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APPENDICES

APPENDIX A

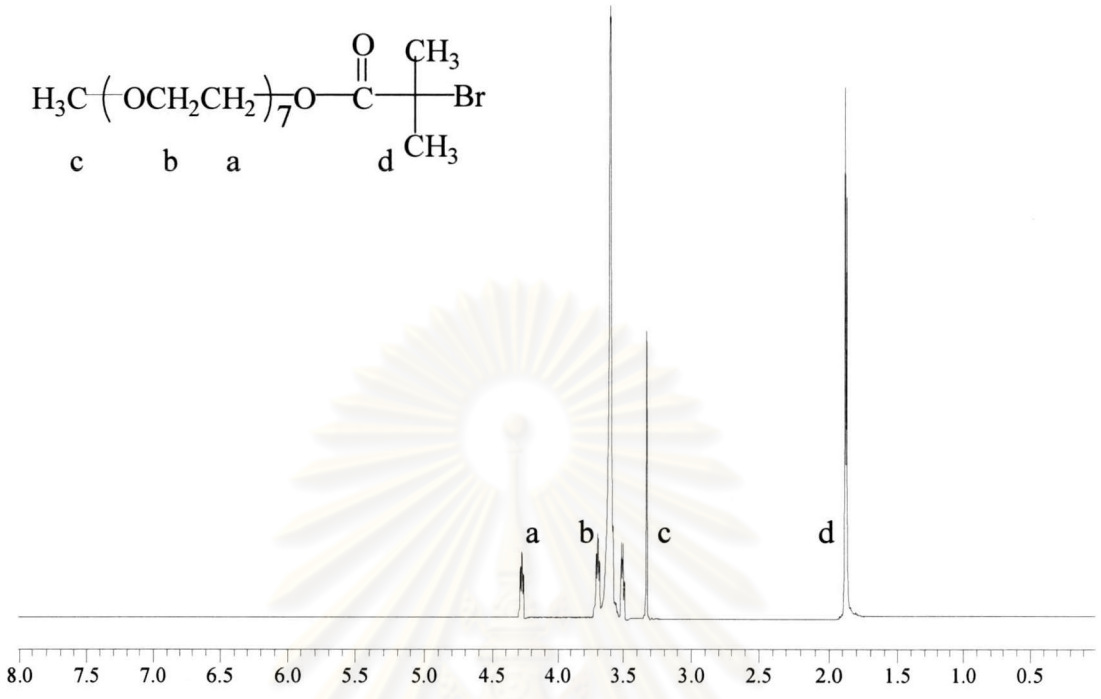

Figure A-1 The ^{1}H-NMR (400 MHz, $CDCl_3$) of methoxy-capped oligo(ethylene glycol)-2-bromoisobutylrate initiator: OEGBr **(1)**.

