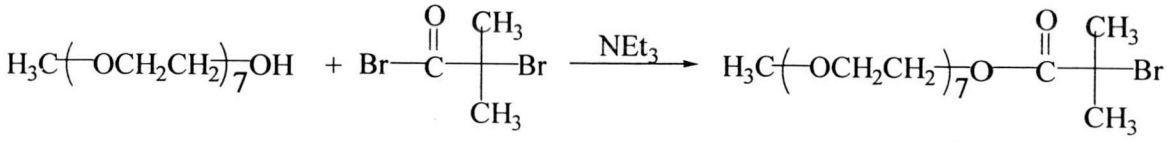
CHAPTER IV

RESULTS AND DISCUSSION

4.1 Synthesis of PMPC in solution

In general, the molecular weight of polymer synthesized by polyaddition can be controlled by the ratio between monomer and initiator. This is not really the case for the synthesis of polymer brush whose initiation begins from the initiator being attached to the surface. The concentration of initiation site is usually low in comparison with the monomer added. As a result, reaction time seems to be a more suitable parameter that can be used for controlling the length or the molecular weight of polymer brush. This part of study was conducted with an objective to determine the effect of solvent polarity on the rate of monomer conversion in solution in order to find the most efficient solvent for polymer brush formation. We hypothesize that the length or the molecular weight of polymer brush should be linearly dependent on the monomer conversion and the molecular weight of polymer in solution. Polymerization of MPC was carried out in solution using methoxy-capped oligo(ethylene glycol)-2-bromoisobutyrate: OEGBr as an initiator in the presence of CuBr/bpy.

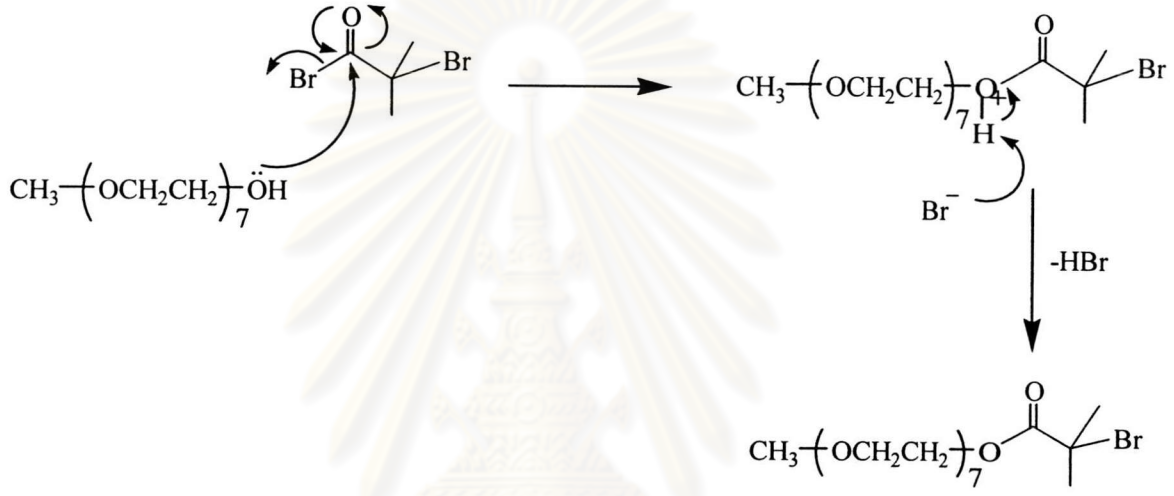
4.1.1 Synthesis of Methoxy-capped Oligo(ethylene glycol)-2-bromoiso butyrate initiator: OEGBr (1)



1

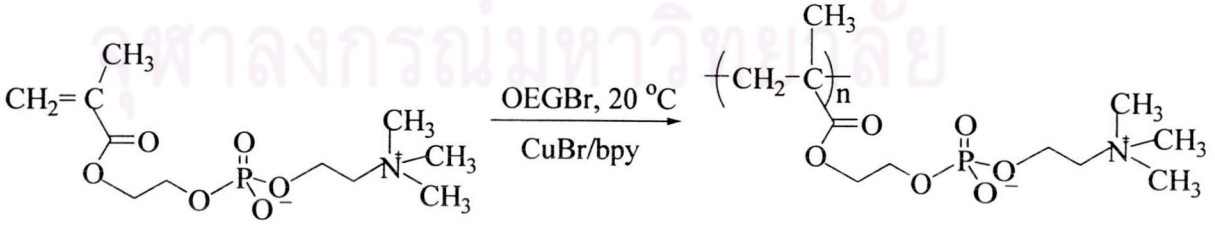
The nucleophilic acyl substitution of poly(ethylene glycol) 350 monoethyl ether with 2-bromoisobutyryl bromide in tetrahydrofuran (Scheme 4.1) gave an

orange viscous liquid of methoxy-capped oligo(ethylene glycol)-2-bromoisobutyrate: OEGBr **(1)** (80 %yield), which was sufficiently pure for the next synthesis without further purification after the work-up process. The structure of product **(1)** was confirmed by ^{1}H-NMR, which revealed a singlet signal of the methyl proton $C(C\underline{H}_3)_2$ at 1.88 ppm indicating the attachment of 2-bromoisobutyrate group. The ^{1}H-NMR spectrum is shown in Figure A.1.



Scheme 4.1 The mechanism of the nucleophilic acyl substitution reaction between poly(ethylene glycol) 350 monoethyl ether with 2-bromoisobutyryl bromide.

4.1.2 Synthesis of PMPC



MPC **PMPC**

The polymerization of MPC monomer by atom-transfer radical polymerization using OEGBr **(1)** as an initiator in the presence of CuBr/bpy was

carried out at room temperature. %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 PMPC and ^{1}H-NMR peak at 1.80 ppm corresponding to the α-methyl proton (α–CH$_3$) of MPC monomer. The ^{1}H-NMR spectra of MPC and PMPC mixed with the unreacted MPC synthesized by ATRP using [OEGBr]:[MPC] = 1:20 (a fixed target DP$_n$ of 20 ($\overline{M}_n$ = 6,000)), are shown in Figure 4.1.

