Preparation of Poly(trimethylene carbonate) Derivatives with Oligo(ethylene glycol) and Their Thermosensitive and Degradable Properties

CHANTHASET Nalinthip

A thesis submitted in fulfillment of the requirements for

the degree of

Doctor of Philosophy (Engineering)

September 2018

Materials Science

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(NAIST)

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Chapter 3

Investigation on Polymerization of 5-[2-{2-(2-methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one by Organometallic Catalysts

General Introduction

The development of clinical materials especially soft biomaterials has been widely studied in polymeric network materials[1] for potential medical treatment such as scaffold for vascular engineering[2], scaffold for drug delivery system[3] and thermoresponsive hydrogels for cardiac therapy (Figure 1).[4] Particularly, mechanical property and biological compatibility of soft materials have been considered and determined as application demanding.[5] There are plenty of polymeric candidates which have been studied over the past decades such as poly(acrylamide) (PAAm),[6-7] poly(*N*-isopropyl acrylamide) (PNIPAM),[8] poly(dimethyl siloxane) (PDMS),[9] poly(acrylic acid),[10] and poly(lactic acid).[11]

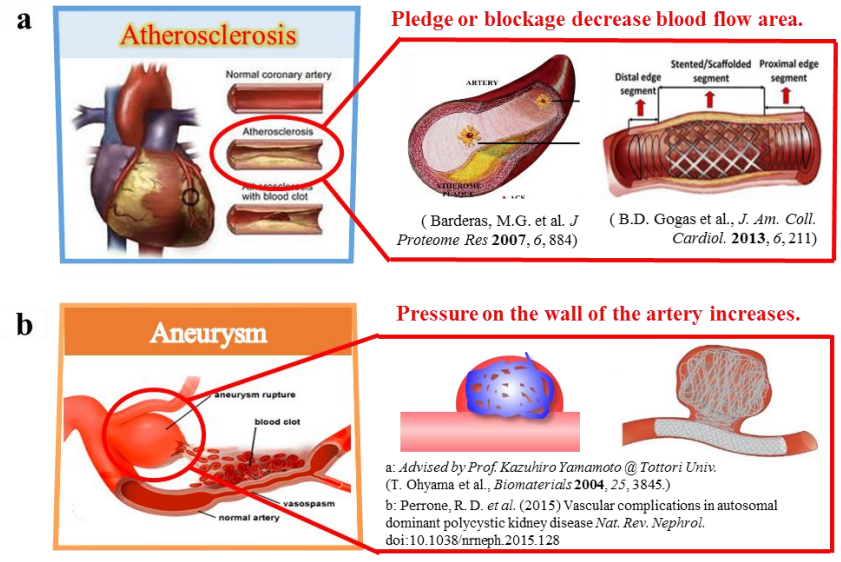


Figure 1. Soft biodegradable hydrogel for circular organ application: Atherosclerosis treatment (a) and brain aneurysm treatment (b).

By now, the conventional responsive homopolymer such as PNIPAM and PAA were limited due to their non-degradable and weakly acidic structure. Now that copolymers such as PNIPAM and natural components which are available in pH,[12] light,[13] thermal stimuli responsive, [14] and mechanical control[14] were reported. Thus, the well-known aliphatic degradable polymers have been studied for example poly(carprolactone) (PCL),[15] poly (lactic acid) (PLA),[15,16] poly(trimethylene carbonate) (PTMC)[16] and so on. Up to the present, the field has gradually broadened as PLA are the most dominant candidate employed for biomaterials as its stiffness and biocompatibility.[17] However, there has been less previous evidence in PLLA biodegradability.

Moreover, there are growing appeals for cyclic carbonate derivative as one of the candidates to overcome the material problems because of the ester-free structure based monomers.[18] Recently, the thermoresponsive trimethylene carbonate (TMC) were designed and successfully synthesized by our group.[19] Homopolymers of TMC modified with oligo(ethylene glycol), 5-[2-{2-(2-methoxyethoxy)ethoxy}ethoxymethyl]-5-methyl-[1,3]-dioxa-2-one (TMC M-MOE3OM) (Figure 2), were prepared and investigated thermoresponsive ability by organcatalyst, 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU).[19] It is interesting to note that, PTMCM-MOE3OM exhibited the lower critical solution (LCST) at around 33˚C (body temperature) and PTMCM-MOE4OM at 72˚C in aqueous (Figure 2).

