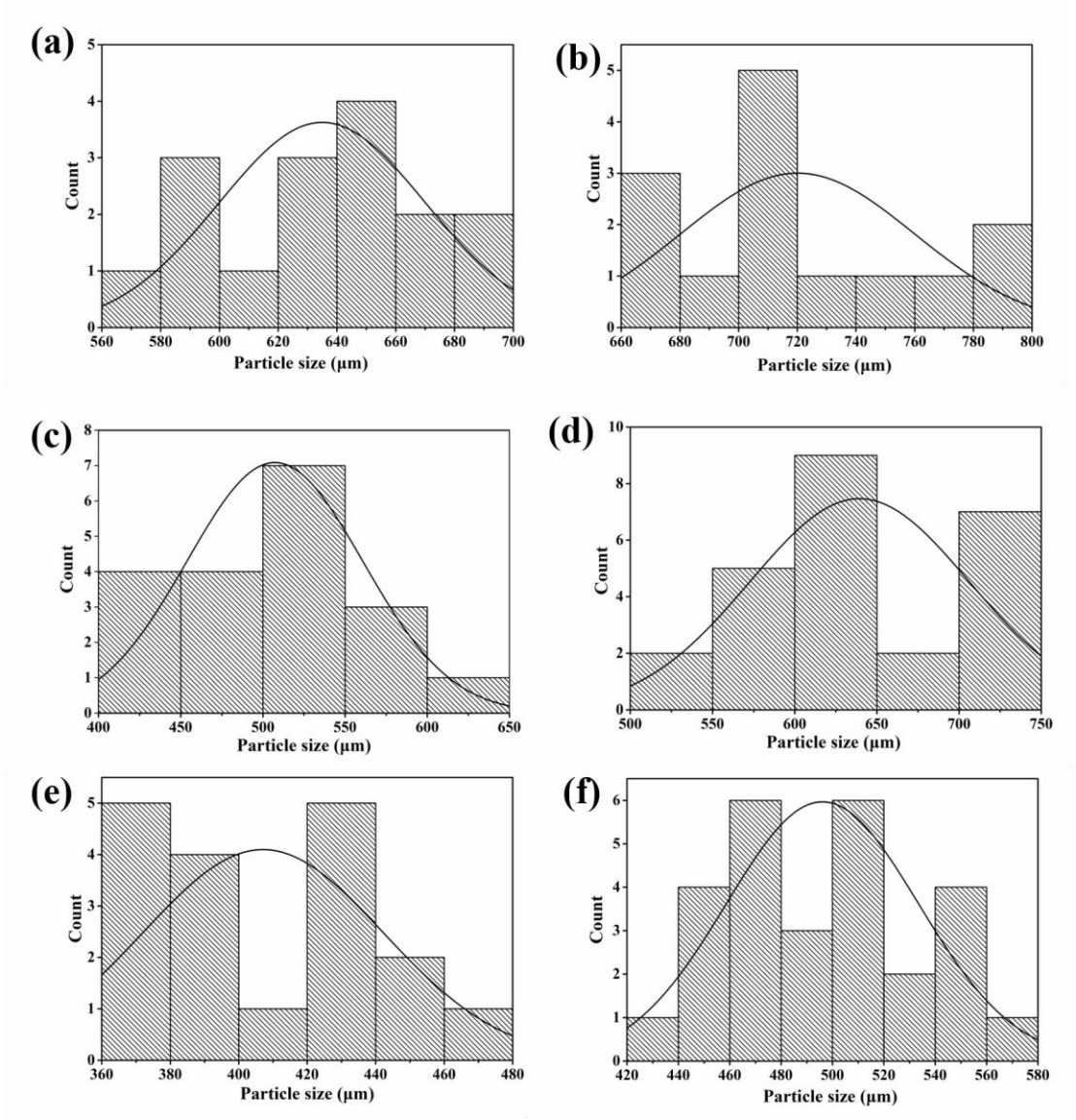

Fig. S5 The particle size distribution of PAHM1, PAHM2, PAHM3 before (a, c, e) and after (b, d, f) swelling