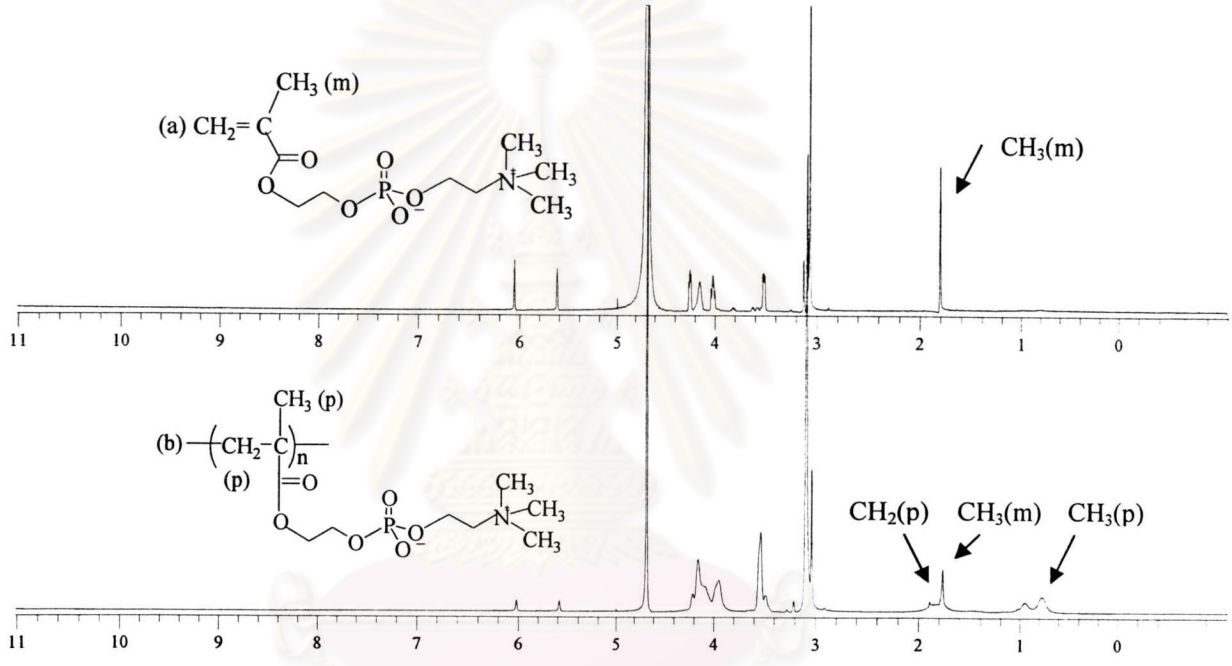


Figure 4.1 ^{1}H NMR spectra of (a) MPC and (b) PMPC mixed with the unreacted MPC synthesized by ATRP using [OEGBr]:[MPC] = 1:20.

Polymerization was carried out in H_2O, 50%MeOH/H_2O and 80%MeOH/H_2O. The rate of monomer conversion was followed by analyzing the solution withdrawn from the mixture during polymerization. The results shown in Figure 4.2 suggested that the rate of monomer conversion increased as the polarity of solvent increased. The polymerization in aqueous has reached almost 100% conversion within 150 min of reaction. The rate of monomer conversion was significantly reduced in the presence of methanol which is a less polar solvent in comparison with water. These results agree very well with the previous data reported

by Armes and coworkers [40]. They explained that MPC can be polymerized to high conversion in both water and methanol at ambient temperature via atom transfer radical polymerization (ATRP). The narrow polydispersities ($\overline{M}_w/\overline{M}_n$ = 1.15-1.35) were obtained in both aqueous and alcoholic media at 20 °C. However, slower polymerizations and narrower polydispersities were always obtained in alcoholic solution, and chain extension experiments indicated significantly greater living character under these conditions. Moreover, these results support that the Cu(I)Br/bpy complexes can well solvate in polar solvent [17]. Nanda and Matyjaszewski studied the effect of polarity of the medium on the activation rate constants (k_{act}) in atom transfer radical polymerization. They found the k_{act} was larger in more polar solvents compared to less polar solvent because of the effect of [CuBr]/[bpy] complex. In more polar media, the bromide anion is sufficiently stable and well solvated, resulting in $Cu(bpy)_2^+Br^-$ species. However, in less polar media, Br^- is destabilized and concurrently binds much stronger to Cu(I)Br than bpy does, resulting in $Cu(bpy)_2^+CuBr_2^-$ species. However, there are data suggest that the aqueous ATRP of MPC has less than ideal living character [40]. Armes and coworkers believe that aqueous ATRP is not well-suited for synthesis of controlled-structure block copolymer. There are two plausible explanations for the reduced living character encountered in aqueous ATRP syntheses. First, the copper-halogen bond of the Cu(II) complex is likely to be less covalent (more ionic) in aqueous media, which could reduce the efficiency of polymer radical deactivation. Second, the terminal halogen atom on the polymer chain ends may be prone to hydrolysis.

