Electronic Supplementary Information (ESI)

EDC/NHS activation mechanism of polymethacrylic acid: anhydride versus NHS-ester

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1 Table S1 Infrared peak assignments in the carbonyl stretching region from 1500 to 2000 cm^{-1}.

Table S1. Infrared peak assignments in the carbonyl stretching region from 1500 to 2000 cm^{-1}.

~2105	ν (azide)	azide stretch
~1738	ν_{as} (imidyl C=O)	NHS-ester imidyl C=O antisymmetric stretch
~1780	ν_{s} (imidyl C=O)	NHS-ester imidyl C=O symmetric stretch
~1810	ν (ester C=O)	NHS-ester carbonyl stretch
~1760	ν_{as} (anhydride C=O)	anhydride C=O antisymmetric stretch
~1802	ν_{s} (anhydride C=O)	anhydride C=O symmetric stretch
~1714	ν (acid C=O)	carboxylic acid C=O stretch
~1570	ν (COO^-)	carboxylate (COO^-) stretch
~1650	ν (amide C=O)	amide I C=O stretch
~1529	ν (amide N-H)	amide II N-H bend*

*For the polymer blend of PMAA/PNIPAM, amide II is at 1535 cm^{-1}.

2 Table S2 Molar percentage of anhydride, NHS-ester, and acid residue in **0.5G**, **1.5G**, **2.5G** calculated according to equations 1~3 in the main body.

Table S2. Molar percentage of anhydride, NHS-ester, and acid residue in **0.5G**, **1.5G**, **2.5G** as a carboxylic acid as the unit.

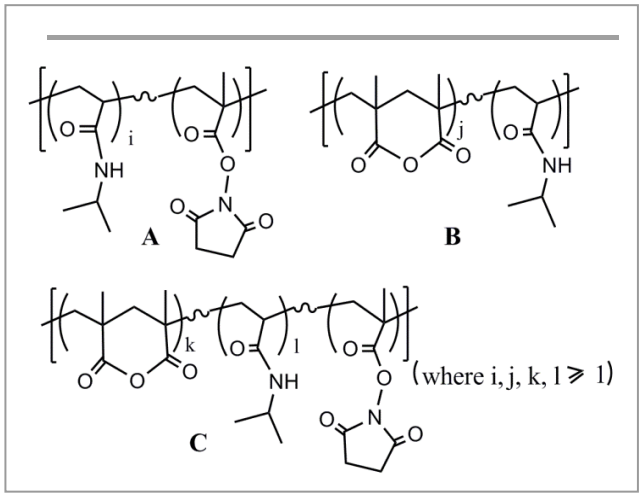
	$\beta_{anhydride}$	$\beta_{NHS\text{-}ester}$	β_{acid}
0.5 G	83	10	7
1.5 G	52	28	20
2.5 G	17	46	37

3 Table S3 Combined molar percentages of anhydride, NHS-ester, acid residue, and amide in **0.5G**, **1.0G**, **1.5G**, **2.0G**, **2.5G**, and **3.0G** respectively according to equations 1~4 in the main body.

Table S2. Combined molar percentages of anhydride, NHS-ester, acid residue, and amide in **0.5G**, **1.0G**, **1.5G**, **2.0G**, **2.5G**, and **3.0G** respectively as a carboxylic acid as the unit ($\beta_{anhydride}$, $\beta_{NHS\text{-}ester}$, β_{acid} in Table S2 correspond to Table S1 respectively) .

	$\alpha_{anhydride}$ (%)	$\alpha_{NHS\text{-}ester}$ (%)	α_{acid} (%)	α_{amide}
0.5G	83 (=$\beta_{anhydride}$)	10 (=$\beta_{NHS\text{-}ester}$)	7 (=β_{acid})	0
1.0G	0	0	68	32
1.5G	35 (=0.68$\beta_{anhydride}$)	19 (=0.68$\beta_{NHS\text{-}ester}$)	14 (=0.68β_{acid})	32
2.0G	0	0	52	48
2.5G	9 (=0.52$\beta_{anhydride}$)	24 (=0.52$\beta_{NHS\text{-}ester}$)	19 (=0.52β_{acid})	48
3.0G	0	0	38	62

4 Scheme S1 Schematic drawing of possible molecular structures after EDC/NHS activation of PMAA/PNIPAM blends by ignoring the acid residues.