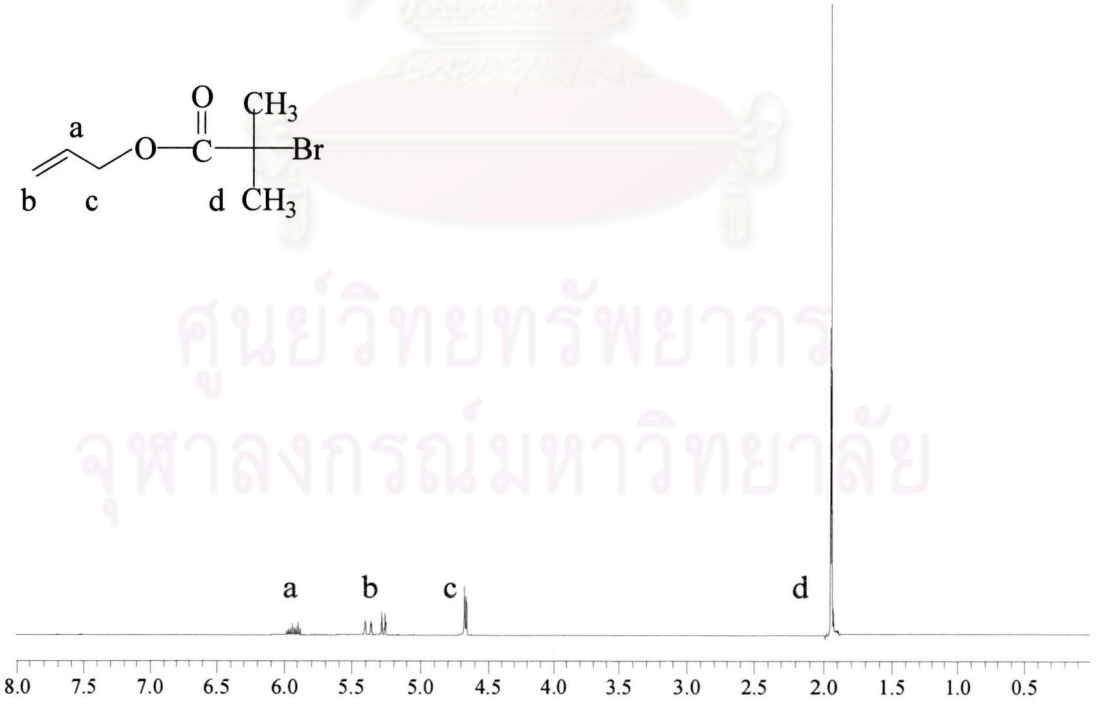

Figure A-2 The ^{1}H-NMR (400 MHz, $CDCl_3$) of prop-2'-enyl 2-bromo-2-methylpropinoate **(2)**.

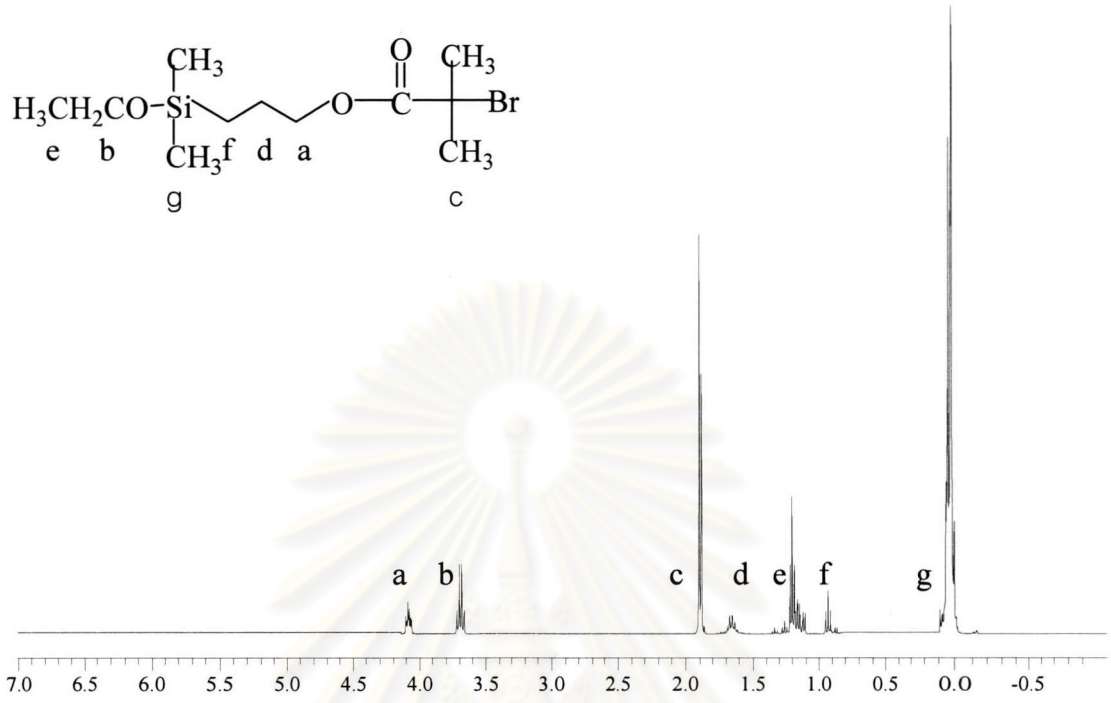

Figure A-3 The ^{1}H-NMR (400 MHz, $CDCl_3$) of 3-(dimthylethoxysilyl)propyl-2-bromoisobutyrate **(3)**.