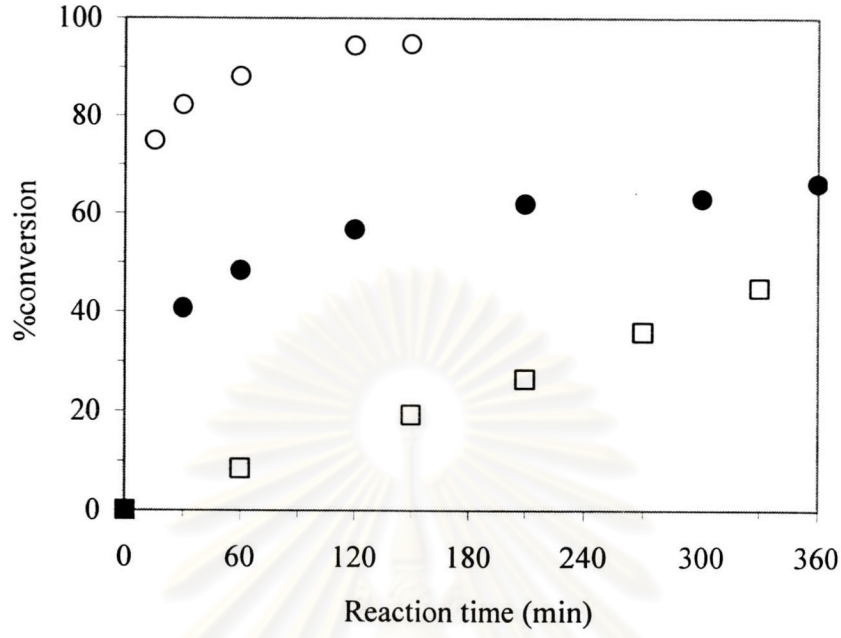
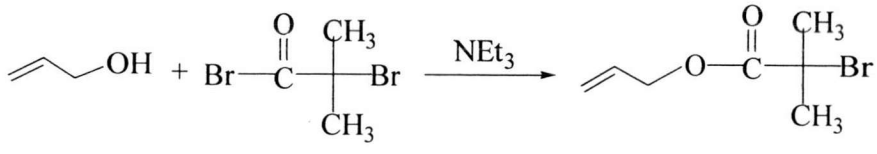


Figure 4.2 % Monomer conversion as a function of reaction time: water (○), 50%MeOH/water (●), and 80%MeOH/water (□).

4.2 Preparation of PMPC Brushes

PMPC brushes was synthesized by surface-initiated atom transfer radical polymerization from surface grafted ∝-bromoester initiators. The effect of solvent polarity and reaction time on the thickness of polymer brush was investigated.

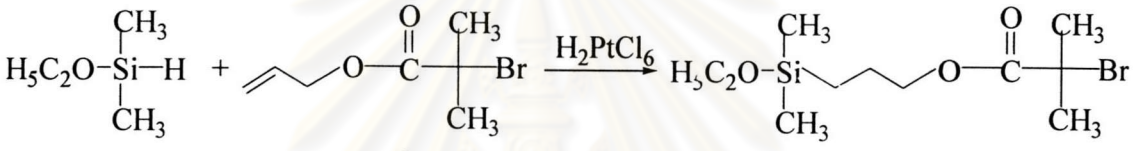
4.2.1 Synthesis of Prop-2'-enyl 2-bromo-2-methyl propinoate (2)



2

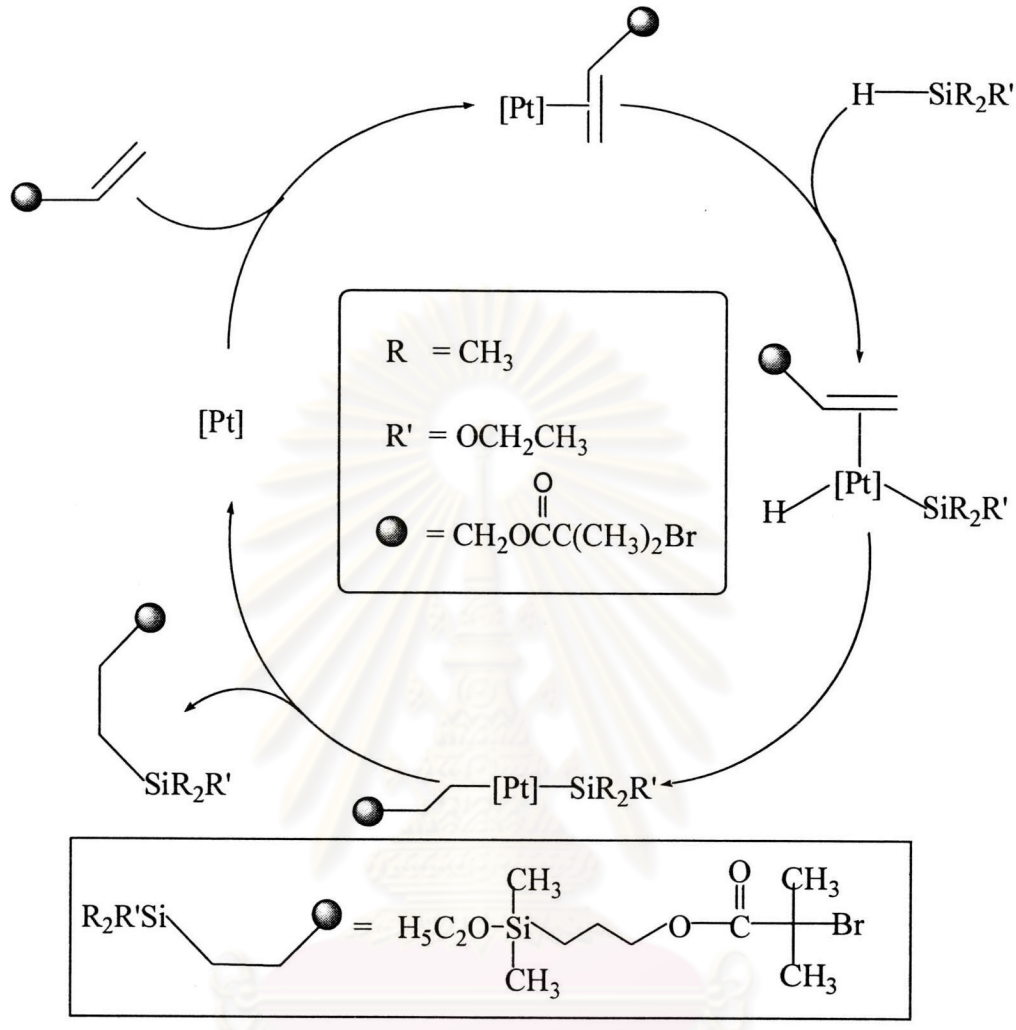
The nucleophilic substitution of allyl alcohol with 2-bromoisobutyryl bromide in tetrahydrofuran gave prop-2'-enyl 2-bromo-2-methylpropinoate **(2)** as a pale yellow viscous liquid (90 %yield), which was sufficiently pure for the next synthesis without further purification after the work-up process. The ^{1}H-NMR (Figure A.2) of product **(2)** shows a singlet signal of the methyl proton C(C$\underline{H}_3$)$_2$ at 1.94 ppm indicating the attachment of allyl alcohol with 2-bromoisobutyryl bromide.