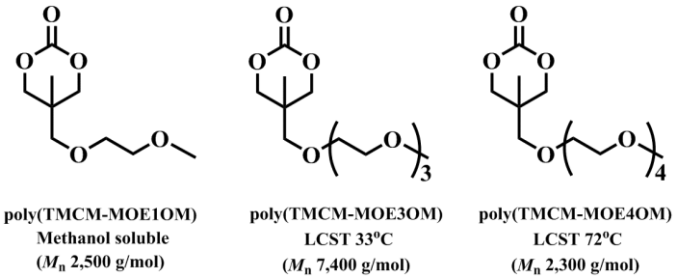


Figure 2. TMCM-MOE*m*OM monomer derivatives.[19]

Widely considered a good way for more application, the various stimuli responsivities are particularly focused such as pH-, light- and so on. Therefore, a common strategy used to extend the polymeric functionality is to copolymerize with another responsive molecules. Corresponding to biomedical application, drug delivery system[20] dealing with pH responsivity are approached for the induced positive/negative charge form.[21,22] Additionally, deprotonation form such as carboxylate ion was examined as ester bond stimulation for the further gelation. Several studies suggest that electrostatically charge existence, alcohol-included solvent, ions in salt solution, and tuning pH-solution are recognized for pharmaceutical system and smart materials.[21,23] The pH responsive with electrostatic charge side chain such as carboxylic moiety[27] and photo responsive molecule with coumarin,[24] spironpyran,[25,27] azobenzene,[26] *o*-nitrobenzyl ester,[27] cinnamic ester[27] were observed by LCST change along copolymerization.

Furthermore, to achieve the purpose of biomaterials research, there are growing appeals for physical and chemical crosslink enhancing. As light-induced crosslinking, photo responsive moiety possess the several effect such as photoisomerzation, photocleavage, and photocycloaddition.[27] Among the well-known photoresponsive molecules, it is of interest to know that photoisomerization of spiropyrans[25,27] undergoing with it hydrophilic zwitterionic

ability and azobenzene[26] responding by trans-cis of nitrogen–nitrogen double bond (N=N) with reversibility. While, *o*-nitrobenzyl ester[27] undergo with irreversible photocleavage into carboxylic acids. In addition to the photocycloaddition, cinnamic ester (cynamoyl group)[27] endures along [2+2] photocycloaddition upon UV irradiation as well as coumarin moiety.[24] However, coumarin molecules was employed to form physical bonding under irradiation (>310 nm) via π-π stacking UV-promoted cycloaddition with reversibility cleavage at 254 nm. By the way, the conjugated chromophore was assigned and show the significant region in optical absorption at around 310-320 nm. Recently, this was successfully established as described that poly(*N*-vinylacetamide) bearing coumarin moiety was indicated the photoresponsive providing dimerization and gelation under rapidly irradiation.[28] Nevertheless, inadequate crosslinked point and limitation of photo crosslinked agent synthesis, chemical crosslinker were introduced. Eventually, three-dimension co-polymeric gel performance base on TMC and CL with synthetic crosslink agent 2,2'-bis(trimethylene carbonate-5-yl)-butylether (BTB) has been successfully fabricated.[29] As desired mechanical and thermal properties were received with an equally good result *in vitro* degradation by lipase.[29]