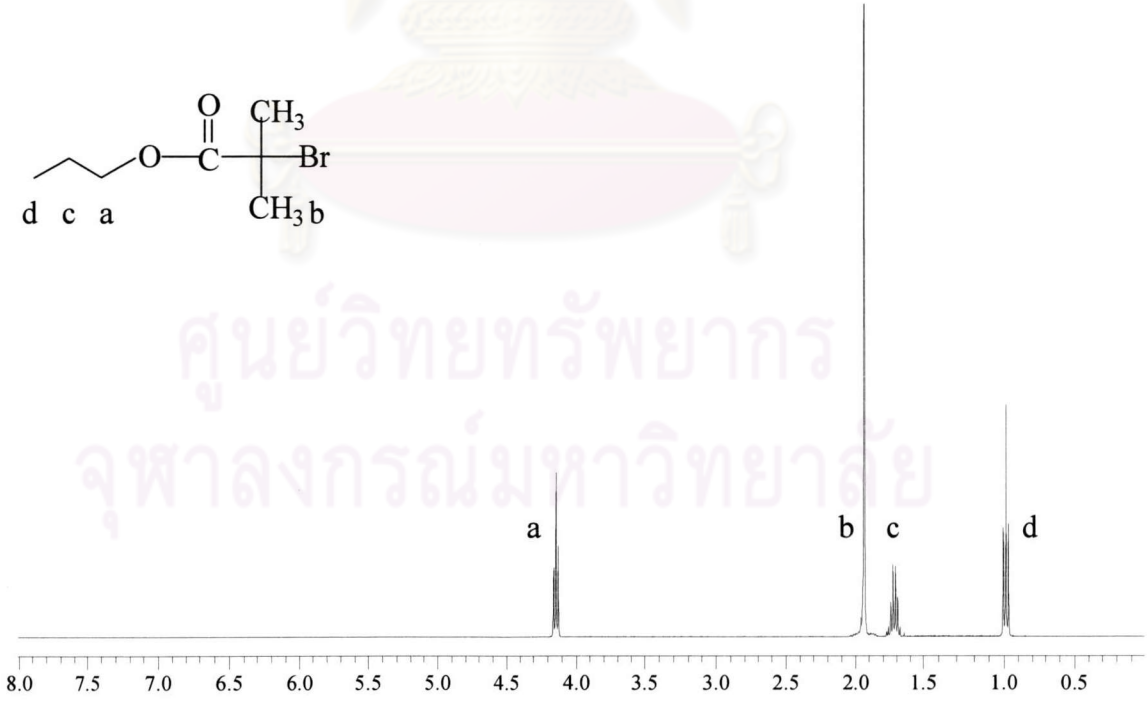

Figure A-4 The ^{1}H-NMR (400 MHz, $CDCl_3$) of prop-2-bromo-2-methylpropinoate **(5)**.