Scheme S1. Schematic drawing of possible molecular structures after EDC/NHS activation of PMAA/PNIPAM blends by ignoring the acid residues. The structures in parentheses are subunits while in brackets a unit for the polymer blend. Curved lines represent connections, but not only in this order; subunits with subscripts in alphabets (i, j, k, l) are variable in integers, meaning the subunit can be lonely or next to each other themselves; while subunits in parentheses without subscript labeling represent they are only lonely and cannot be next to each other themselves. **A** represents the product structure when PMAA is less than 30%, while **B** the product structure when PMAA is over 70%, and **C** the structure when PMAA is between 30% and 70%.

5 Fig. S1 The different EDC/NHS activation performance of glutaric acid, 2,2-dimethyl glutaric acid, succinic acid, and 2,2-dimethyl succinic acid.

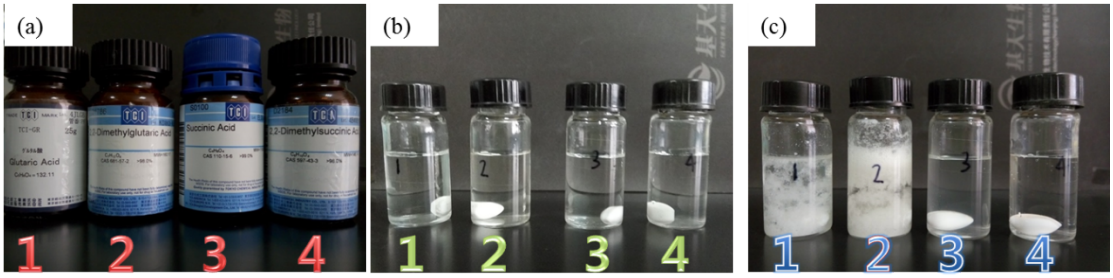


Fig. S1 (a) 1. glutaric acid, 2. 2,2-dimethyl glutaric acid, 3. succinic acid, 4. 2,2-dimethylsuccinic acid; (b) at 0 min after addition of NHS and EDC to the corresponding dicarboxylic acids in 0.1 mol/L MES buffer; (c) 30 min later after addition of NHS and EDC to the corresponding dicarboxylic acids in 0.1 mol/L MES buffer, 1. GA-NHS precipitate, 2. DMGA-NHS precipitate, 3. clear solution of succinic acid, NHS, and EDC, 4. clear solution of 2,2-dimethylsucinic acid, NHS, and EDC.

6 Fig. S2 Infrared spectra of glutaric acid di-succinimidyl ester (GA-NHS) and 2,2-dimethyl glutaric acid di-succinimidyl ester (DMGA-NHS).