4.2.2 Synthesis of 3-(Dimethylethoxysilyl)propyl-2-bromoisobutyrate (3)



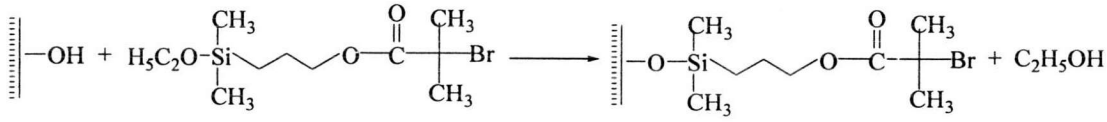
2 **3**

Hydrosilylation of dimethylethoxysilane with **2** was carried out in the dark in the presence of chloroplatinic acid, H_2PtCl_6 at room temperature for 24 h. The crude product of 3-(dimethylethoxysilyl)propyl-2-bromoisobutyrate **(3)** was a yellow viscous liquid (70 %yield), which was sufficiently pure for the next synthesis without further purification after the work-up process. The mechanism of hydrosilylation is shown in Scheme 4.2. The ^{1}H-NMR spectrum (Figure A.3) of product **(3)** shows a triplet signal and multiplet signal of the methylene proton (SiC$\underline{H}_2$CH$_2$CH$_2$O) and (SiCH$_2$C$\underline{H}_2$CH$_2$O) at 0.93 and 1.66 ppm, respectively. While a multiplet peak of alkene proton (C$\underline{H}_2$=CH) and (CH$_2$=C$\underline{H}$) at 5.25-5.40 and 5.88-5.98 ppm, respectively of starting alkene derivative **(2)** were disappeared indicating the completion of reaction.



Scheme 4.2 The mechanism of the hydrosilylation reaction between dimethylethoxysilane with **2**.

4.2.3 Preparation of Surface Grafted ∝-Bromoester Initiators (4)



3 **4**

The substrates used for PMPC brush formation were silicon oxide substrates that were chemically modified by incorporating α-bromoester functionality in its surface region. Silicon oxide reacts with **3** to form α-bromoester functionalized

surface **(4)**. Contact angle measurements and ellipsometry were used as tools to determine an appropriate reaction time that results in the maximum graft density of initiator.

Figure 4.3 shows water contact angle data. This data suggested that hydrophobization has reached its maximum within 6-12 h of reaction. According to the result from ellipsometric analysis (Figure 4.4), a monolayer of α-bromoester initiator **(4)** was readily formed with a thickness of ~10Å on the top of the silicon oxide layer (~34 Å) within 18-24 h. To obtain the maximum graft density 24 h was selected. Upon the formation of α-bromoester monolayer, it is obvious that the surface hydrophobicity was increased. Water contact angle of the surface grafted α-bromoester increased from 34°/15° of the cleaned silicon oxide to 71°/62° of the silicon oxide containing α-bromoester groups.

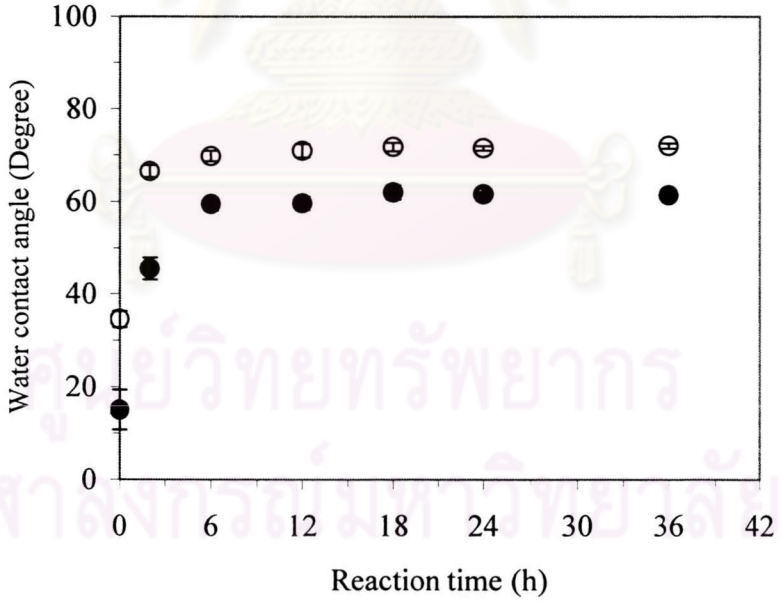


Figure 4.3 Advancing (○) and receding (●) water contact angle data of silicon oxide surface after a reaction with **4** as a function of reaction time.