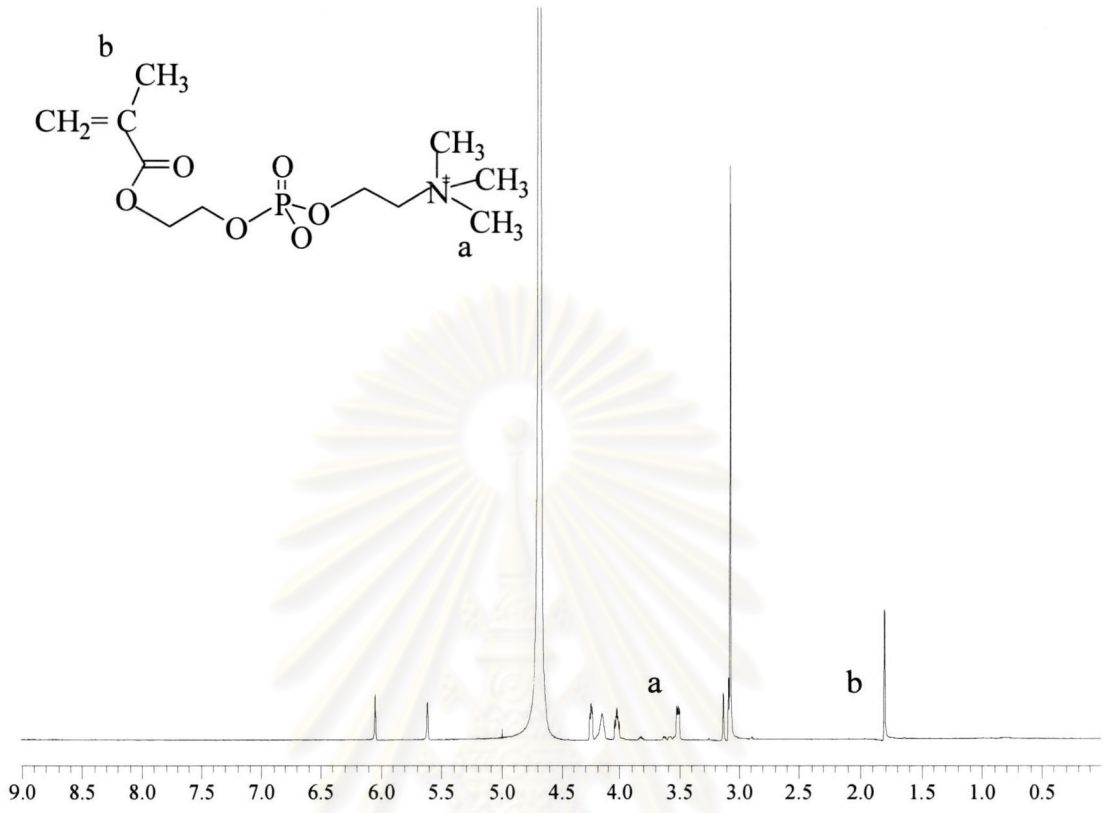

Figure A-5 The ^{1}H-NMR (400 MHz, D_2O) of MPC monomer.

