Electronic Supplementary Material (ESI) for Chemical Communications

PolyPEGA with predetermined molecular weights from enzyme-mediated radical polymerization in water

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Experimental section

Materials

Catalase from bovine liver (CBL, lyophilized powder, 2000-5000 units/mg, Aldrich), laccase from *T. versicolor* (LTV, 21.8 units/mg, Sigma), peroxidase from horseradish (HRP, Type II, essentially salt-free, lyophilized powder, Aldrich), L-(+)-ascorbic acid (AA, 99+%, Alfa Aesar), ethyl α-bromoisobutyrate (EBiB, assay 98%, Aldrich), 2-bromopropionitrile (BPN, 97%, Aldrich), iodoacetonitrile (IAN, 98%, Aldrich) were used as received. Water was dispensed from an AquaMAX^{TM} deionized water purification system. Poly(ethylene glycol) methyl ether acrylate (PEGA, M_n=454 g/mol, Aldrich) was passed before use through a short column of alumina to remove the inhibitor.

Radical polymerization of PEGA in the presence of CBL

In a typical reaction, 0.5 mL of PEGA was mixed with 2 mL of deionized water to form a homogeneous solution. The mixture was deoxygenated by bubbling argon for ca. 45 min. CBL (20mg) and AA (50 mg) were placed in a Schlenk flask. The flask was sealed with a rubber septum and three vacuum/argon cycles were done. The PEGA/water mixture was transferred into the Schlenk flask using an argon purged syringe. Subsequently, 3 μL of

BPN was syringed into the flask and the resulting mixture was heated to 60ºC in an oil bath.

Radical polymerization of PEGA premixing CBL and AA

In a typical experiment, 0.5 mL of PEGA were mixed with 0.5 mL of deionized water to form a homogeneous solution. The mixture was deoxygenated by bubbling argon for ca. 45 min. CBL (20mg) and AA (50 mg) were placed in a Schlenk flask. The flask was sealed with a rubber septum and three vacuum/argon cycles were done. 1.5 mL of deionized water was then transferred into the Schlenk flask using an argon purged syringe. The homogeneous mixture was stirred at 60ºC in an oil bath for 1 h. Subsequently, the PEGA/water mixture was transferred into the Schlenk flask using an argon purged syringe. 3 μL of BPN was syringed into the flask and the resulting mixture in order to initiate the polymerization.

***In-situ* chain extension experiment**

Deionized water was deoxygenated under argon for 1 h before use. 0.5 mL of PEGA was mixed with 0.5 mL of deionized water to form a homogeneous solution. The mixture was deoxygenated by bubbling argon for ca. 45 min. CBL (40mg) and AA (100 mg) were placed in a Schlenk flask. The flask was sealed with a rubber septum and three vacuum/argon cycles were done. 1.5 mL of deionized water was then transferred into the Schlenk flask using an argon purged syringe. The homogeneous mixture was stirred at 60ºC in an oil bath for 1 h. Subsequently, the PEGA/water mixture was transferred into the Schlenk flask using an argon purged syringe. 5 μL of EBiB was syringed into the

flask and the resulting mixture in order to initiate the polymerization. After 6 h, sample was taken from the reaction mixture via an argon-purged syringe for the determination of monomer conversion. 0.2 mL of deoxygenated PEGA was added as second batch monomer and the polymerization was continued for another 20 h.

