

Electronic Supplementary Information

Rapid bactericidal activity of amphiphilic polyacrylate terpolymer system comprised of same centered comonomers with 2-carbon and 6-carbon spacer arms and an uncharged repeating unit

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S 1. ¹H NMR spectra of monomers and copolymers

6-((tert-butoxycarbonyl)amino)hexyl acrylate (Monomer M6) ¹H NMR (600 MHz, CDCl₃): δ 1.28-1.36 (m, 4H), 1.37-1.42 (s, 9H), 1.42-1.48 (m, 2H), 1.63 (m, 2H), 3.06 (s, 2H), 4.10 (t, 2H), 5.77 (dd, 1H), 6.07 (q, 1H), 6.35 (dd, 1H).

2-((tert-butoxycarbonyl)methylamino)ethyl acrylate (Monomer M2) ¹H NMR (600 MHz, CDCl₃): δ 1.41 (s, 9H), 2.88 (s, 3H), 3.48 (s, 2H), 4.23 (s, 2H), 5.81 (d, 1H), 6.09 (q, 1H), 6.38 (d, 1H).

PolyM6-M2 ¹H NMR (600 MHz, D₂O): δ 1.36-1.47 (bs, 75H), 1.56-2.6 (bm, 162H), 2.72 (s, 2H), 2.80-2.86 (bs, 33H), 2.99-3.04 (bs, 36H), 3.37-3.45 (bs, 22H), 3.74 (s, 3H), 4.00-4.21 (bs, 36H), and 4.31-4.84 (bs, 22H).

PolyM-4% ¹H NMR (600 MHz, D₂O): δ 1.29-1.39 (bs, 60H), 1.49-2.54 (bm, 135H), 2.64 (s, 2H), 2.71-2.78 (bs, 25H), 2.91-2.97 (bs, 28H), 3.30-3.38 (bs, 16H), 3.61-3.64 (bs, 2H), 3.67 (s, 3H), 3.96-4.12 (bs, 28H), and 4.23-4.37 (bs, 16H).

PolyM-12% ¹H NMR (600 MHz, D₂O): δ 1.29-1.39 (bs, 65H), 1.49-2.54 (bm, 142H), 2.64 (s, 2H), 2.72-2.79(bs, 21H), 2.91-2.97 (bs, 31H), 3.30-3.38 (bs, 14H), 3.61-3.66(bs, 7H), 3.67 (s, 3H), 3.94-4.12 (bs, 31H), and 4.23-4.37 (bs, 14H).

PolyM-24% ¹H NMR (600 MHz, D₂O): δ 1.29-1.42 (bs, 65H), 1.49-2.54 (bm, 143H), 2.65 (s, 2H), 2.72-2.79(bs, 12H), 2.91-2.97 (bs, 30H), 3.30-3.38 (bs, 7H), 3.61-3.66(bs, 15H), 3.67 (s, 3H), 3.94-4.12 (bs, 30H), and 4.23-4.37 (bs, 7H).

PolyE-4% ¹H NMR (600 MHz, D₂O): δ 1.23-1.32 (bs, 3H) δ 1.36-1.44 (bs, 66H), 1.58-2.58 (bm, 145H), 2.71 (s, 2H), 2.79-2.83(bs, 26H), 2.96-3.02 (bs, 33H), 3.36-3.44 (bs, 17H), 3.72 (s, 3H), 4.01-4.19 (bs, 33H), and 4.30-4.43 (bs, 17H).

PolyE-24% ¹H NMR (600 MHz, D₂O): δ 1.21-1.32 (bs, 20H) δ 1.34-1.46 (bs, 92H), 1.51-2.51 (bm, 198H), 2.70 (s, 2H), 2.77-2.81(bs, 16H), 2.97-3.04 (bs, 40H), 3.36-3.42 (bs, 10H), 3.72 (s, 3H), 3.98-4.21 (bs, 57), and 4.29-4.43 (bs, 10H).

PolyE-24% ¹H NMR (600 MHz, D₂O): δ 1.23-1.32 (bs, 21H) δ 1.36-1.47 (bs, 88H), 1.62-2.74 (bm, 177H), 2.76 (s, 2H), 2.79-2.85(bs, 17H), 2.97-3.04 (bs, 42H), 3.37-3.45 (bs, 10H), 3.73 (s, 3H), 4.01-4.22 (bs, 55H), and 4.30-4.40 (bs, 10H).

PolyB-4% ¹H NMR (600 MHz, D₂O): δ 0.93-1.00 (bs, 3H) δ 1.36-1.47 (bs, 61H), 1.56-2.60 (bm, 136H), 2.79 (s, 2H), 2.79-2.87 (bs, 27H), 2.98-3.05 (bs, 30H), 3.36-3.48 (bs, 18H), 3.74 (s, 3H), 4.01-4.21 (bs, 31H), and 4.31-4.47 (bs, 18H).

PolyB-12% ¹H NMR (600 MHz, D₂O): δ 0.93-1.00 (bs, 10H) δ 1.34-1.47 (bs, 83H), 1.52-2.60 (bm, 171H), 2.79 (s, 2H), 2.79-2.84(bs, 24H), 2.96-3.03 (bs, 37H), 3.34-3.44 (bs, 14H), 3.72 (s, 3H), 4.01-4.19 (bs ,44H), and 4.29-4.44 (bs, 14H).

PolyB-24% ¹H NMR (600 MHz, D₂O): δ 0.91-1.03 (bs, 22H) δ 1.34-1.47 (bs, 92H), 1.49-2.57 (bm, 181H), 2.70 (s, 2H), 2.78-2.84(bs, 15H), 2.96-3.03 (bs, 37H), 3.33-3.45 (bs, 9H), 3.72 (s, 3H), 3.96-4.21 (bs, 52H), and 4.29-4.43 (bs, 9H).