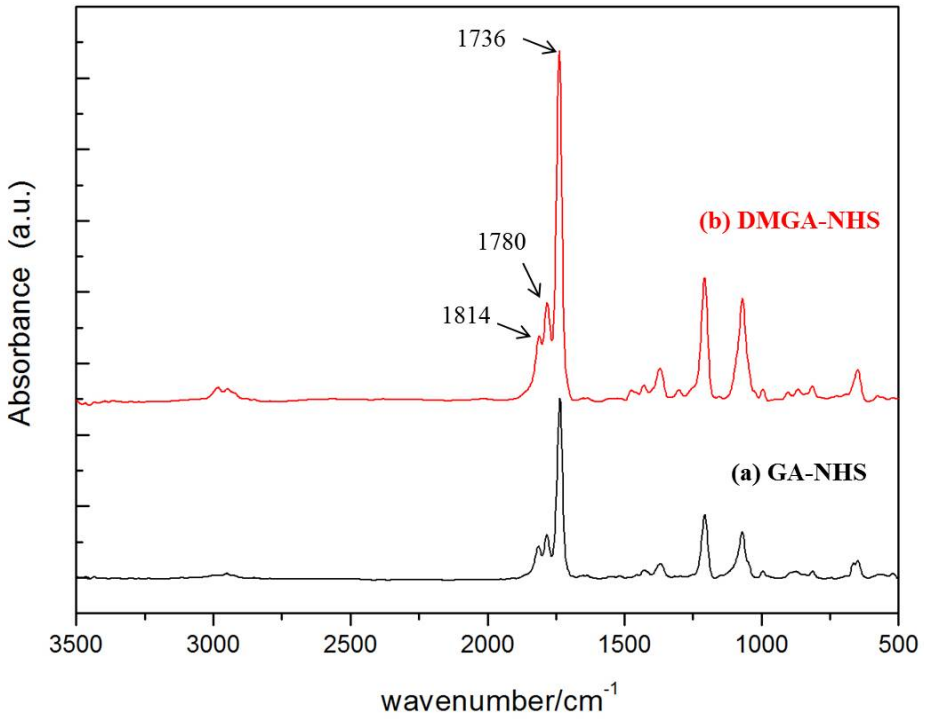


Fig. S2 Infrared spectra of (a) GA-NHS and (b) DMGA-NHS, where the associated triplex bands of NHS-ester at 1736, 1780, and 1814 cm^{-1} are obvious for both GA-NHS and DMGA-NHS.

7 Fig. S3 Mass spectrogram of DMGA-NHS

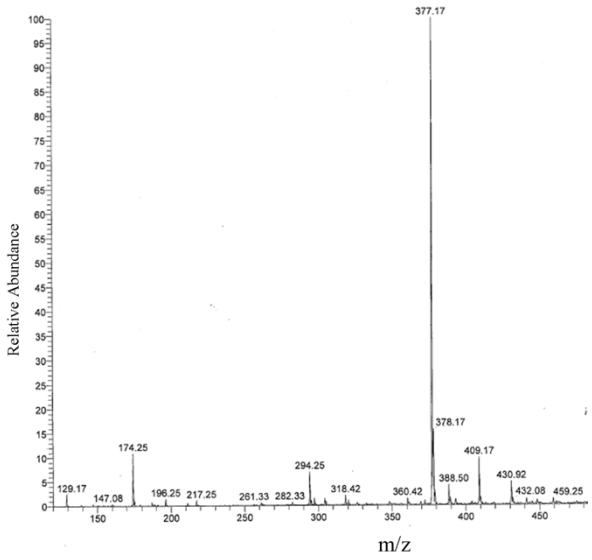


Fig. S3 ESI (LCQ Fleet) mass spectrogram of DMGA-NHS in the positive-ion mode: $(M + Na)^+$ at m/z of 377.17 (354+23).