Characterization

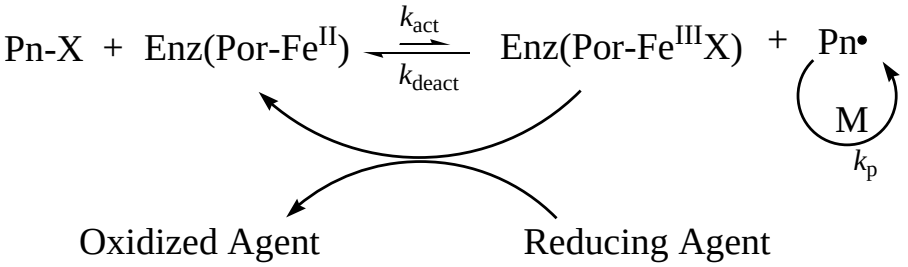
Monomer conversion was determined by ^{1}H NMR spectroscopy performed on a Bruker UltraShield spectrometer operated at 400 MHz. The number- and weight-average molecular weights (M_n and M_w, respectively) as well as polydispersity indices (M_w/M_n, PDI) of polymers were measured by size exclusion chromatography (SEC) system equipped with a Waters 515 HPLC pump, a 717plus autosampler, a 2414 refractive-index detector, and the following Styragel® columns arranged in series: guard, HR5E (4.6 mm ID × 300 mm), HR1 and HR0.5, using THF as an eluent, operated at 0.3 mL/min and 40ºC. The instrument was calibrated with poly(methyl methacrylate) standards.

Table 1S

Entry[a]	*Initiator*[b]	*Enzyme*[c] *(mg)*	*AA (mg)*	*Conv.%*[d]	M_n	M_w/M_n	$M_{n,theo}$
1	BPN	20	50	89.7	9926	1.48	14392
2	BPN	20	20	93.8	8872	1.47	15036
3	BPN	20	10	95.9 (5h)	8698	1.47	15366
4	BPN	20	50	80.8	12027	1.65	12974
5	BPN	20	50	53.7	8776	1.62	8666
6	BPN	10	20	56.3	9457	1.63	9076
7	BPN	10	50	36.5	8781	1.60	5935
8	IAN	20	50	26.7	10682	1.55	4377
9	EBiB	20	50	49.9	8545	1.61	8132
10	BPN	10 (LTV)	50	69.6	11357	1.68	11194
11	BPN	10 (HRP)	50	95.6 (5h)	10156	1.58	15328
12	BPN	2 (HRP)	50	62.3	9550	1.53	10034

PEGA/Initiator=35/1, PEGA=0.5mL, Water=2mL

a. For Entry 4-10, polymerization was carried out with premixed enzyme and CBL.
b. BPN= 2-bromopropionitrile(3μL), EBiB= ethyl 2-bromoisobutyrate(5μL), IAN= iodoacetonitrile(2.5μL).
c. The enzyme used was CBL, unless specified otherwise.
d. Conversion was estimated at 8 h, unless specified otherwise.



