Electronic Supplementary Information for

Microgels formed by enzyme-mediated polymerization in reverse micelles with tunable activity and high stability

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1. Materials and reagents

Horseradish peroxidase (HRP, EC 1.11.1.7, ME = 40 kDa, 300 U mg^{-1}) was purchased from Shanghai Baoman Biotechnology Co., Ltd. Hydrogen peroxide (H_2O_2, 30%) was obtained from Sinopharm Chemical Reagent Co., Ltd. *N,N*-dimethylacrylamide (DMAA ⩾ 99.0%) and *N,N'*-methylenebisacrylamide (MBA ⩾ 99.0%) were bought from TCI Shanghai and used without further purification. Acetylacetone (ACAC), surfactants Tween 20 and Span 80 were got from Alfa Aesar and used as received. All other chemical reagents and solvents were of the highest purity commercially available and were used as received.

2. Instruments and characterizations

Two instruments were employed for characterization of the morphologies of PDMAA microgel particles: one was a field-emission scanning electron microscopy (SEM, hitachi S-4800, JEOL, Japan) with the accelerating voltage of 1.0 kV for vacuum-dried microgel particles; another was optical microscope (Inverted Microscope, EVOS xl, AMG Co., USA) for microgel particles in distilled water under bright field.

Besides, size distribution was conducted by Laser Diffraction Particle Size Analyzer (Mastersizer 3000, Malvern, UK) for microgel particles in distilled water.

3. Preparation of PDMAA microgel

For a typical example of hydrogelation, monomer DMAA (310 μL), crosslinker MBA (7.2 mg) and enzyme HRP (9 mg) were dissolved in deionized water (3.37 mL) to form a clear homogeneous solution. Meanwhile, ACAC (130 μL), surfactants including Tween 20 (1.2 g) and Span 80 (2.4 g) were dissolved in octane (16.4 g) to form organic phase and purged with argon for 30 min. Then, the aqueous solution was injected dropwise into organic phase by a syringe under stirring. The mixture was initiated by adding H_2O_2 solution (320 μL, 0.2 M) slowly at room temperature. After given time (6 h), the reaction mixtures were demulsified by excess ethanol twice, and then washed twice with distilled water, thus the light-brown microgel was obtained. For catalysis study, the as-prepared microgel was dispersed in corresponding solvent to obtain dispersed suspension, whose concentration was based on swelling ratio. It's worth noting that microgel particles couldn't be produced without purging argon by our preliminary experiments.

3. Calculation of theoretical average mesh size

Mesh size of microgel could be theoretically deduced according to the network structure model. Based on this similar model, microgel can be considered as a macroscopical cube with side length of *a* (Fig. S1, ESI†). The crosslink points were supposed to be evenly dispersed in three-dimensional space, so the number (*n*) of crosslink points in one dimension could be obtained by equation (1):

$$n = \left(N \times \mathrm{N_A}\right)^{1/3} \tag{1}$$

where *N* denotes the number of moles of crosslinking molecules and $\mathrm{N_A}$ stands for Avogadro constant. For a cube model, its volume (*V*) is equal to a^3. Therefore, the shortest distance (*d*) between two adjacent crosslink points, namely the value of theoretical mesh size of microgel, can be deduced by the following equation (2):

$$d = \frac{a}{n} = \frac{V^{1/3}}{\left(N \times \mathrm{N_A}\right)^{1/3}} = \frac{1}{\frac{N}{V} \times \mathrm{N_A}}^{1/3} = \frac{1}{C \times \mathrm{N_A}}^{1/3} \tag{2}$$

where *C* represents the molar concentration of crosslinker.

4. Calculation of swelling ratio (S_R)

The gravimetric method was employed to calculate the swelling ratio of microgels. The mass of freshly prepared light-brown microgel was recorded as W_S. After dried in a drying oven for 40 min at 220 °C, the mass of dried microgel was recorded as W_d. The swelling ratio of microgel was subjectively defined and expressed by equation (3):

$$S_R = \frac{W_S - W_d}{W_d} \tag{3}$$

The data were averaged from three independent measurements.

5. Determination of microgel yield

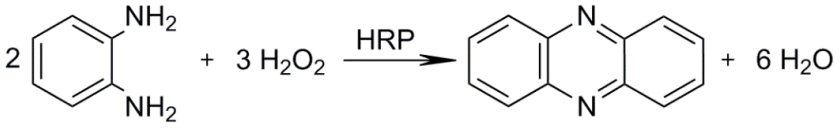
In theory, the mass of dry micrgel particles (W_{d1}) should be the incorporated total mass of DMAA, MBA and HRP, i.e., $W_{d1} = W_{DMAA} + W_{MBA} + W_{HRP}$, where W_{DMAA}, W_{MBA} and W_{HRP} are their respective initial weight. During the process of HRP-mediated polymerization, the mass of actual obtained dry microgel could be

calculated according to the swelling ratio of microgel and abbreviated as W_{d2}. Therefore, the yield of microgel in inverse emulsion could be written as equation (4):

$$\text{Yield } \% = \frac{W_{d2}}{W_{d1}} \times 100\% \qquad (4)$$

6. Test of catalytic activity

The reaction of *o*-phenlenediamine (OPD) oxided by H_2O_2 was selected as a model to characterize the catalytic activity of $HRP_{(U)}$ and $HRP_{(I)}$. The concentration of the product was corrected according to the molar extinction coefficients in aqueous buffer.