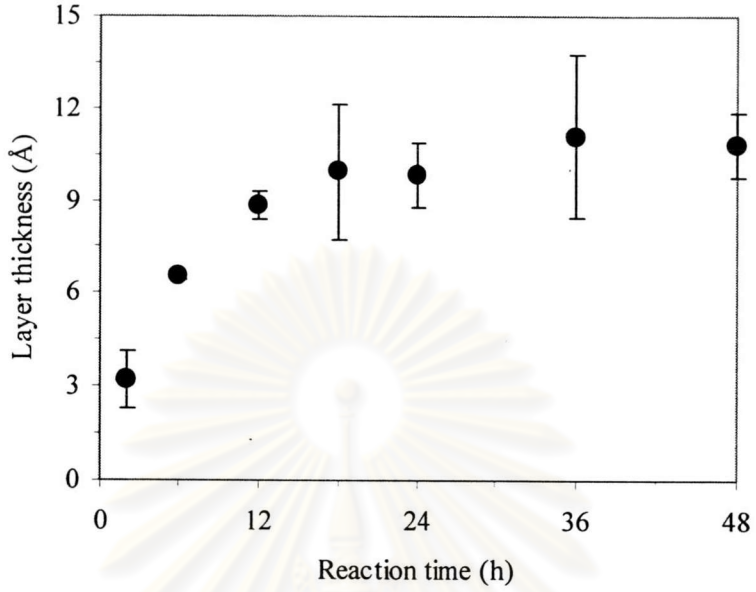
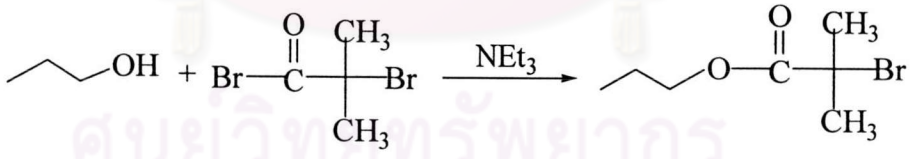


Figure 4.4 The thickness of the surface grafted α-bromoester initiator as a function of reaction time.

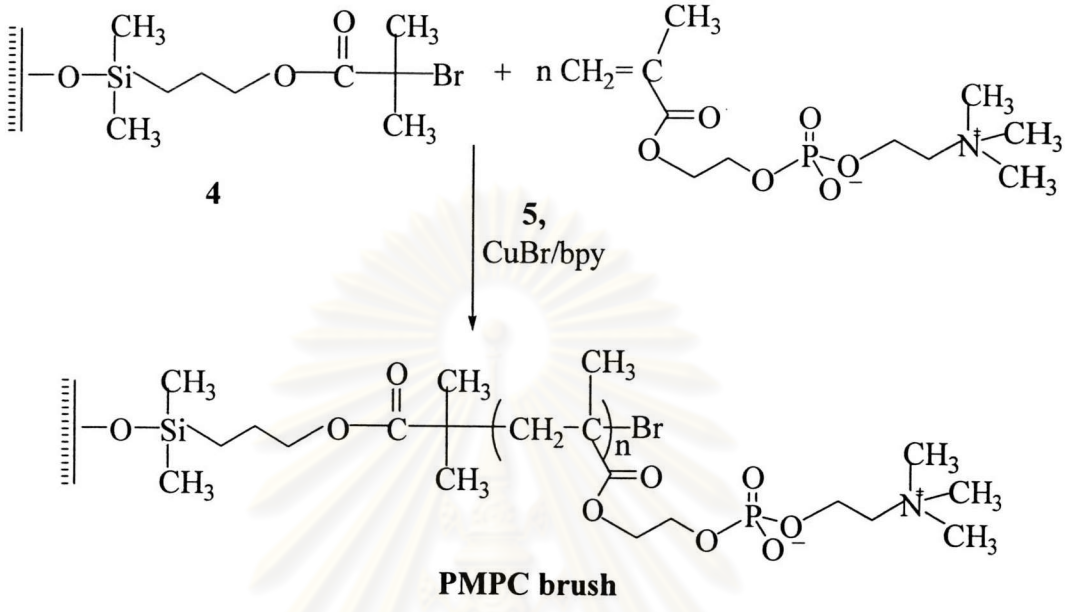
4.2.4 Synthesis of Prop-2-bromo-2-methylpropinoate (5)



5

The nucleophilic substitution of 1-propanol with 2-bromoisobutyryl bromide in tetrahydrofuran gave prop-2-bromo-2-methylpropinoate **(5)** as a pale yellow viscous liquid (90 %yield), which was sufficiently pure for the next synthesis without further purification after the work-up process. The ^{1}H-NMR (Figure A.4) of product **(5)** showed a singlet signal of the methyl proton C($C\underline{H}_3$)$_2$ at 1.91 ppm indicating the success of reaction between 1-propanol and 2-bromoisobutyryl bromide. This product was used as an "added" or controlling initiator for the polymerization of polymer brushes.

4.3 Preparation of PMPC Brushes



4.3.1 Effect of Solvent on Thickness

PMPC brushes were grown from the surface bearing α-bromoester group **(4)** via ATRP mechanism in the presence of CuBr/bpy using H_2O or isopropanol (IPA) as a reaction media. IPA is selected as an alcoholic media for polymerization since it is much more environmentally friendly than methanol. There is a second advantage in using IPA rather than methanol as an ATRP solvent. With certain hydrophilic methacrylates, Armes and coworkers have observed some degree of transesterification occurring during methanolic ATRP, even for polymerizations conducted at ambient temperature [40]. This unwanted side reaction is suppressed in IPA, which makes this solvent the preferred choice for the synthesis of certain MPC-based diblock copolymers [75]. Figure 4.5 shows the relationship between PMPC brush thickness and reaction time. It was found that polymer brushes grow more rapidly in a more polar aqueous media in comparison with a less polar alcoholic media which is in good agreement with the data obtained in solution as previously described. The polar media, especially water facilitates the CuBr catalyst solubility and dissociation and thus accelerates the polymerization [19, 26]. Such a media,

however, deteriorates the livingness of reaction and may cause premature termination as can be evidenced from the thickness of PMPC reaching its maximum of ~50 Å after 3 h.