Scheme 1S

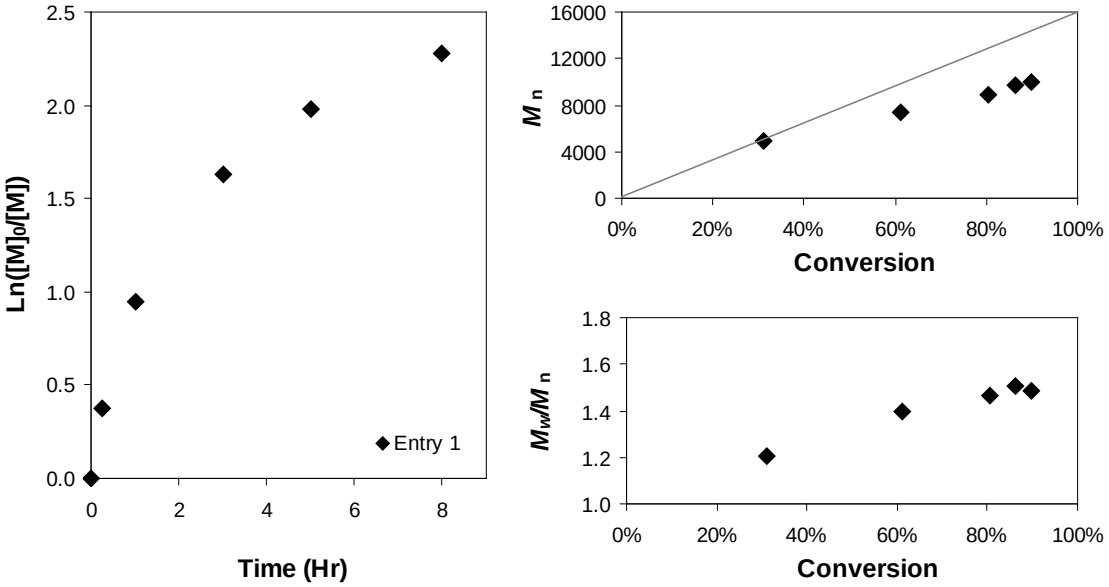


Fig. 1S Kinetic plot (left) and number-average molecular weight (M_n) and M_w/M_n of polyPEGA *vs*. percent monomer conversion. See Tables 1S and 2S for experimental conditions.

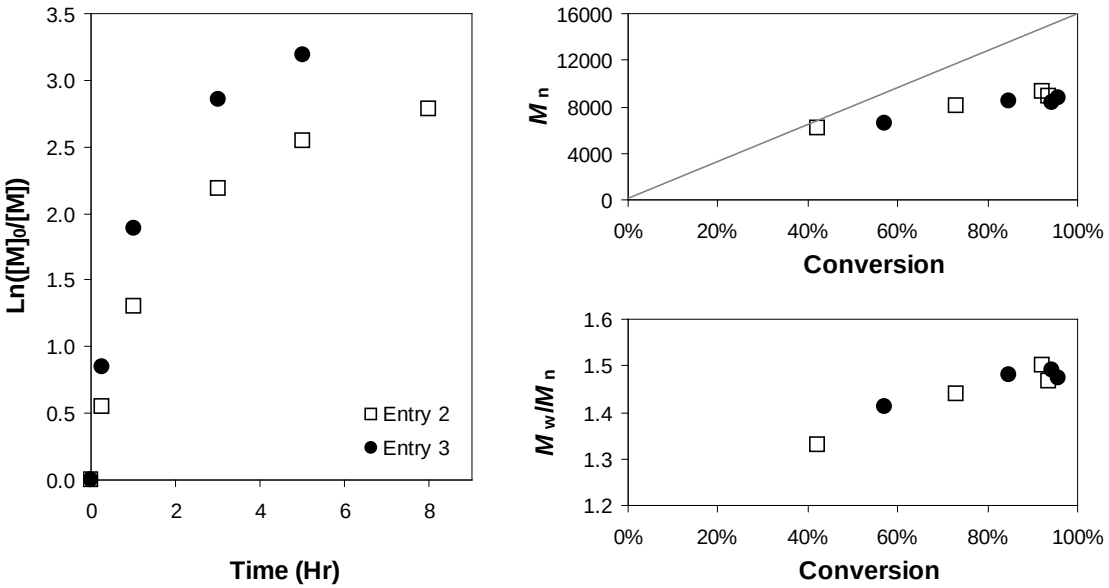


Fig. 2S Kinetic plot (left) and number-average molecular weight (M_n) and M_w/M_n of polyPEGA *vs*. percent monomer conversion. See Tables 1S and 2S for experimental conditions.