$\varepsilon_{450\ nm}$ is 16300 M^{-1} cm^{-1} in phosphate buffer (50 mM, pH 7.0).

For enzyme catalytic reaction in aqueous solution, the volume of the mixture containing substrate of different concentration and H_2O_2 of 50 mM was fixed to 2.0 mL, and the absorbance of product was measured with 6 s interval in the first minute. During the process, the reaction mixture was slightly stirred at room temperature.

The colorful products were calculated by their molar extinction coefficient at 450 nm. The initial reaction rate (V) was obtained by linear fitting the product concentration against time since the enzyme catalytic reaction displayed zero kinetics in the starting period. At saturated concentration of H_2O_2, a series of initial reaction rates was obtained by adjusting OPD concentrations from 10.0 mM to 1.0 mM. According to the steady-state Michaelis-Mentan equation, the dynamic constants includes the maximum reaction rate (V_{max}) and enzyme Michaelis-Menten constant (K_m) can be obtained by plotting $1/V$ against $1/[S]$ (Lineweaver-burk Plot). The Michaelis-Menten equation is expressed by equation (5) as follows:

$$V = \frac{V_{max}[S]}{[S] + K_m} \qquad (5)$$

The lineweaver-burk plot is written by equation (6):

$$\frac{1}{V} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \quad (6)$$

where [*S*] denotes the initial molar concentration of the substrate (OPD). The turnover number, $k_{cat} = V_{max}/[Enzyme]$, suggesting the activity of the enzyme, is independent of the concentration of substrate and enzyme.

8. Tables

Table S1. Properties of microgels under various concentration of MBA

Sample	MBA (wt%)	Swelling Ratio	Conversion (%)	Average Mesh Size (nm)
I-1	0.90	4.6 ± 0.1	62 ± 2	3.1
I-2	0.45	7.0 ± 0.2	55 ± 2	3.8
I-3	0.24	10.1 ± 0.2	40 ± 1	4.7
I-4	0.21	11.1 ± 0.3	37 ± 1	5.0
I-5	0.18	14.5 ± 0.2	30 ± 1	5.2
I-6	0.15	17.5 ± 0.4	27 ± 1	5.5
I-7	0.09	-	-	6.6

Table S2. Kinetics parameters of $HRP_{(I)}$ under various concentration of MBA and its comparison to $HRP_{(U)}$ in aqueous solution

Sample	MBA (wt%)	V_{max} ($\mu M\ s^{-1}$)	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}\ s^{-1}$)
$HRP_{(U)}$	-	0.993	2.13	198 ± 3	93.2
I-1	0.90	0.132	2.04	26 ± 1	12.9
I-2	0.45	0.139	2.13	28 ± 1	13.1
I-3	0.24	0.284	1.10	57 ± 2	51.5
I-4	0.21	0.429	1.35	86 ± 2	63.4
I-5	0.18	0.742	1.55	148 ± 3	95.6
I-6	0.15	0.629	1.60	126 ± 3	78.7

9. Figures

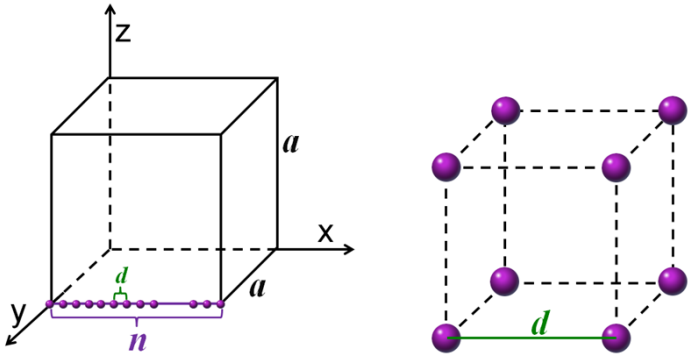


Fig. S1 Structure model for the calculation of theoretical average mesh size.