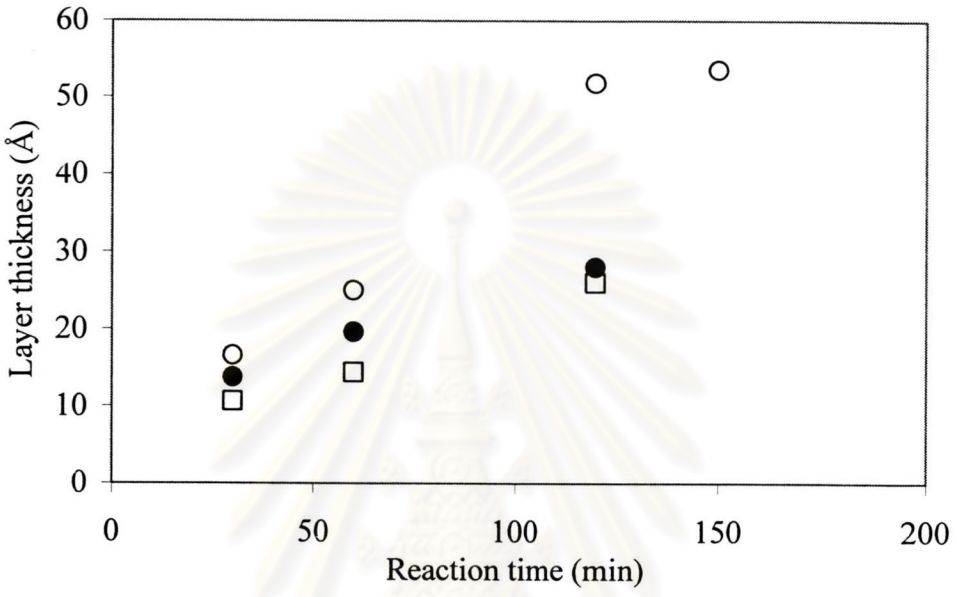


Figure 4.5 Ellipsometric thickness of PMPC brushes prepared in water (○), 50%IPA/water (●), and IPA (□).

4.3.2 Molecular Weight and Graft Density of PMPC brushes

Because of the difficulty to obtain the molecular weight of the polymer brush directly, we used the "free" polymer formed by "added" initiator in solution to monitor the surface-grafted polymerization process. The free initiator plays a role not only as an indicator of the polymerization but also as a controller for the ATRP on the surface. In the polymerization without an additional initiator, the concentration of the Cu^{II} complex produced from the reaction at the substrate surface is too low to reversibly deactivate polymer radicals with a sufficiently high rate. The additional initiator would increase and adjust the concentration of the Cu^{II} complex as in a free ATRP system. Alternatively, the adjustment of the Cu^{II} concentration could be made by directly adding an appropriate amount of the Cu^{II} complex [68,

76]. Therefore, since the amount of polymer on the silicon wafer is too small to degraft and analyze, the polymer in solution may provide an indication of the properties of the polymer on the surface. Another advantage of the additional initiator is that it produces free polymers, which can be used as a measure of the molecular weight and molecular weight distribution of the graft chains.

A grafting density (σ) which is a unit per cross-sectional area (A_x) per chain can be determined from the corresponding film thickness (t) and the molecular weight of the chain (M) from the following equation by

$$\sigma = \frac{t\rho N_A}{M} = \frac{1}{A_x} \quad (5)$$

where ρ is the mass density (1.19 g/cm^3 for PMPC) and N_A is Avogadro's number. For example, a molecular weight of PMPC ~1900 g, a thickness layer ~25.7 Å, an average value of σ ca. 0.95 chain/nm^2 is found.

As characterized by Atomic absorption, the purified PMPC contains 0.01-0.05 ppm of Cu. This minute content is acceptable for biomedical applications.

4.3.3 Confirmation of PMPC Brush Formation by XPS

XPS was used to confirm the formation of the initiator monolayer and the growth of PMPC brushes. The result is outlined in Table 4.1. The α-bromoester layer can be indicated by the signal of Br_{3d}. The N_{1s} and P_{2p} data suggest that phosphatidylcholine analogous groups are present on the surface of PMPC brushes. The N/P ratios varying in the range of 0.7-0.8 reasonably agree with the stoichiometric ratio of MPC unit. Due to a partial X-ray damage of bromide during XPS analysiis, the percentage of Br_{3d} is neither quantitative nor consistent with the percentages of N_{1s} and P_{2p}. The surfaces having polymer brushes were more hydrophilic than the surface bearing α-bromoester groups (θ_A/θ_R ~71/62°) which is an indication of hydrophilic phosphorylcholine moieties. The independence of water

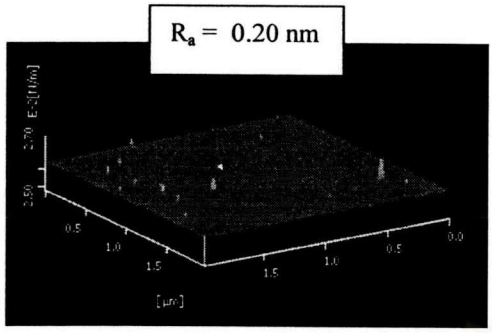
contact angles on the thickness of PMPC brushes evidently implied that the growing of each polymer brush is simultaneous and living in character.

Table 4.1 XPS elemental surface composition (%) and water contact angle of PMPC brushes.

