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Supplementary Information for

Efficient and Controllable Synthesis of Highly Substituted Gelatin Methacrylamide for Mechanically Stiff Hydrogels

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Experimental

Synthesis of gelatin-MA.

Type A gelatin (175 bloom) derived from acid-cured porcine skin tissue was dissolved at 10% w/v at 60 °C in two buffer systems (PBS and 0.1 M CB buffer (3.18 g sodium carbonate and 5.86 g sodium bicarbonate in 1 L distilled water)). Gelatin-MA was prepared by reaction of free amino groups of lysine/hydoxylysine amino acids in the gelatin with MAA (94%) at 0.1 mL per gram of gelatin at 50 °C for 3 hours. One-sixth of 1 mL MAA (167 µL) was added every 30 minutes for 3 hours in a drop-wise format to the gelatin solutions with or without pH adjustment at 600 rpm stirring speed. After 3 hours of reaction, the solutions were readjusted to a pH of 7.4, filtered with standard filter paper and membrane filter with 0.2 µm pore diameter, dialyzed using a PALL Minimate TFF Capsule (with 10 kDa molecular weight cut-off) at 40 °C for 1 day, lyophilized, and stored at -20 °C until further use. MAA concentration-dependent experiments were also conducted with different feed ratios of MAA (ml) to gelatin (g) ranging from 0.0125:1 to 0.2:1 were added into 10% w/v gelatin in CB buffer. The synthesis condition was the same as in CB with pH maintenance at 9.0. In order to investigate the effect of pH on the degree of substitution in CB buffer solutions, pH dependent experiments were carried out at the fixed feed ratio of MAA (0.1 mL) to gelatin (1 g) in different pHs (7, 8, 9, 10, and 11) of CB buffer solutions. The yield was around 67~73% for all the groups. All chemicals were purchased from Sigma-Aldrich.

Quantification of gelatin-MA substitution.

For quantification of the DS, 2,4,6-trinitrobenzene-sulfonic acid (TNBS), which can react with primary amino groups, was used as follows: Gelatin and gelatin-MA samples were separately dissolved in 0.1 M sodium bicarbonate buffer (pH 8.5) at a concentration of 1.6 mg/ml. Then, 0.5 mL of 0.01% TNBS solution was added to 0.5 mL of each sample solution. The sample solutions were mixed well and incubated at 37 °C for 2 hours. Subsequently, 0.5 mL of 10% w/v sodium dodecyl sulfate (SDS) and 0.25 mL of 1N HCl were added to each sample in order to stop the reaction and the absorbance of each solution was measured at 335 nm. The molar concentration of primary amino groups in each gelatin-MA solution was determined by comparison with glycine standard solutions,[1] which were prepared at 0, 0.8, 8 16, 32, and 64 µg/mL.

^{1}H-NMR (400 MHz Varian) experiments were conducted in order to directly verify the DS of gelatin-MA. Around 50 mg of each lyophilized gelatin-MA sample was dissolved in 1 mL of deuterium oxide (D_2O) at 40 °C. The peak area of aromatic acids in the gelatin-MA samples was employed as a reference in each spectrum. The peak area of lysine methylene protons appearing at around 2.8 ppm was used for calculation of the DS as DS (%)=(1 - the area of lysine methylene of gelatin-MA/ the area of lysine methylene of gelatin)x100.[2]

Gelation of gelatin-MA.

The gelation properties of gelatin-MA samples (30% w/v in distilled water) containing 1% of 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone were characterized using steady shear and sinusoidal shear rheometry. Time-sweep and frequency-sweep measurements were performed using an Anton Paar Physica MCR 501 instrument equipped with a Peltier temperature-controlled transparent glass plate and connected to a UV curing system (365nm, 150 mW/cm^2) through an 8 mm light guide and 25 mm cone-plate geometry with a cone angle of 1 degree. The testing conditions for all measurements were 2% strain amplitude at an oscillation frequency of 0.1–10 Hz within the linear viscoelastic regime. The UV irradiation was conducted 30 seconds after running the rheometer. The temperature was maintained at 37 °C throughout the measurements. The gross pictures of gelatin-MA samples (30% w/v), cured in the silicone tube molds with 0.6 mm-diameters, were taken for the purpose of demonstration.

Table S1. Effect of the feed ratio of gelatin to MAA on the degree of substitution in the CB buffer system with pH 9 maintenance.

Group	0.275	0.55	0.825	1.1	1.375	1.65	1.925	2.2	4.4
Gelatin (% w/v)	10	10	10	10	10	10	10	10	10
MAA (% v/v)	0.125	0.25	0.375	0.5	0.625	0.75	0.875	1	2
MAA (mL)/ Gelatin (g)	0.0125/ 1	0.025/1	0.0375/ 1	0.05/1	0.0625/ 1	0.075/1	0.0875/ 1	0.1/1	0.2/1
Molar ratio (MAA/amine)	0.275	0.55	0.825	1.1	1.375	1.65	1.925	2.2	4.4
Buffer	CB	CB	CB	CB	CB	CB	CB	CB	CB
pH adjustment	6 times at pH 9.0	6 times at pH 9.0	6 times at pH 9.0	6 times at pH 9.0	6 times at pH 9.0	6 times at pH 9.0	6 times at pH 9.0	6 times at pH 9.0	6 times at pH 9.0
DS* (%)	22.32 ± 1.27	36.18 ± 0.35	52.95 ± 0.55	66.02 ± 0.80	85.66 ± 0.16	93.67 ± 0.45	97.24 ± 0.25	97.20 ± 0.28	99.00 ± 0.20

* determined by TNBS assay.

Table S2. Effect of pH on the degree of substitution in the CB buffer system with different pH maintenance.

Group	pH 7	pH 8	pH 9	pH 10	pH 11
Gelatin (% w/v)	10	10	10	10	10
MAA (% v/v)	1	1	1	1	1
MAA (mL)/ gelatin (g)	0.1/1	0.1/1	0.1/1	0.1/1	0.1/1
Molar ratio (MAA/amine)	2.2	2.2	2.2	2.2	2.2
Buffer	CB	CB	CB	CB	CB
pH adjustment	6 times at pH 7.0	6 times at pH 8.0	6 times at pH 9.0	6 times at pH 10.0	6 times at pH 11.0
DS* (%)	79.61 ± 0.32	91.11 ± 0.52	97.20 ± 0.28	88.68 ± 0.30	76.95 ± 0.17

* determined by TNBS assay.

References

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2. E. Hoch, T. Hirth, G. E. M. Tovar and K. Borchers, *Journal of Materials Chemistry B*, 2013, **1**, 5675-5685.