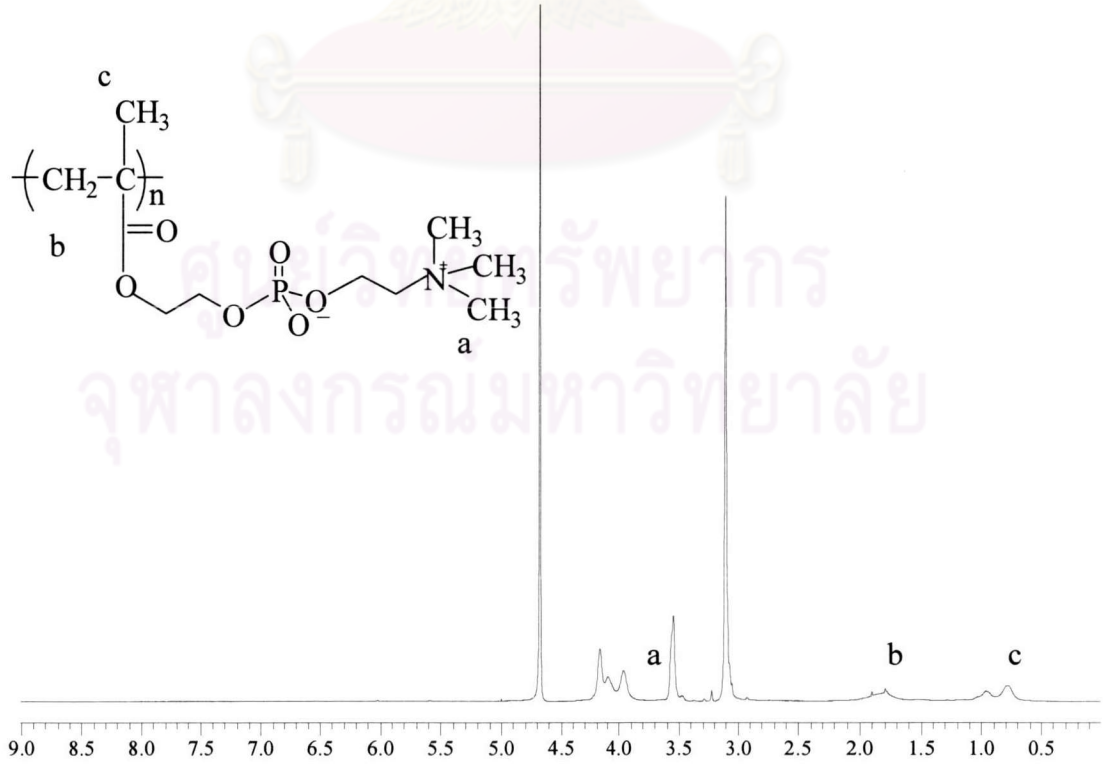

Figure A-6 The ^{1}H-NMR (400 MHz, D_2O) of PMPC.

APPENDIX B

Calculation of % monomer conversion by ^{1}H NMR analysis

% Conversion of MPC monomer was calculated from the ratio between ^{1}H-NMR peak at 0.96 ppm corresponding to the α-methyl proton (α–C<u>H</u>$_3$) of poly(MPC) and ^{1}H-NMR peak at 1.80 ppm corresponding to the α-methyl proton (α–CH_3) of MPC monomer.

$$\%\text{conversion} = \frac{\text{peak area of } \alpha\text{-}CH_3 \text{ of polymer}}{\text{peak area of } \alpha\text{-}CH_3 \text{ of monomer + polymer}} \times 100 \qquad (6)$$

Table B-1 % Monomer conversion as a function of reaction time.

Water		**50%MeOH/water**		**80%MeOH/water**	
Time (min)	**%Monomer conversion**	**Time (min)**	**%Monomer conversion**	**Time (min)**	**%Monomer conversion**
0	0	0	0	0	0
15	74.9	30	40.7	60	8.5
30	82.2	60	48.4	150	19.3
60	88.1	120	56.7	210	26.5
120	94.5	210	62	270	36
150	94.8	300	63	330	44.9

Table B-2 The average thickness of surface grafted α-bromoester initiator calculated from ellipsometric data and advancing and receding water contact angle as a function of time.

Time (h)	Average thickness (Å)	θ_A/θ_R (°)
0	0	34.57 ± 1.75/15.07 ± 4.30
2	3.20 ± 0.94	66.50 ± 1.29/45.50 ± 2.37
6	6.54 ± 0.09	69.80 ± 1.14/59.39 ± 1.38
12	8.82 ± 0.45	70.98 ± 1.48/59.60 ± 1.48
18	9.94 ± 2.19	71.80 ± 0.90/62.01 ± 1.51
24	9.83 ± 1.02	71.50 ± 0.50/61.60± 0.55
36	11.1 ± 2.68	72.05 ± 0.54/61.40 ± 1.17

Table B-3 The average thickness of PMPC brushes without "added" initiator calculated from ellipsometric data as a function of time.

Time (min)	Average thickness (Å)		
	Water	50%IPA/water	IPA
30	16.6	13.8	10.7
60	25.0	19.6	14.4
120	51.8	28.1	26.2
150	53.6	-	-

Table B-4 The average molecular weight and molecular weight distribution of PMPC brushes analyzed by GPC and the graft layer thickness of PMPC brushes calculated from ellipsometric data as a function of time (Solvent: water).