S 2. Determination of actual mole percentage of the repeating units in polymer

S 2a. PolyM6-M2: In Figure S 2a, a broad single peak at δ 2.99-3.04 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.37-3.45. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

$$\text{Mol\% of M6 repeat unit: } (36/2) \div (18 + 11) = 62\%$$

$$\text{Mol\% of M2 repeat unit: } (22/2) \div (18 + 11) = 38\%$$

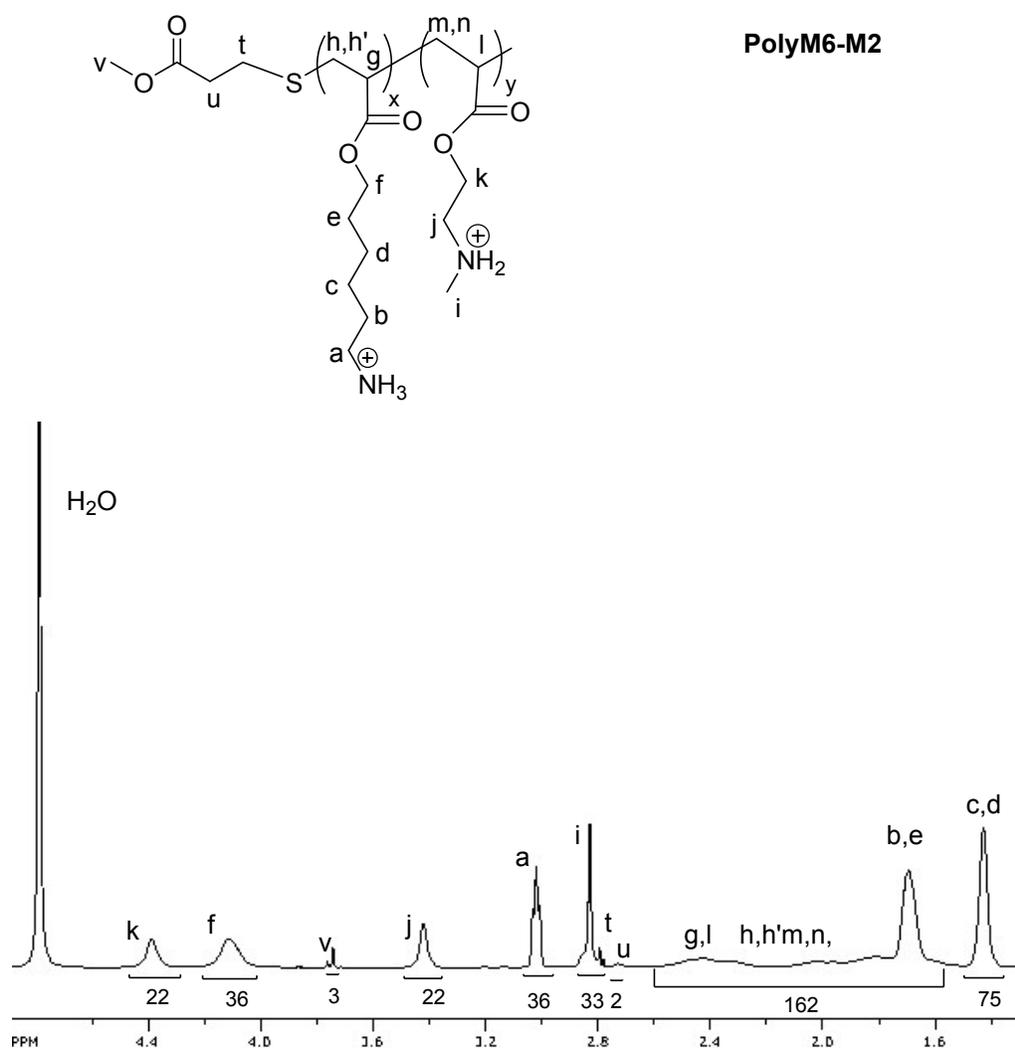


Figure S 2a. ¹H NMR spectrum of PolyM6-M2

S 2b. PolyM-4%: In Figure S 2b, a broad single peak at δ 2.91-2.97 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.30-3.38. A broad single peak at δ 3.61-3.64 corresponds to methoxy protons (p) in methyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

$$\text{Mol\% of M6 repeat unit: } (28/2) \div (14 + 8 + 0.67) = 62\%$$

$$\text{Mol\% of M2 repeat unit: } (16/2) \div (14 + 8 + 0.67) = 35\%$$

$$\text{Mol\% of methyl acrylate repeat unit: } 2/3 \div (14 + 8 + 0.67) = 3\%$$

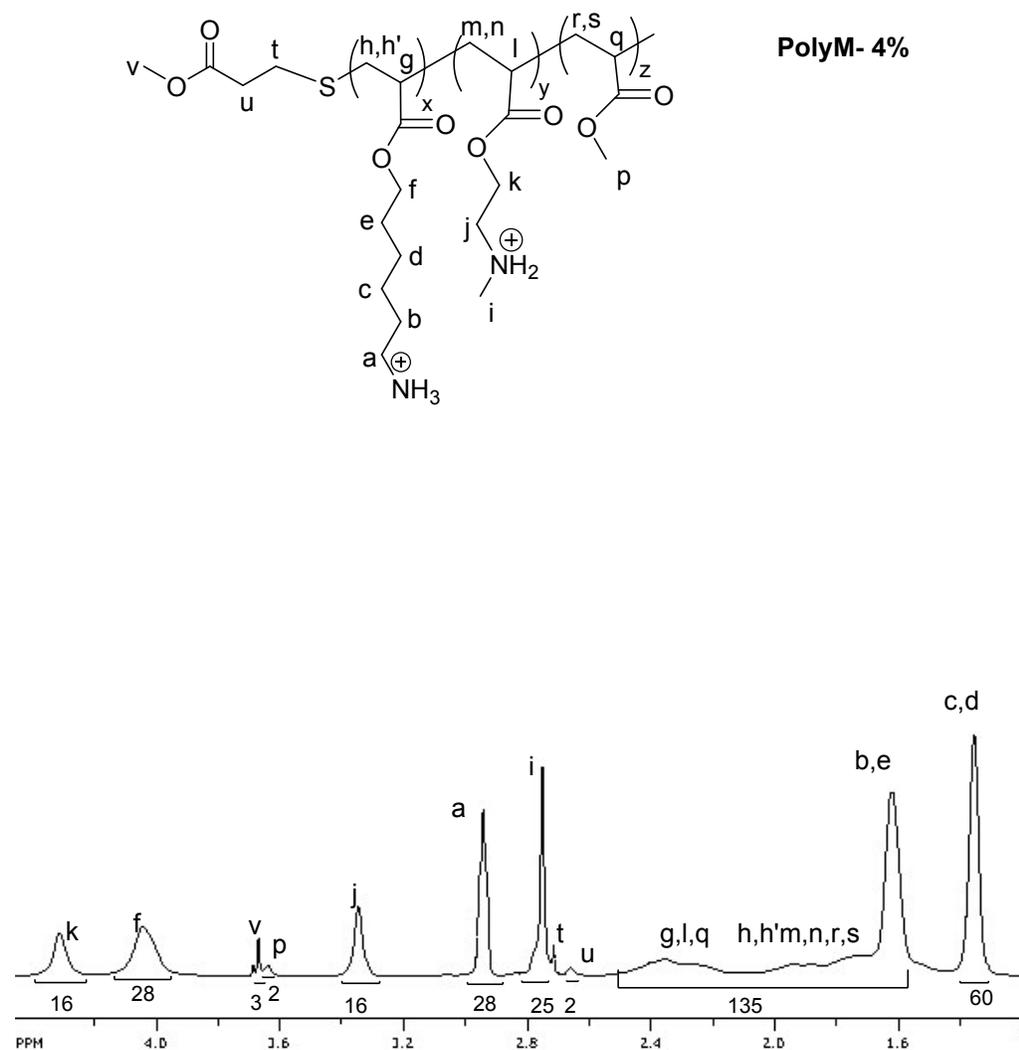


Figure S 2b. ^1H NMR spectrum of PolyM-4%

S 2c. PolyM-12%: In Figure S 2c, a broad single peak at δ 2.91-2.97 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.30-3.38. A broad single peak at δ 3.61-3.66 corresponds to methoxy protons (p) in methyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