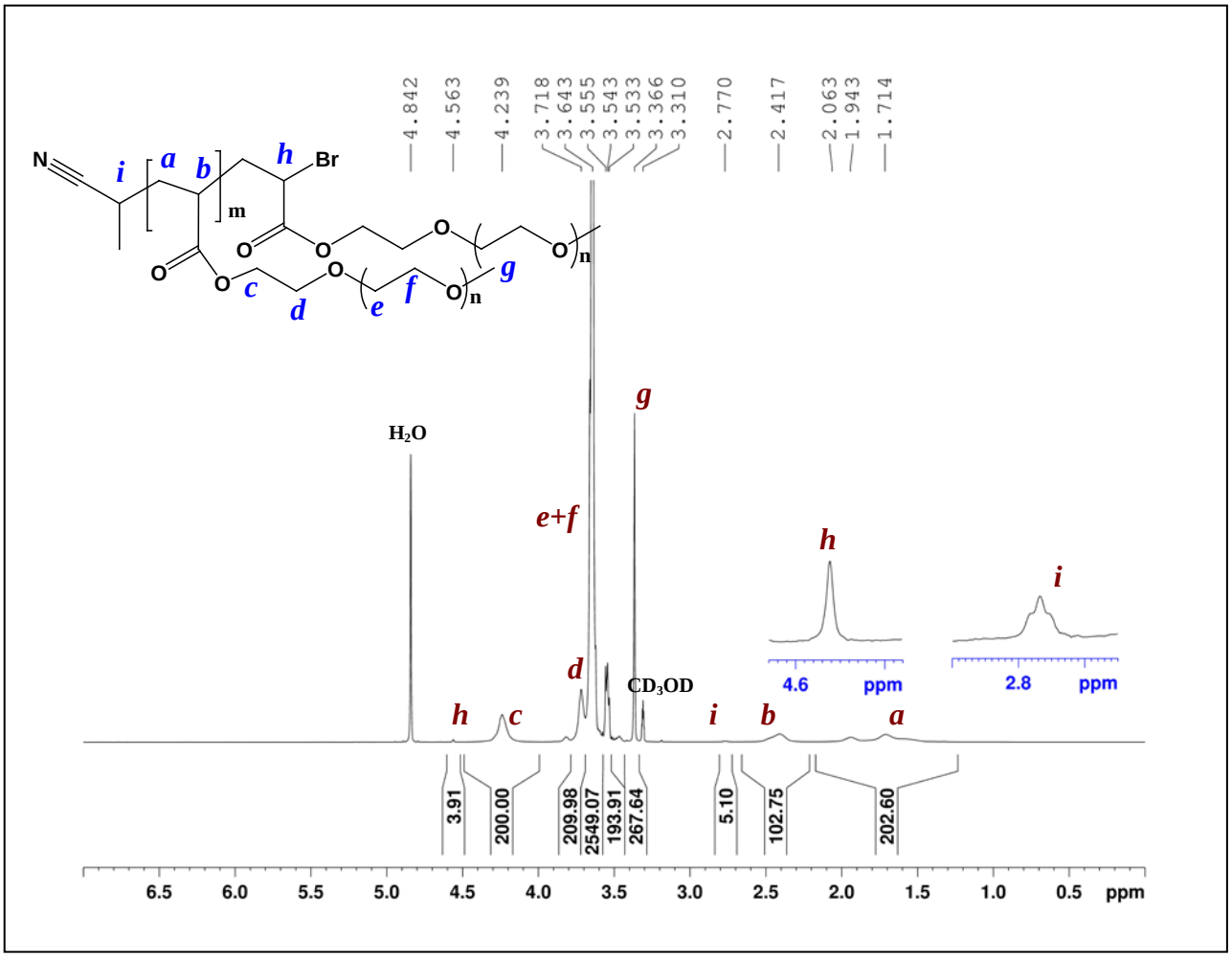


Fig. 3S. ^{1}H NMR spectrum of polyPEGA produced in Entry 4 (Tables 1 and 2).