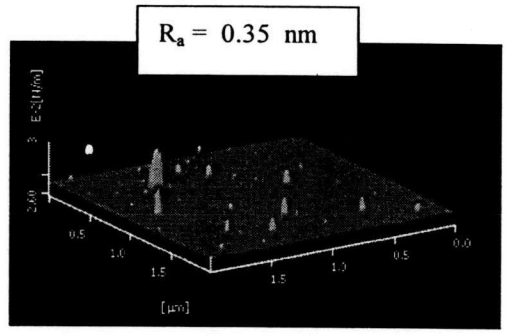
Surface	Condition	Si_{2p}	C_{1s}	O_{1s}	Br_{3d}	P_{2p}	N_{1s}	θ_A/θ_R (°)
Silicon oxide	-	40.50	29.75	29.75	-	-	-	34/15
α-bromoester surface	-	36.37	34.34	28.79	0.49	-	-	71/62
PMPC brushes	2 h in H_2O	25.78	48.89	23.13	0.27	1.12	0.81	44/25
	3 h in H_2O	18.07	61.49	18.13	0.35	1.08	0.88	44/25

4.3.4 Surface Topography of PMPC Brushes

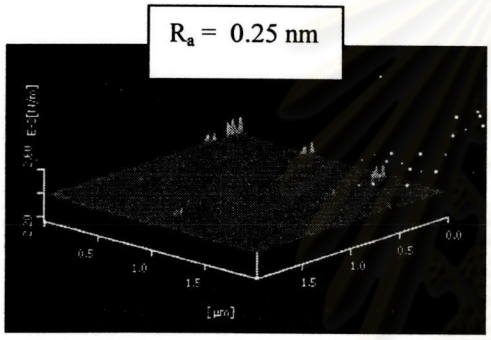
The surface lopography of PMPC brushes was analyzed by AFM. The AFM micrographs as shown in Figure 4.8 suggest that all surfaces bearing PMPC brushes having the thickness in the range of ~10-50 Å are quite smooth and have an average roughness (R_a) in the range of 0.25-0.30 nm.



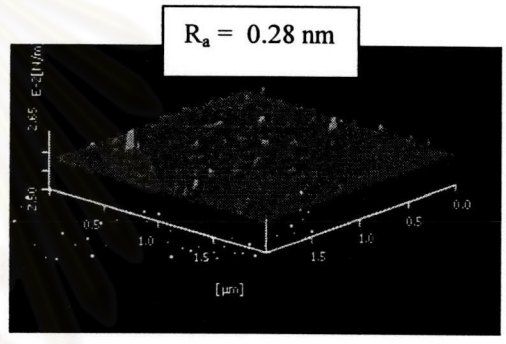
(a) silicon oxide surface



(b) α-bromoester surface



(c) PMPC brushes (1 h, H_2O,~14 Å)



(d) PMPC brushes (5 h, H_2O,~52 Å)

Figure 4.6 AFM micrographes of silicon oxide and PMPC brushes.

4.4 Blood Compatibility of PMPC Brushes

In this study, the blood compatibility of polymer brushes was determined with an attention to investigate the interaction between the surface bearing PMPC brushes with blood components which are, plasma proteins from platelet-poor plasma (PPP) and platelets from platelet-rich plasma (PRP). The effect of the thickness PMPC brushes on blood compatibility was investigated.

4.4.1 Plasma Protein Adsorption

The amount of plasma adsorbed on a material surface is a primary factor in evaluating the blood compatibility of the material. When a blood-incompatible material is in contact with blood, a surface induced thrombosis which is initiated by the adsorption of plasma protein, followed by adhesion and activation of platelets. Here we determine the amount of plasma protein adsorption using BCA microassay. The calibration curve using albumin as a standard is displayed in Figure 4.7.

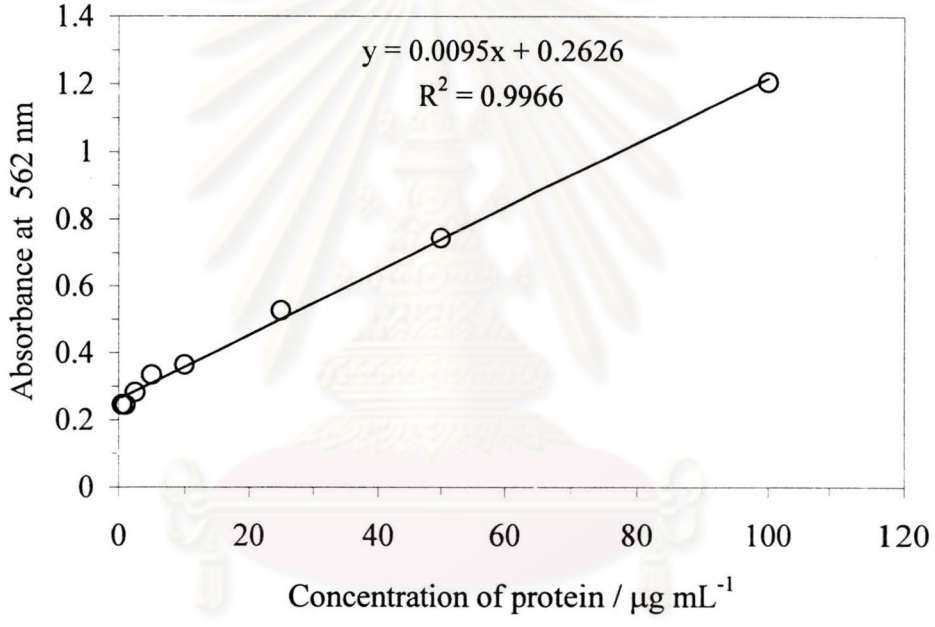


Figure 4.7 A calibration curve of the amount of albumin adsorbed and the absorbance obtained from BCA microassay.

Table 4.2 shows the amount of plasma protein adsorbed on the surfaces bearing PMPC brushes having various thickness. It can be seen that the amount of protein adsorbed on the surfaces having PMPC brushes are essentially the same independence of the solvent used and the thickness of polymer brushes.

Table 4.2 The amount of plasma protein adsorbed on the surfaces bearing PMPC brushes.