$$\text{Mol\% of M6 repeat unit: } (31/2) \div (15.5 + 7 + 2.33) = 62\%$$

$$\text{Mol\% of M2 repeat unit: } (14/2) \div (15.5 + 7 + 2.33) = 28\%$$

$$\text{Mol\% of methyl acrylate repeat unit: } 7/3 \div (15.5 + 7 + 2.33) = 10\%$$

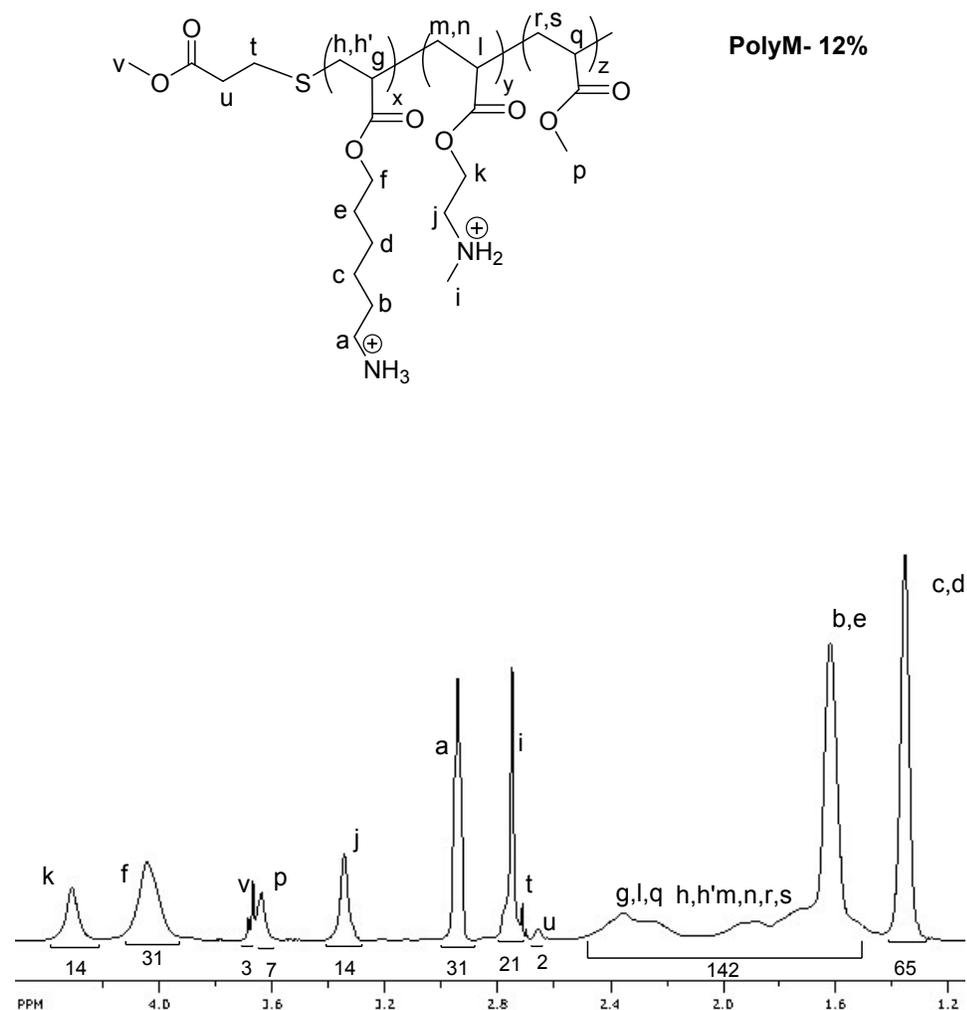


Figure S 2c. ¹H NMR spectrum of PolyM-12%

S 2d. PolyM-24%: In Figure S 2d, a broad single peak at δ 2.91-2.97 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.30-3.38. A broad single peak at δ 3.61-3.66 corresponds to methoxy protons (p) in methyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

$$\text{Mol\% of M6 repeat unit: } (30/2) \div (15 + 3.5 + 5) = 64\%$$

$$\text{Mol\% of M2 repeat unit: } (7/2) \div (15 + 3.5 + 5) = 15\%$$

$$\text{Mol\% of methyl acrylate repeat unit: } 15/3 \div (15 + 3.5 + 5) = 21\%$$