8 Fig. S4 ^{1}H-NMR Spectra of GA-NHS

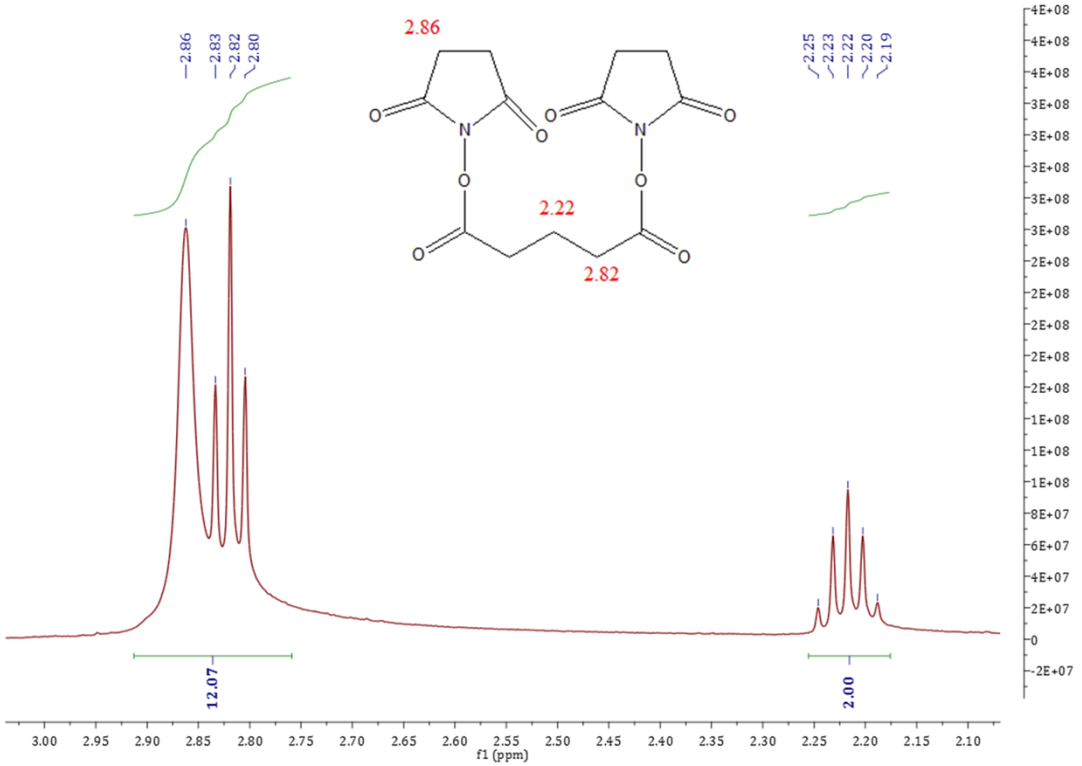


Fig. S4 ^{1}H-NMR Spectra of GA-NHS. It is the same as reported in reference 1.

9 Fig. S5 ^{1}H-NMR Spectra of DMGA-NHS.

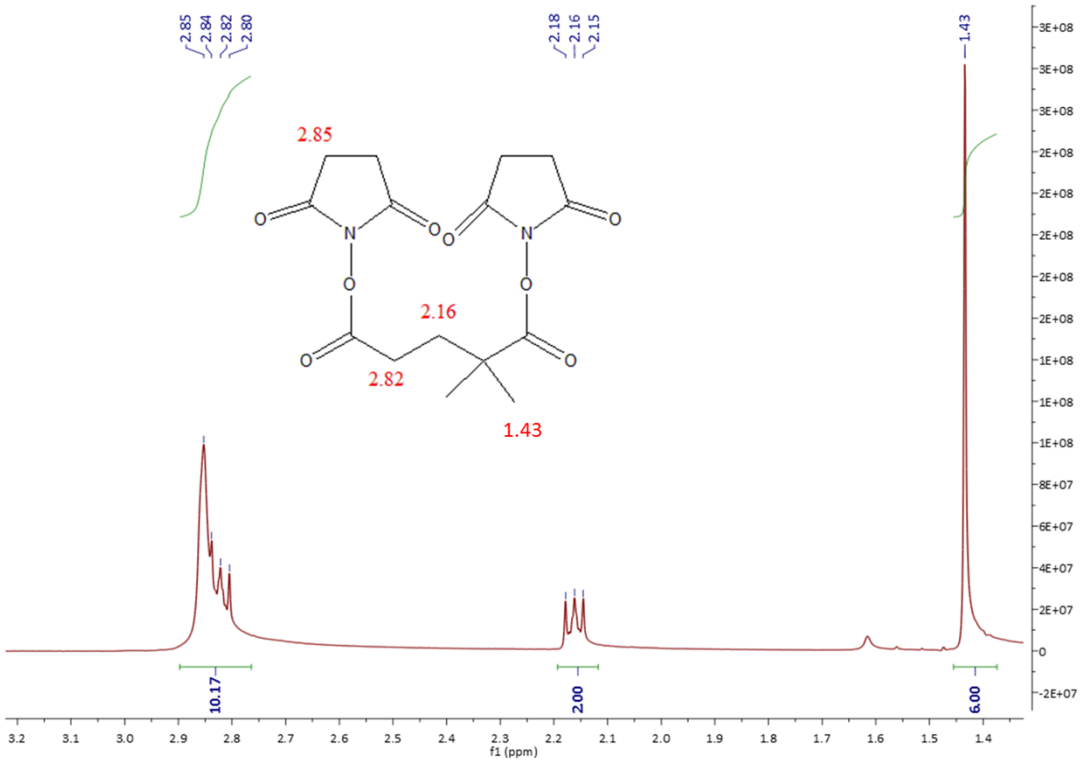


Fig. S5 ^{1}H-NMR Spectra of DMGA-NHS.