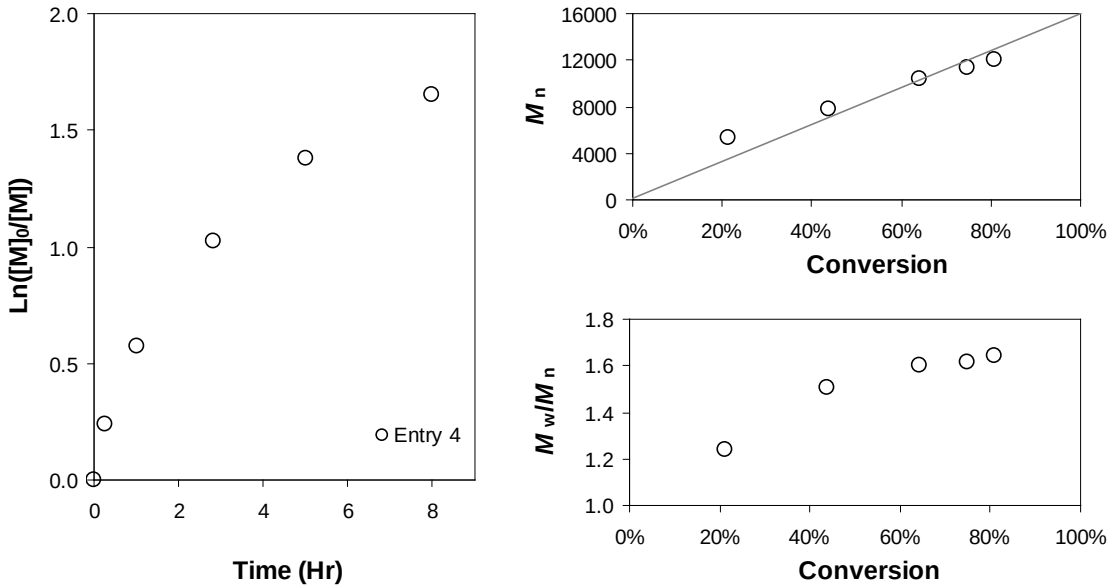


Fig. 4S Kinetic plot (left) and number-average molecular weight (M_n) and M_w/M_n of polyPEGA *vs*. percent monomer conversion. See Tables 1S and 2S for experimental conditions.

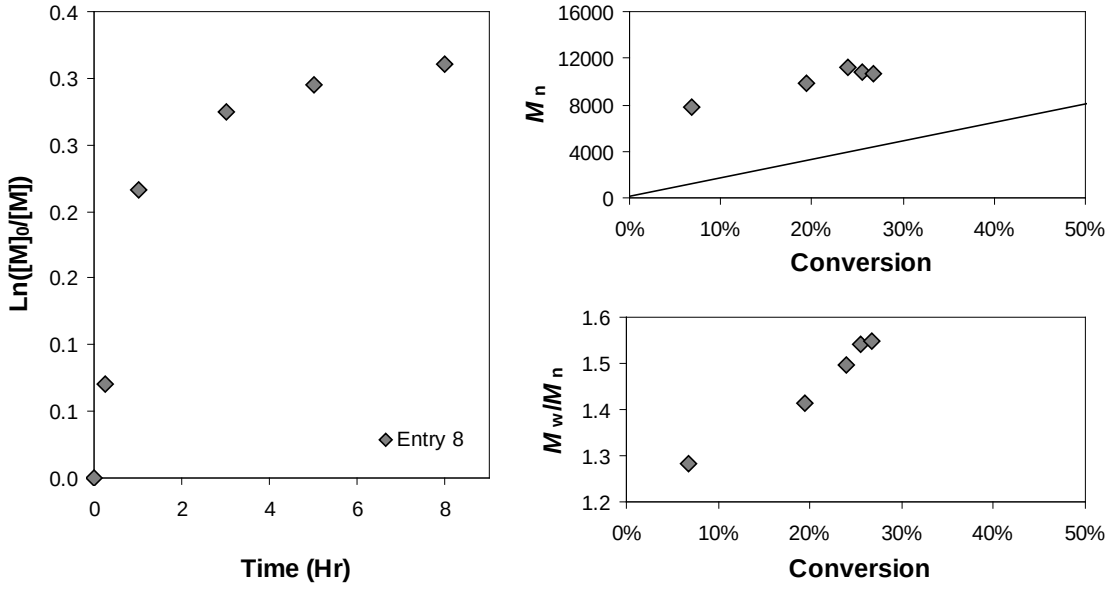


Fig. 5S Kinetic plot (left) and number-average molecular weight (M_n) and M_w/M_n of polyPEGA *vs*. percent monomer conversion. See Tables 1S and 2S for experimental conditions.