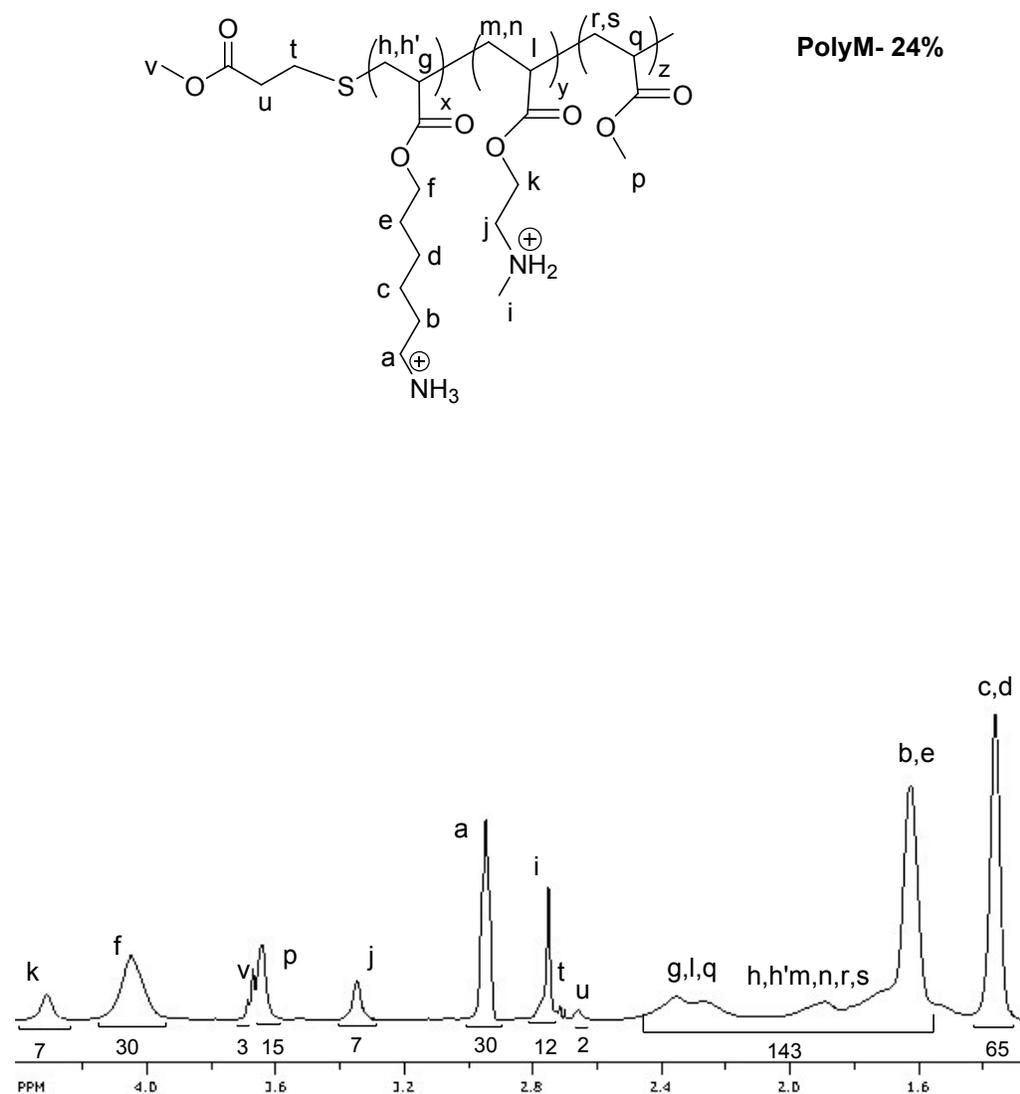


Figure S 2d. ¹H NMR spectrum of PolyM-24%

S 2e. PolyE-4%: In Figure S 2e, a broad single peak at δ 2.96-3.02 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.36-3.44. A broad single peak at δ 1.23-1.32 corresponds to methyl protons (p) in ethyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

$$\text{Mol\% of M6 repeat unit: } (33/2) \div (16.5 + 8.5 + 1) = 63\%$$

$$\text{Mol\% of M2 repeat unit: } (17/2) \div (16.5 + 8.5 + 1) = 33\%$$

$$\text{Mol\% of ethyl acrylate repeat unit: } 3/3 \div (16.5 + 8.5 + 1) = 4\%$$

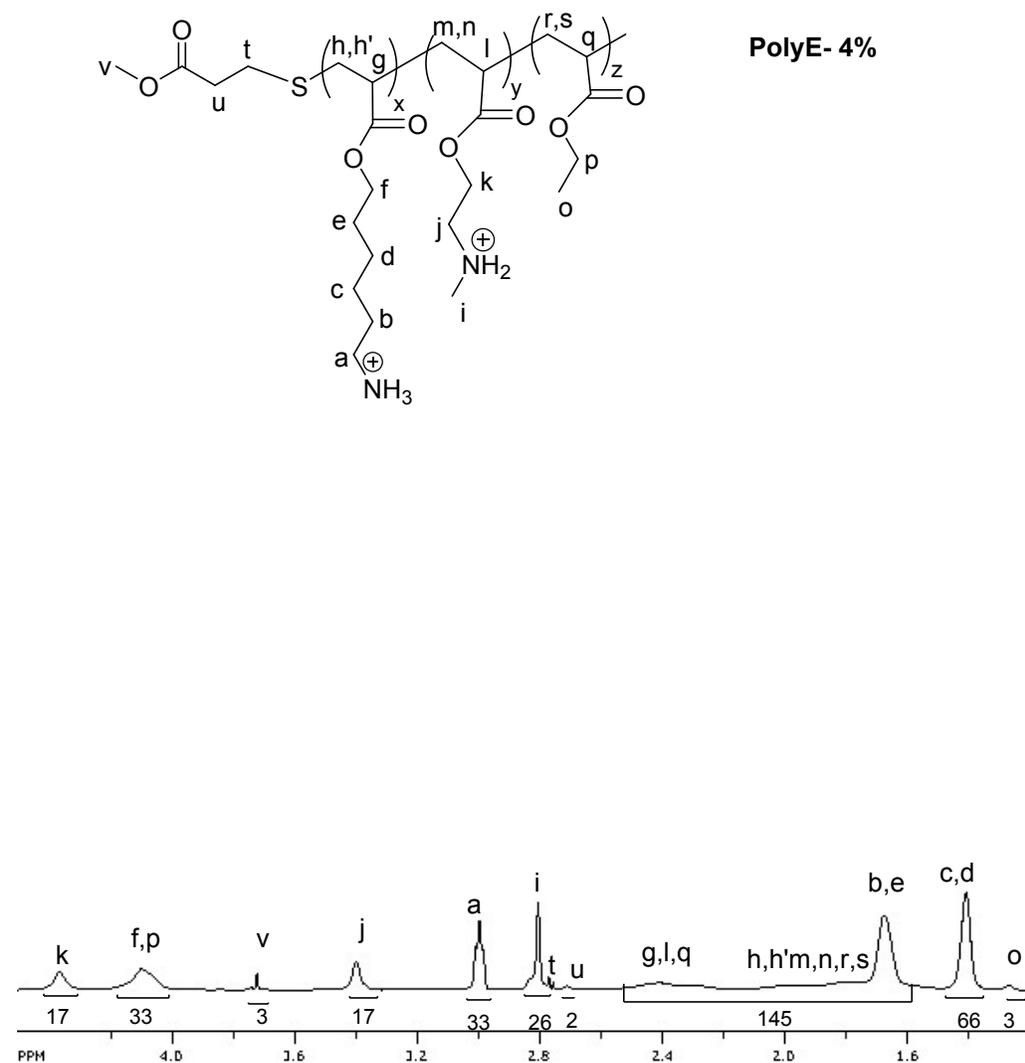


Figure S 2e. ^1H NMR spectrum of PolyE-4%

S 2f. PolyE-12%: In Figure S 2f, a broad single peak at δ 2.97-3.03 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.36-3.44. A broad single peak at δ 1.23-1.32 corresponds to methyl protons (p) in ethyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

$$\text{Mol\% of M6 repeat unit: } (46/2) \div (23 + 9.5 + 4.33) = 62\%$$

$$\text{Mol\% of M2 repeat unit: } (19/2) \div (16.5 + 8.5 + 1) = 26\%$$

$$\text{Mol\% of ethyl acrylate repeat unit: } 13/3 \div (16.5 + 8.5 + 1) = 12\%$$