Surface	Solvent	Reaction time (h)	Thickness (Å)	θ_A/θ_R (°)	Amount of protein adsorbed ($\mu g/cm^2$)
Silicon oxide surface	-	-	-	34/15	0.32 ± 0.12
α-Bromoester surface	-	-	-	71/62	0.65 ± 0.30
PMPC brush	H_2O	0.5	17	44/24	0.36 ± 0.06
		1	25	43/23	0.39 ± 0.12
		2	52	44/25	0.34 ± 0.04
	IPA	0.5	11	44/23	0.41 ± 0.05
		1	14	44/25	0.35 ± 0.07
		2	26	44/25	0.45 ± 0.09
PMMA brush	IPA	1	18	66/54	1.47 ± 0.60
		2	30	67/58	2.40 ± 0.39

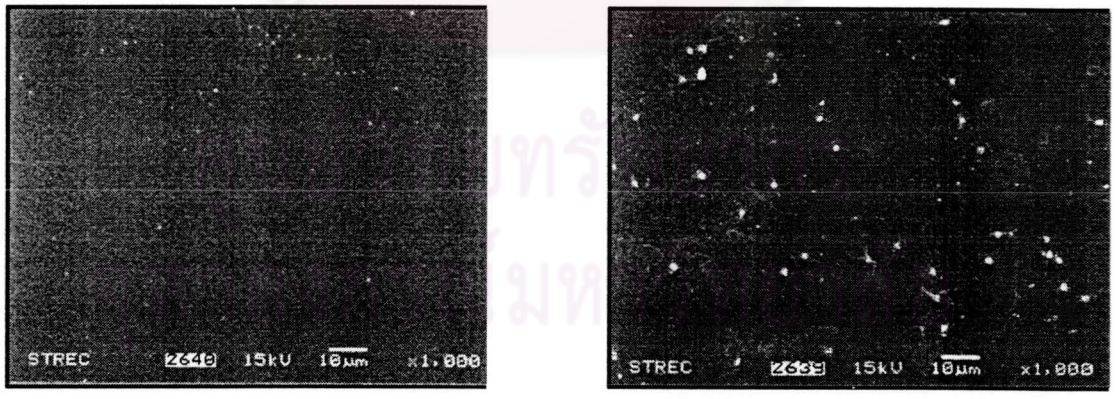
Even though the amount of plasma protein adsorbed on the surfaces having PMPC brushes was lower than those on of the surface grafted with α-bromoester initiator, the difference was not that significant considering the more hydrophilic nature of PMPC in comparison with the surface grafted with α-bromoester initiator. It is quite necessary to find a surface having polymer brushes that is blood incompatible and can be prepared from the same surface grafted with α-bromoester initiator. We then chose to synthesize poly(methyl methacrylate) (PMMA) brushes by surface-initiated atom transfer radical polymerization of methyl methacrylate. The surfaces having PMMA brushes were used as a positive-control surface.

As expected, the amount of protein adsorbed on the surface having PMMA brushes was significantly higher than that on the surface having PMPC brushes. This can be explained by the blood incompatibility of PMMA. This positive controll

study confirmed that the reduction of protein adsorption and the blood compatibility of the surface are really due to the existence of PMPC brushes.

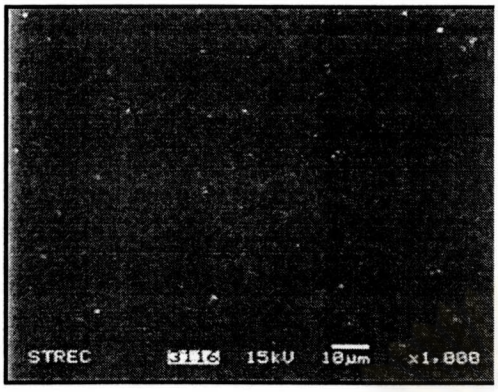
4.4.2 Human Platelet Adhesion

It is well known that platelets also contribute to the thrombus formation. In general, a foreign substrate induces adhesion and activation of platelets with the adsorbed protein layer serving as a controlling factor of the platelet response. Figure 4.8 shows SEM micrographs of silicon oxide surface, surface grafted with α-bromoester initiator, PMMA brushes, and PMPC after contacting with PRP. This result confirms the blood compatibility of PMPC brushes. Many platelets adhered to the PMMA brushes. A few platelets were adhered on the surface grafted α-bromoester but did not adhere on the silicon oxide surface. The thickness layer of PMPC brushes does not seem to influence the adherent platelets. PMPC brushes completely inhibited platelet adhesion. The data from platelet adhesion studies agree very well with the plasma protein adsorption and confirm the blood compatibility of PMPC brushes.

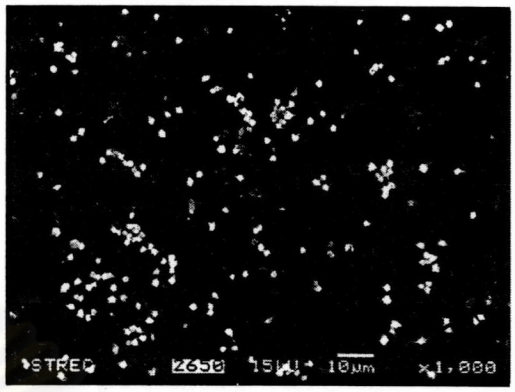


(a) silicon oxide surface (b) α-bromoester surface

Figure 4.8 SEM micrographs of surfaces after contacting with human PRP.

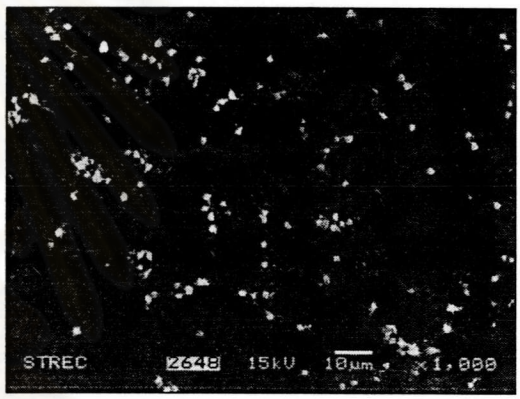


(c) PMPC brushes (~14 Å)



(d) PMMA brushes (~16 Å)

(e) PMPC brushes (~26 Å)



(f) PMMA brushes (~30 Å)

Figure 4.8 (continued).