10 Figure S6 Precipitation kinetics measurement of EDC/NHS activation of PMAA with the optical density at 600 nm by UV-1800 (Shimadzu) equipped with S1700.

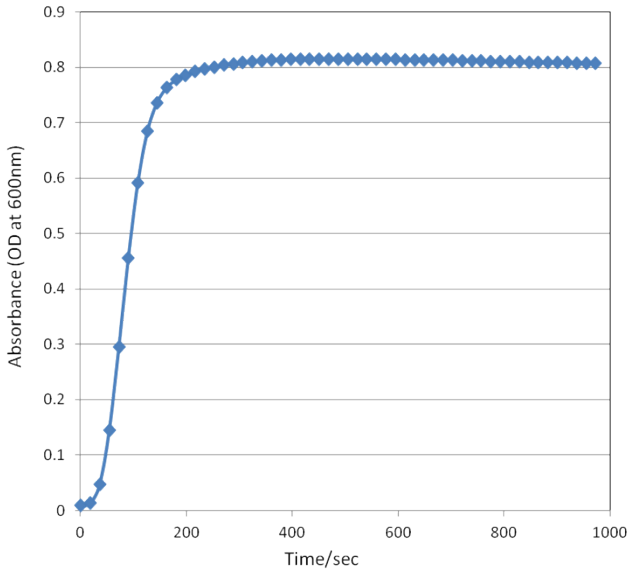


Figure S6 Precipitation rate measurement of EDC/NHS activation of PMAA (0.015 mol/L EDC, 0.015 mol/L NHS, 0.01 mol/L PMAA monomer in 0.1 mol/L MES buffer) with the optical density at 600 nm.

11 Figure S7 Precipitation kinetics measurement of EDC/NHS activation of PAA with the optical density at 600 nm.

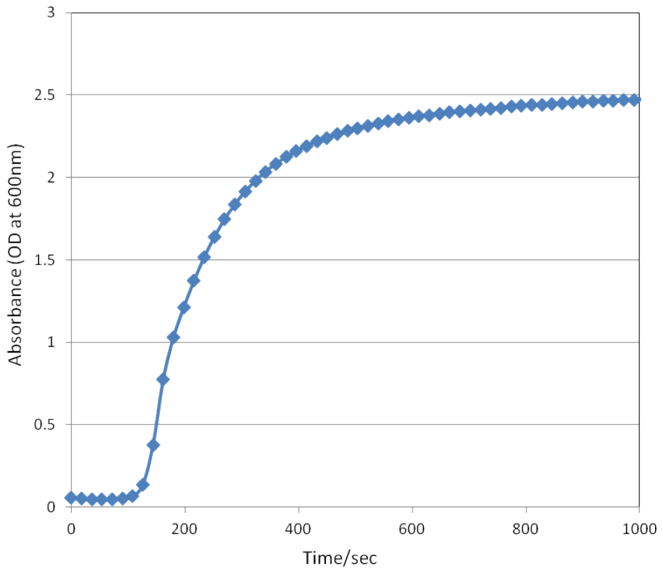


Figure S7 Precipitation rate measurement of EDC/NHS activation of PAA (0.015 mol/L EDC, 0.015 mol/L NHS, 0.01 mol/L PAA monomer in 0.1 mol/L MES buffer) with the optical density at 600 nm.

References

(1) The ^{1}H-NMR spectrum and MS spectrogram of GA-NHS are in ESI of this reference: T. A. van den Berg, B. L. Feringa, G. Roelfes. *Chem. Commun*, 2007 (2): 180-182.