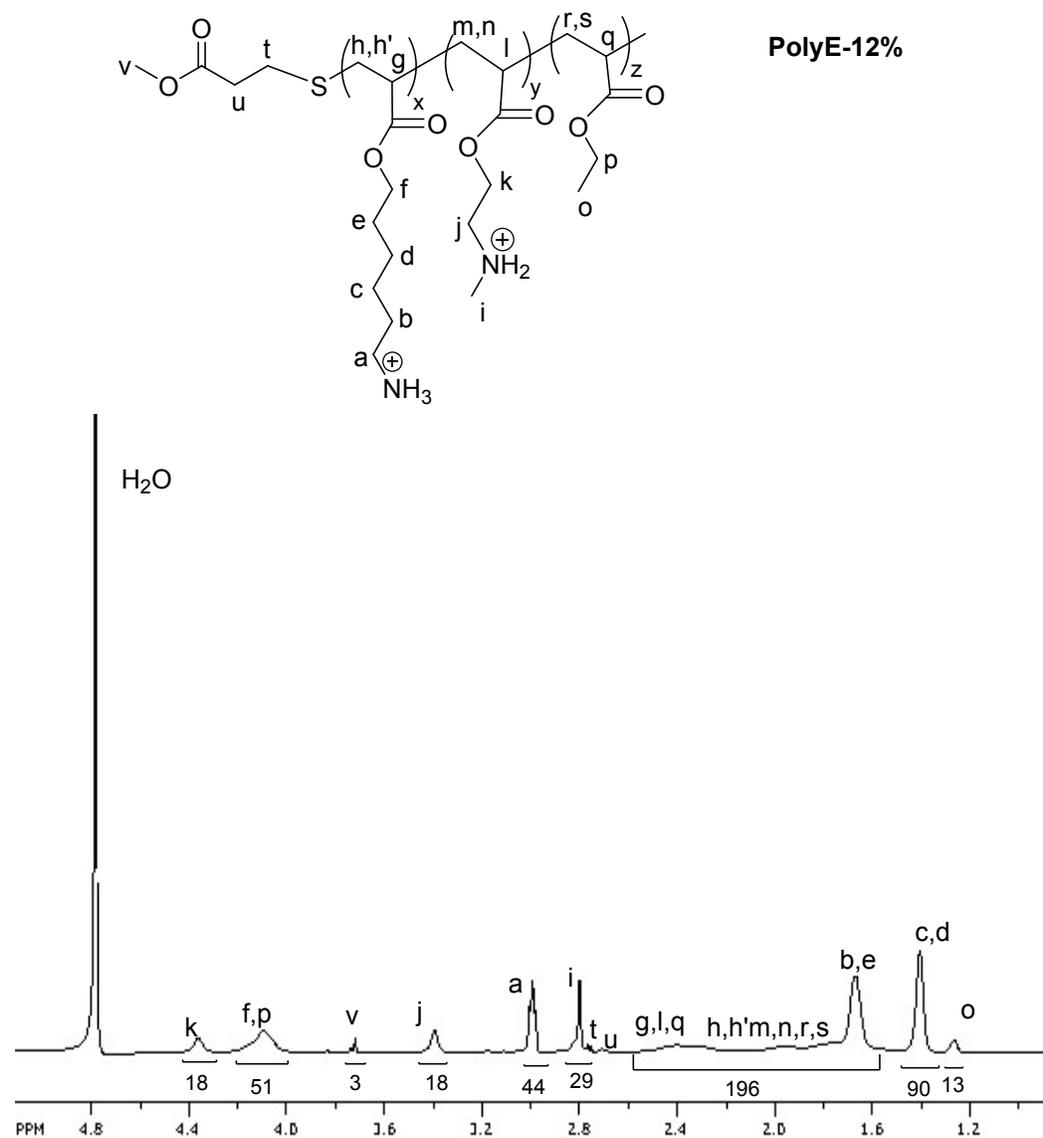


Figure S 2f. ^1H NMR spectrum of PolyE-12%

S 2g. PolyE-12%: In Figure S 2g, a broad single peak at δ 2.97-3.04 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.36-3.42. A broad single peak at δ 1.21-1.32 corresponds to methyl protons (p) in ethyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: $(40/2) \div (20 + 5 + 6.7) = 63\%$