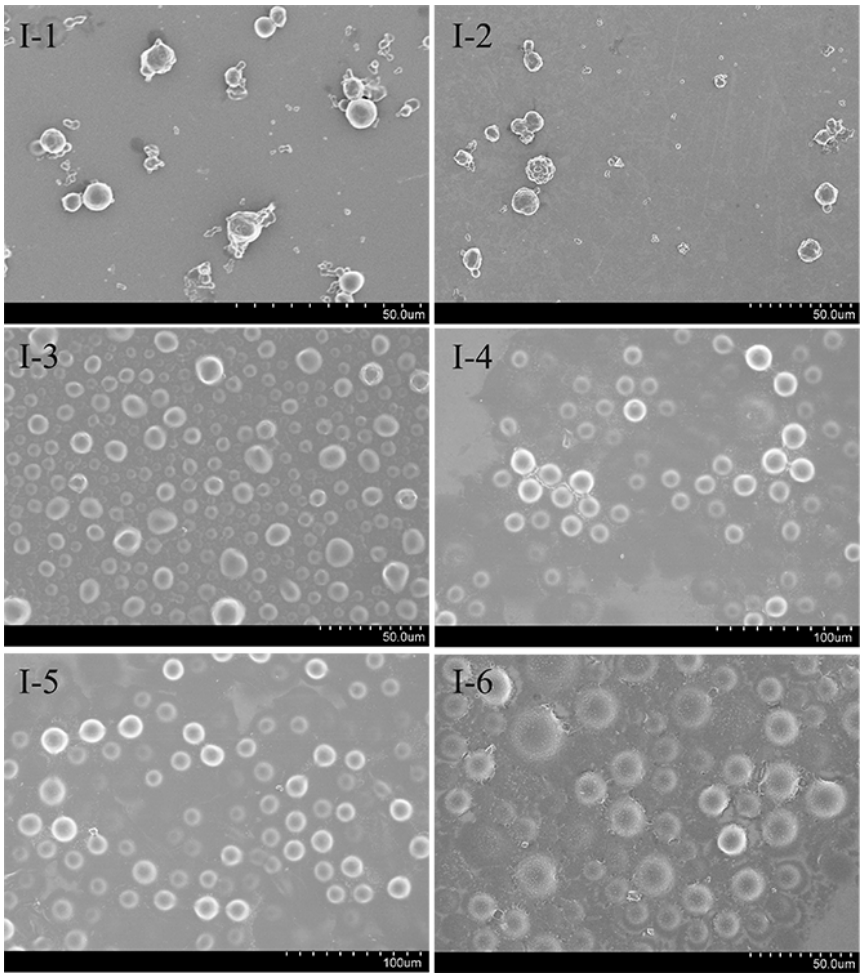


Fig. S2 SEM images of samples under various concentration of MBA. (Sample numbers correspond to those in Table S1)

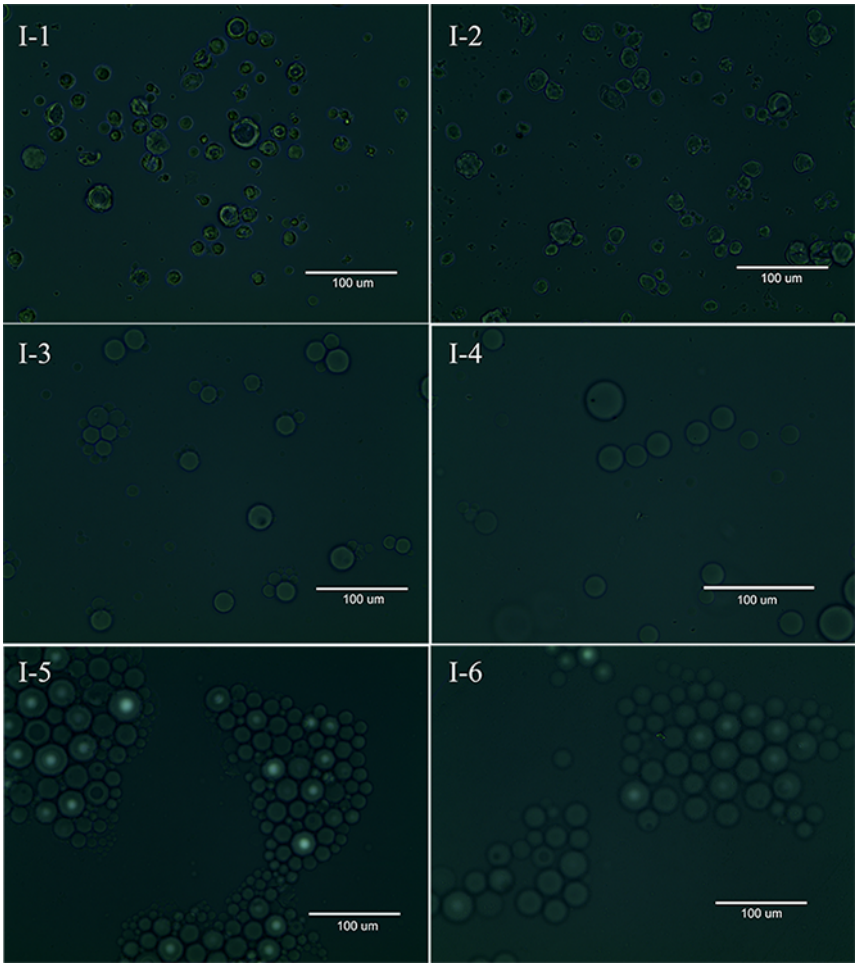


Fig. S3 Inverted microscope images of samples under various concentrations of MBA. (There are light dots in the center of some microgels due to the reflection of light. Sample numbers correspond to those in Table S1.)