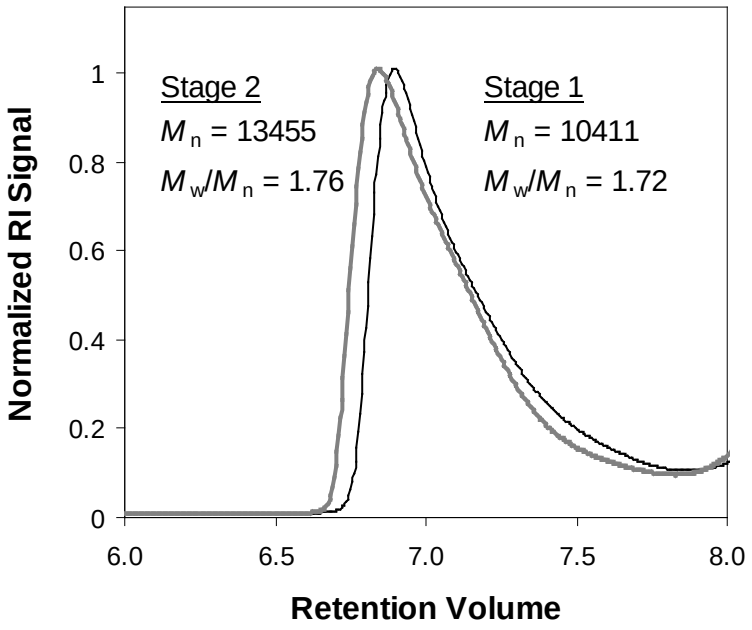


Fig. 6S. *In situ* chain extension of polyPEGA.

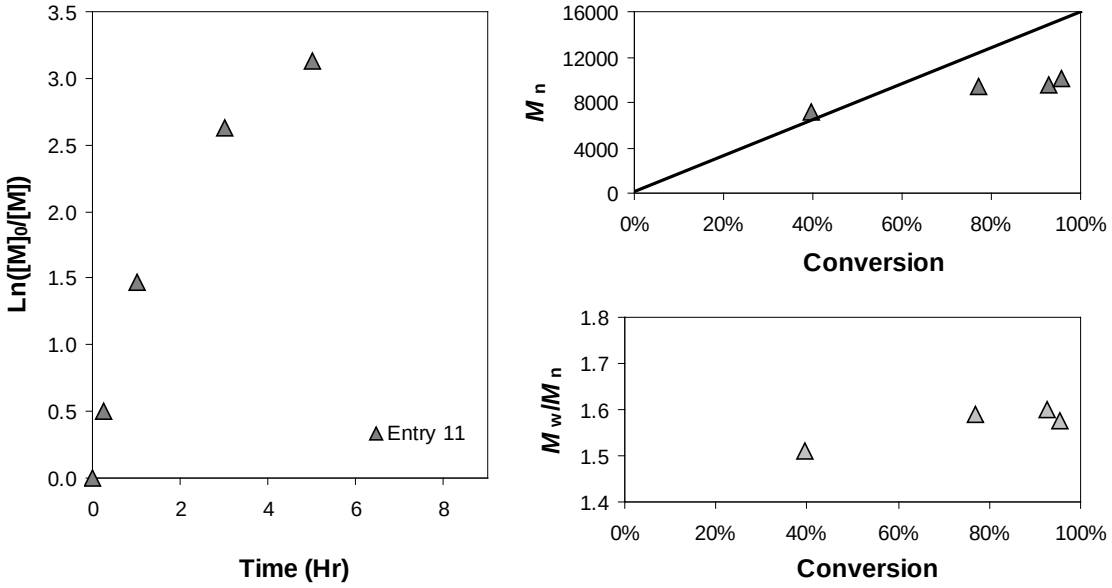


Fig. 7S Kinetic plot (left) and number-average molecular weight (M_n) and M_w/M_n of polyPEGA *vs*. percent monomer conversion. See Tables 1S and 2S for experimental conditions.