Mol% of M2 repeat unit: $(10/2) \div (20 + 5 + 6.7) = 16\%$

Mol% of ethyl acrylate repeat unit: $20/3 \div (20 + 5 + 6.7) = 21\%$

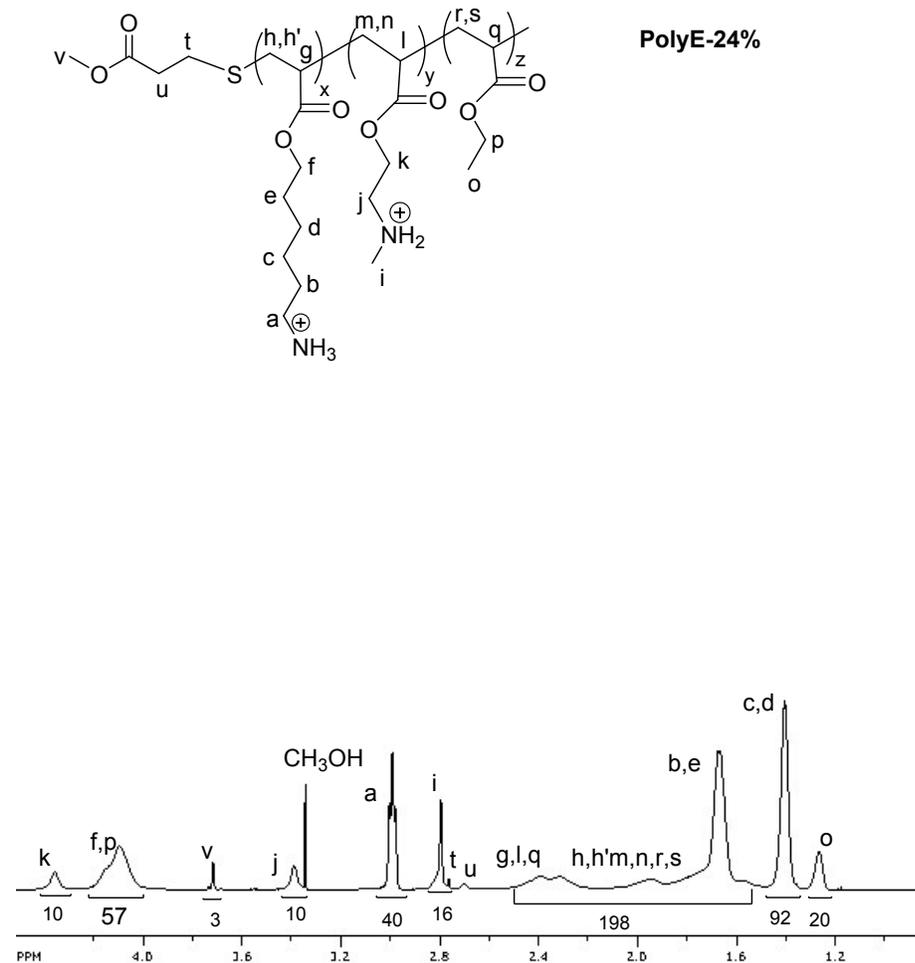


Figure S 2g. ¹H NMR spectrum of PolyE-24%

S 2h. PolyB-4%: In Figure S 2h, a broad single peak at δ 2.98-3.05 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.36-3.48. A broad single peak at δ 0.93-1.00 corresponds to methyl protons (p) in butyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: $(30/2) \div (15 + 9 + 1) = 60\%$

Mol% of M2 repeat unit: $(18/2) \div (15 + 9 + 1) = 36\%$

Mol% of butyl acrylate repeat unit: $3/3 \div (15 + 9 + 1) = 4\%$

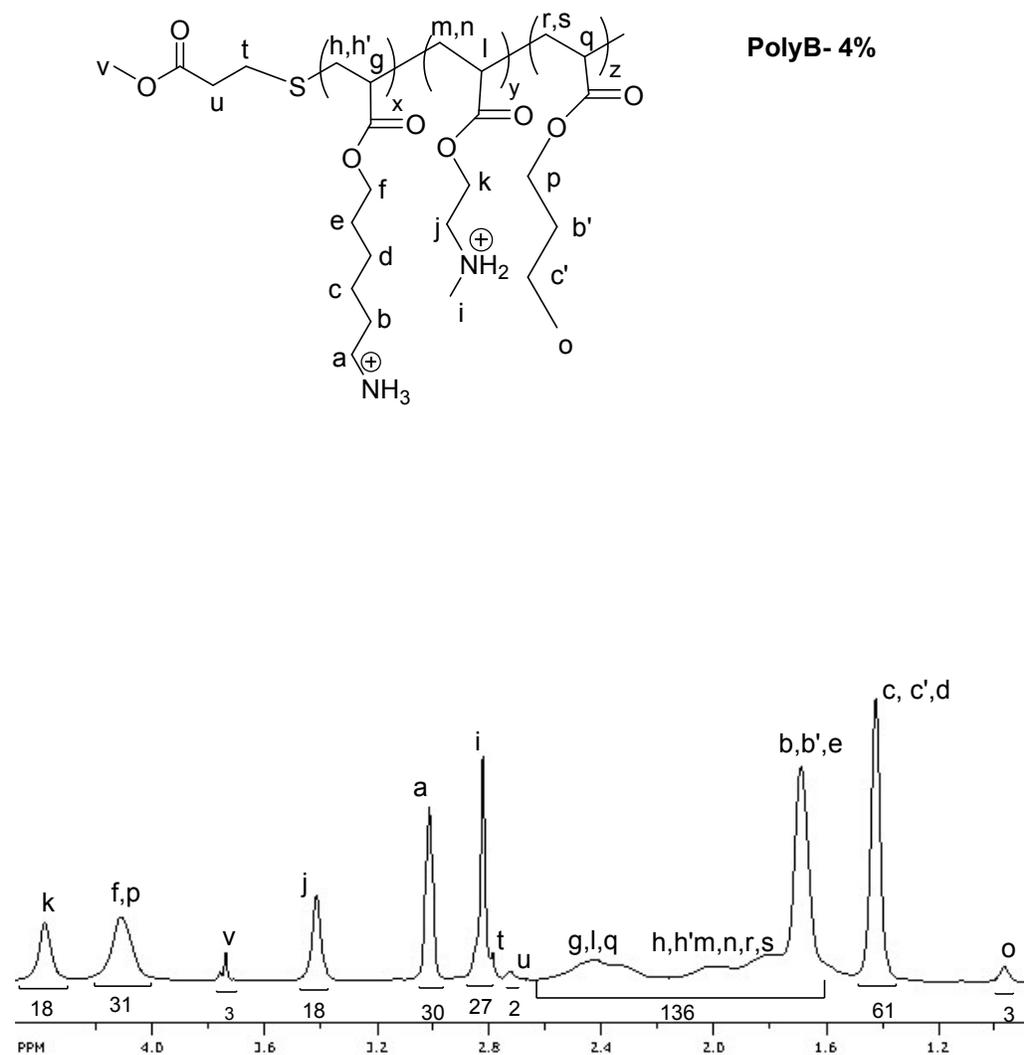


Figure S 2h. ¹H NMR spectrum of PolyB-4%

S 2i. PolyB-12%: In Figure S 2i, a broad single peak at δ 2.96-3.03 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.34-3.44. A broad single peak at δ 0.93-1.00 corresponds to methyl protons (p) in butyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

$$\text{Mol\% of M6 repeat unit: } (37/2) \div (18.5 + 7 + 3.33) = 64\%$$

$$\text{Mol\% of M2 repeat unit: } (14/2) \div (18 + 7.5 + 3.33) = 25\%$$

$$\text{Mol\% of butyl acrylate repeat unit: } 10/3 \div (18 + 7.5 + 3.33) = 11\%$$