Time (min)	thickness (Å)	GPC data		
		$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
60	25.7	2100	1900	1.11
120	35.8	2200	2000	1.10
180	37.6	2500	2400	1.03
300	44.4	2400	2300	1.04

APPENDIX C

Bicinchoninic acid assay (BCA assay)

Bicinchoninic acid assay is a method used for determination of the amount of proteins. The standard reagents used in this method are reagent A, reagent B and reagent C. Reagent A consists of an aqueous solution of Na_2tartrate, Na_2CO_3, $NaHCO_3$ in 0.2 M NaOH, pH 11.25. Reagent B is 4% (W/V) bicinchoninic acid solution, pH 8.5. Reagent C is 4% $CuSO_4 \cdot 5H_2O$ in deionized water.

The principle of the bicinchoninic acid (BCA) relies on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{1+}. The amount of reduction is proportional to protein present. It has been shown that the peptide bond is able to reduce Cu^{2+} to Cu^{1+}. BCA forms a purple-blue complex with Cu^{1+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins.[30] Figure C-1 shows complexation between bicinchoninic acid and Cu^{1+}.

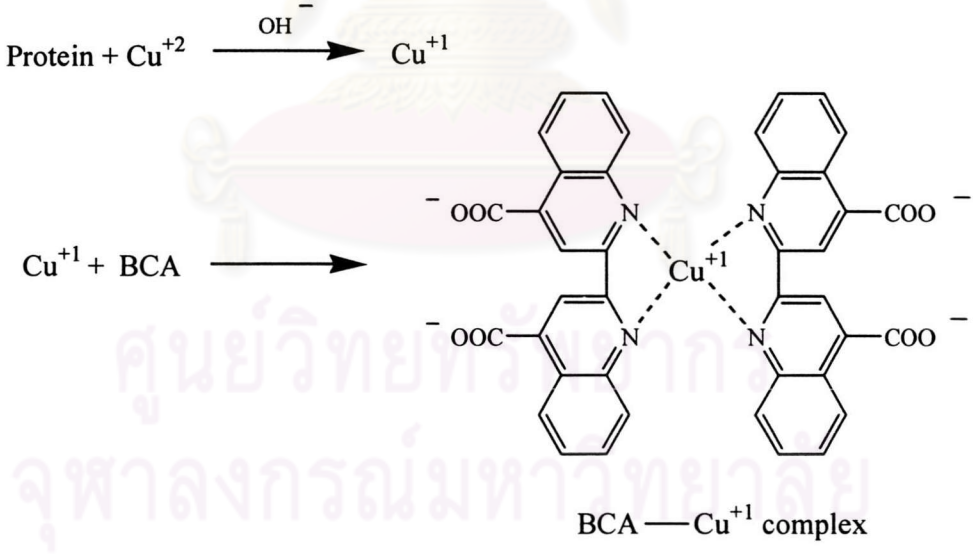

Figure C-1 Formation of purple complex between BCA and cuprous ion generated from the biuret reaction.

The calculated method of the amount of adsorbed protein

1. Read UV absorbance at $\lambda = 562$ nm.

2. Calculate the net absorbance by subtracting the absorbance of the blank (SDS) from the recorded absorbance

$$\textbf{Net } A_{562} = \textbf{recorded } A_{562} - A_{562} \textbf{ (blank)} \quad (7)$$

3. Plot a calibration curve from Net A_{562}

4. Determine the protein concentration (C; μg/mL) in each well from the calibration curve

5. Calculate the total amount of protein (P) in the original solution (1 mL) from the sampling sample (100 μL) + BCA working solution (100 μL)

$$\textbf{Total amount of protein (P)} = \frac{\textbf{C (μg/mL) x 200 (μL)}}{\textbf{1000 (μL/mL)}} \times \frac{\textbf{1000 (μL)}}{\textbf{100 (μL)}} \quad (8)$$

6. Calculate the amount of adsorbed protein/surface area

$$\textbf{Adsorbed protein/surface area } P_{ads} = \textbf{P/surface area (2 sides) } (\mu g/cm^2) \quad (9)$$

VITAE

Miss Piyawan Suk-in was born on February 14, 1980 in Phuket, Thailand. She received a bachelor degree of science from Department of Chemistry, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla Thailland in 2001. In the same year she was admitted to a Master's Degree in Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University and completed program in 2004.