Table 2S. Detailed kinetic data

Entry 1- CBL=20mg, AA=50mg, BPN=3μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	31.0	4986	1.20	5063
1	61.2	7321	1.40	9854
3	80.4	8857	1.46	12915
5	86.2	9708	1.50	13837
8	89.7	9926	1.48	14392

Entry 2- CBL=20mg, AA=20mg, BPN=3μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	42.2	6094	1.33	6842
1	72.9	8053	1.44	11723
3	88.7			14229
5	92.2	9298	1.50	14777
8	93.8	8872	1.47	15036

Entry 3- CBL=20mg, AA=10mg, BPN=3μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	57.1	6593	1.41	9202
1	84.8	8478	1.48	13612
3	94.2	8351	1.49	15108
5	95.9	8698	1.47	15366

Entry 4- CBL=20mg, AA=50mg, BPN=3μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	21.3	5314	1.24	3517
1	43.8	7744	1.51	7096
2.8	64.2	10364	1.60	10329
5	74.8	11318	1.62	12015
8	80.8	12027	1.65	12974

22	85.5	12794	1.71	13718

Entry 5- CBL=20mg, AA=50mg, BPN=3μL, Temp=40℃

Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	14.1	5067	1.13	2370
1	26.5	5435	1.26	4347
3	40.8	6893	1.48	6623
5	48.3	8234	1.57	7816
8	53.7	8776	1.62	8666
22	61.7	10303	1.68	9932

Entry 6- CBL=10mg, AA=20mg, BPN=3μL, Temp=60℃

Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	20.8	5537	1.28	3443
1	32.9	6872	1.48	5367
3	47.3	8346	1.60	7643
5	54.1	9588	1.62	8731
8	56.3	9457	1.63	9076
22	60.6	10475	1.63	9771

Entry 7- CBL=10mg, AA=50mg, BPN=3μL, Temp=60℃

Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	11.2	5410	1.16	1916
1	20.7	6119	1.32	3424
3	30.7	7892	1.53	5017
5	35.0	9549	1.66	5691
8	36.5	8781	1.60	5935
22	38.9	10242	1.67	6319

Entry 8- CBL=20mg, AA=50mg, IAN=2.5μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	6.8	7795	1.28	1218
1	19.4	9909	1.41	3224
3	24.0	11171	1.50	3949
5	25.6	10771	1.54	4194
8	26.7	10682	1.55	4377
22	30.8			5031

Entry 9- CBL=20mg, AA=50mg, EBiB=5μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	13.8	5967	1.22	2386
1	34.1	7346	1.48	5615
3	46.2	9208	1.64	7537
5	48.8	8351	1.58	7942
8	49.9	8545	1.61	8132
22	54.3			8822

Entry 10- LTV=10mg, AA=50mg, BPN=3μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	13.0	5405	1.21	2193
1	23.9	6664	1.43	3938
3	49.7	9513	1.66	8025
5	59.2	10501	1.66	9535
8	69.6	11357	1.68	11194
22	85.7	12137	1.68	13748

Entry 11- HRP=10mg, AA=50mg, BPN=3μL, Temp=60℃				
Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	39.6	7138	1.51	6424
1	77.0	9350	1.59	12369
3	92.7	9544	1.60	14871

5	95.6	10156	1.58	15328

Entry 12- HRP=2mg, AA=50mg, BPN=3μL, Temp=60℃

Time (hr)	Conv %	M_n	M_w/M_n	$M_{n,theo}$
0.25	14.1	5119	1.23	2372
1	29.6	6241	1.42	4843
3	48.0	8126	1.56	7759
5	56.8	8726	1.58	9162
8	62.3	9550	1.53	10034
22	64.6	9645	1.58	10404