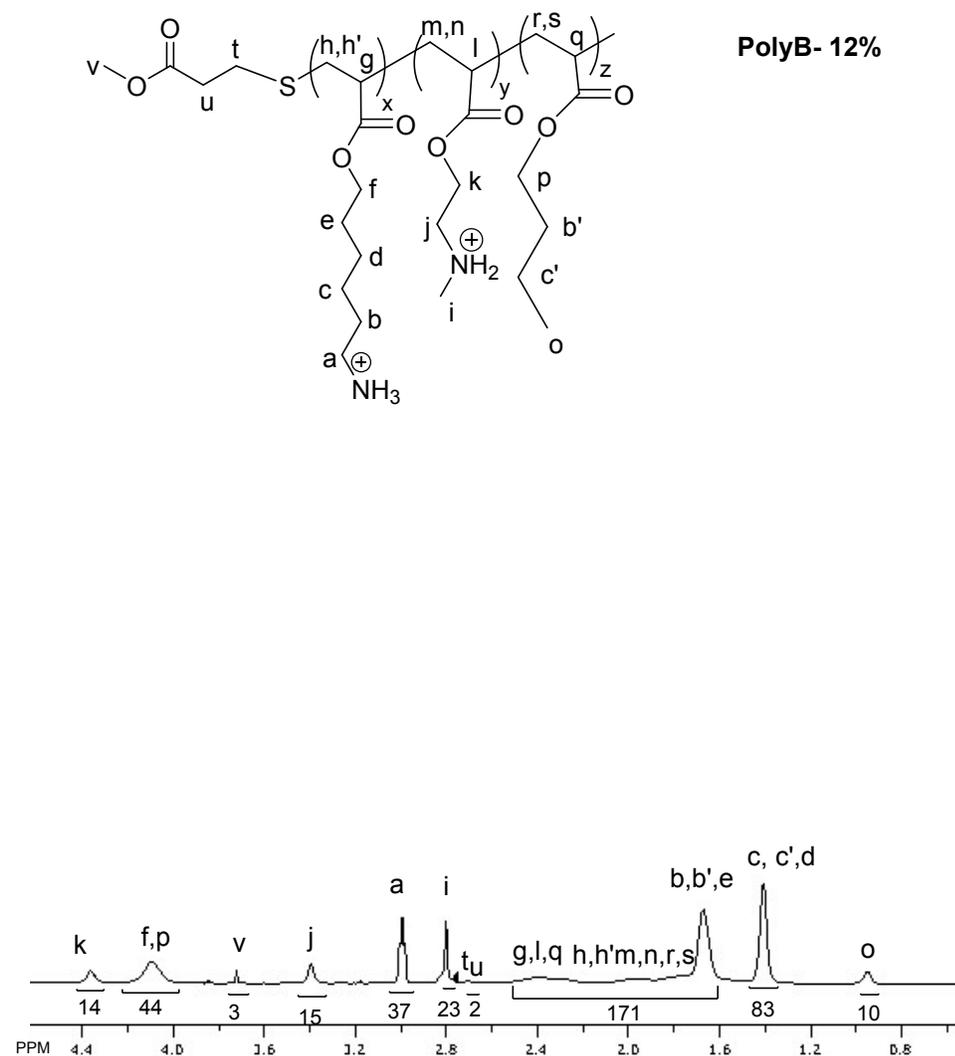


Figure S 2i. ^1H NMR spectrum of PolyB-12%

S 2j. PolyB-24%: In Figure S 2j, a broad single peak at δ 2.96-3.03 (a) belongs to methylene protons in M6 monomer repeating unit. Methylene protons in M2 monomer repeating unit (k) give a chemical shift at δ 3.33-3.45. A broad single peak at δ 0.91-1.03 corresponds to methyl protons (p) in butyl acrylate monomer. Mole percentages of the repeating units are calculated as below assuming chain transfer as mode of termination:

Mol% of M6 repeat unit: $(37/2) \div (18.5 + 4.5 + 7.33) = 61\%$

Mol% of M2 repeat unit: $(9/2) \div (18.5 + 4.5 + 7) = 15\%$

Mol% of butyl acrylate repeat unit: $22/3 \div (18.5 + 4.5 + 7) = 24\%$

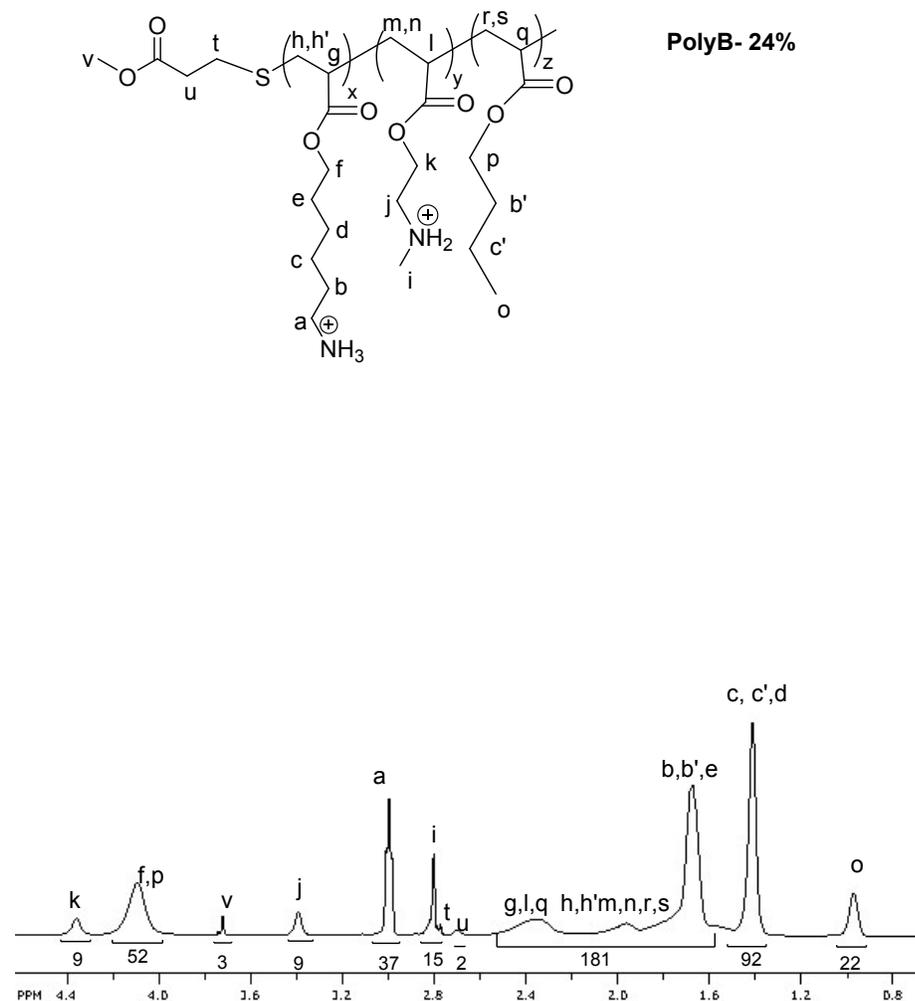


Figure S 2j. ¹H NMR spectrum of PolyB-24%

S 3. Estimation of molecular weight through gel permeation chromatography (GPC)

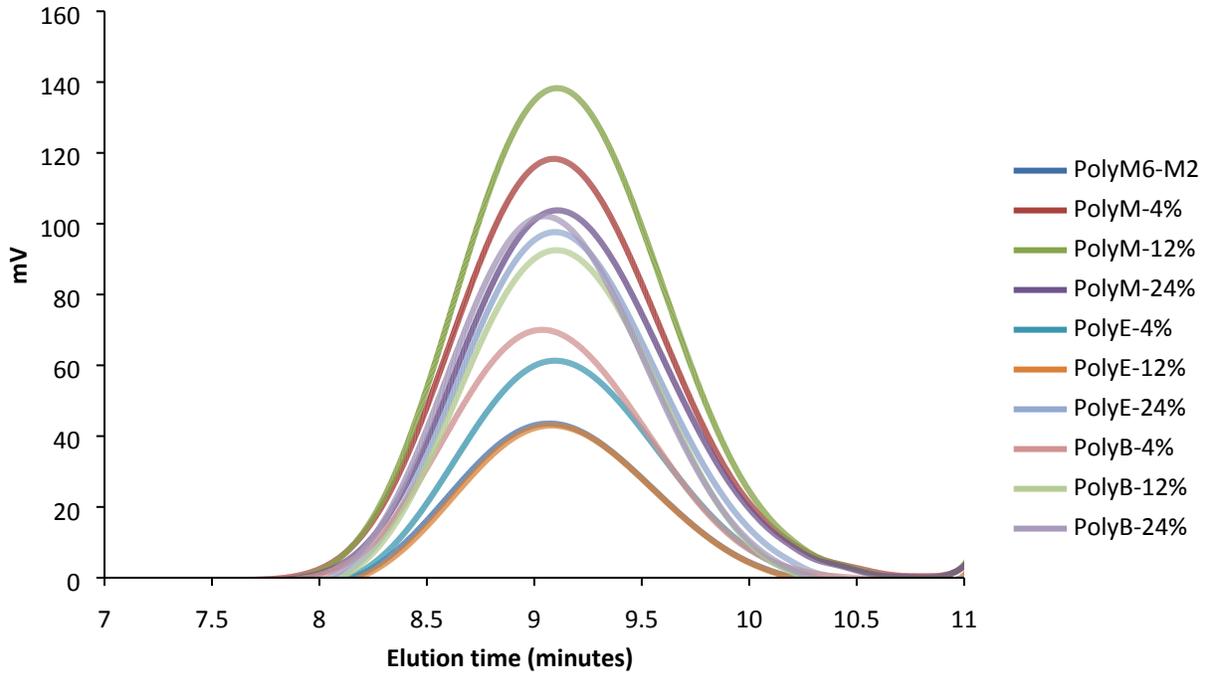


Figure S 3.1. Gel permeation chromatography elution curves of copolymers

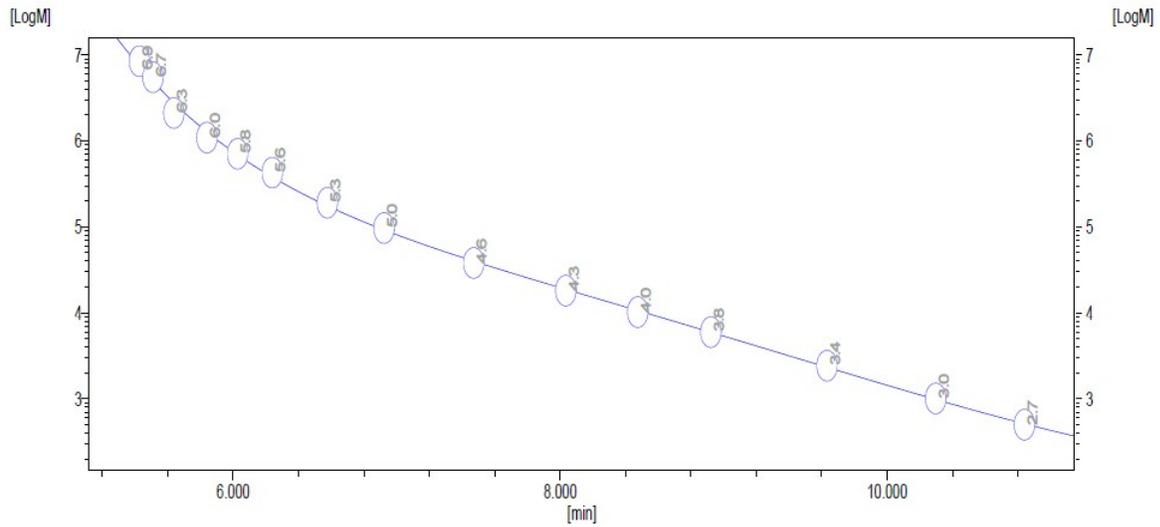


Figure S 3.2. Polystyrene standard calibration curve used for the estimation of molecular weights of copolymer samples by GPC

S 4. Time-dependent bacterial killing efficiency of PolyE-12% copolymer

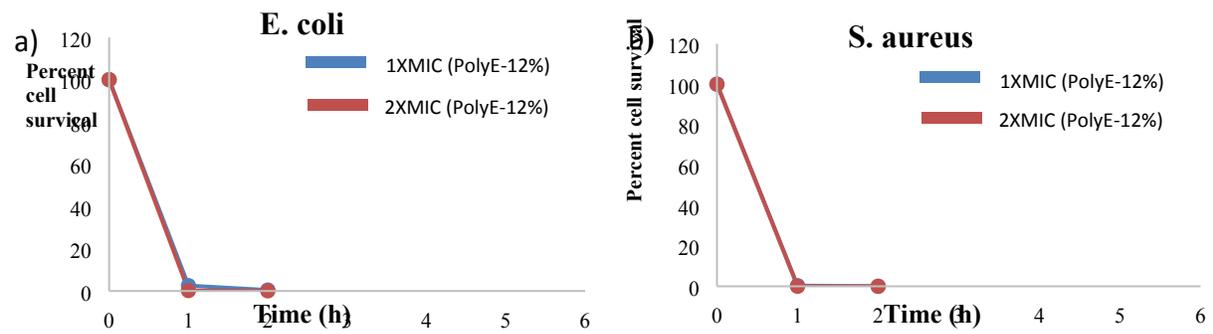


Figure S 4. Time-dependent killing efficiency analysis of PolyE-12% against a) *E. coli* and b) *S. aureus*. Results shown here are the averages of two experiments (size of error bars is negligible).