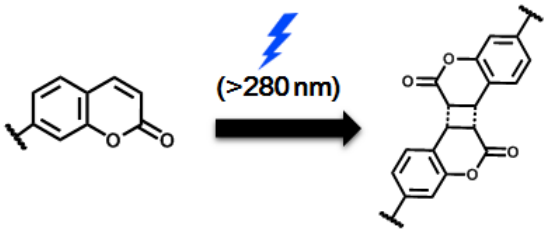
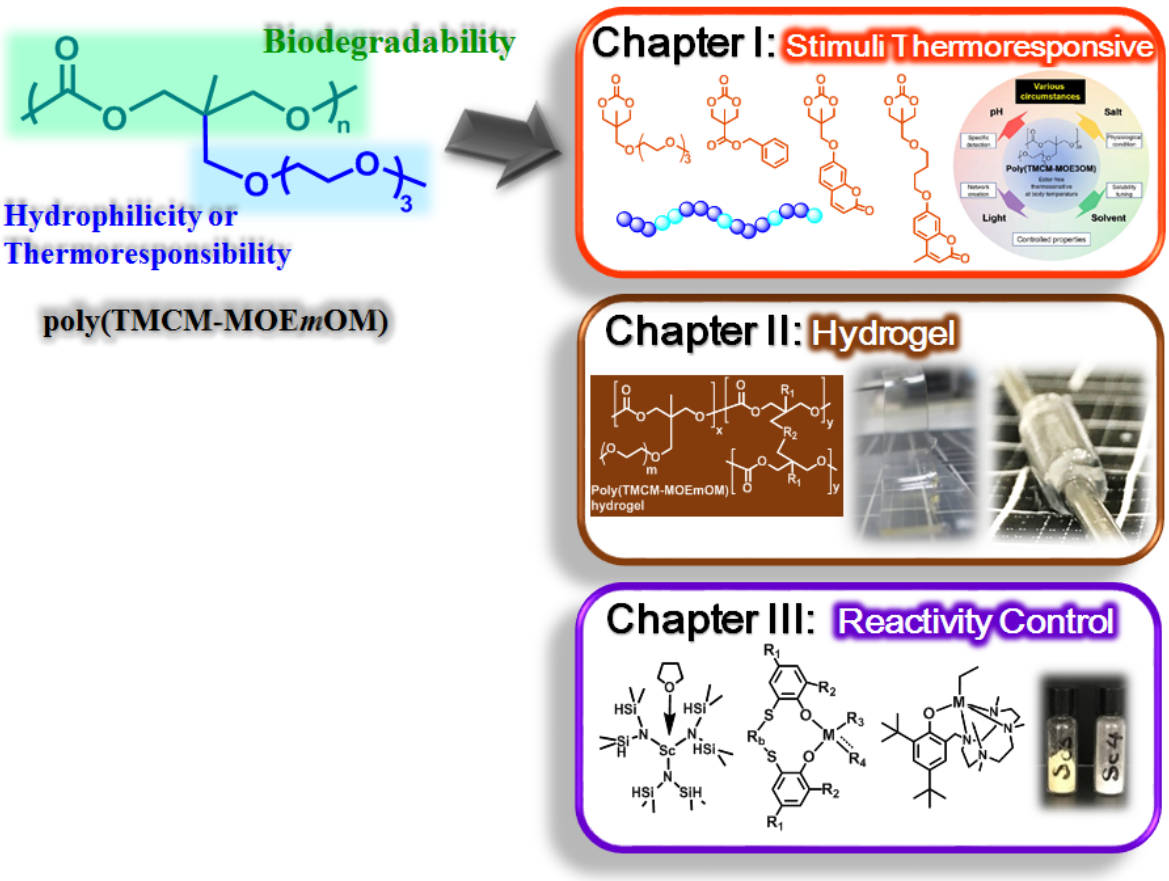


Figure 3. Photodimerization of coumarin moiety[28]

As scope of biomaterials research, biodegradable polymers has become tremendous not only solidification or gel performance but the reactivity control enhancement are also driven in more detailed. For decades, there are growing appeals for ring-opening development, the reaction control is required both of excellent catalyst active and highly selective.[30-31] Regarding to a great cyclic carbonate degradability and biocompatibility, PTMC is one of the most important candidates[32] among ester cyclic polymer such as PLA, CL and so on. Previous studies have emphasized the variety of catalytic compound including enzymatic,[33] metal-based,[34] and organic catalyst especially DBU.[35] In case of organocatalysts mechanism, recent researches suggest that using Tin(II) 2-ethylhexanoate ($SnOct_2$) at high temperature[36] and DBU at ambient could initiated TMC copolymerization with or without alcohol initiator.[37] As far as it is known, organometallic research has investigated many efficiency catalysts for PLA to control stereo selectivity and avoid the transesterification reaction (backbiting).[38] Only a few works demonstrated commercial metal based catalyst initiated to thermoseponsive polymer, PTMCR-MOE*m*OM.

Herein, the objective of the present study is focused on LCST change of thermoresponsive copolymer, hydrogel fabrication and organometallic catalytic reactivity on PTMCM-MOE*m*OM. Our laboratory has preliminarily attempted to examined those important issues and discuss the potential and problematic occurrence. The scope of research studies (Scheme 1) were categorized in 3 chapters as following:



Scheme 1. Overview illustration of TMCM-MOE*m*OM research.

In Chapter 1, TMCM-MOE3OM was used as a principle component along copolymer series. Herein, the series of copolymers with a small additives of various comonomers, including carboxylic acid derivative and coumarin derivatives, were examined for the expanding application under solvent-, pH-, and salt stimuli conditions. Meaningfully, the cloud point or thermal responsive was examined and discussed lower critical solution temperature (LCST) change effects in details in this partition.

In Chapter 2, the novel thermoresponsive hydrogels were prosperously prepared based on poly (TMCM-MOE3OM) with a consistence of TMC biodegradable crosslinked agent. In

principle, TMCM-MOE3OM, and TMCM-MOE4OM monomers were used with the resemble cross linkers BTB and 2,2'-bis(trimethylene carbonate-5-yl)-pentaethylene glycol dimethyl ether (BTP-GDE) to obtain gel via anionic ring opening polymerization in organic solvents, respectively. Thermoresponsive behaviors in linear polymers, as well as volume phase transition temperature (VPTT),[39] were typically observed in swelling ratio. In the aspects of materials characteristic, thermal resistance and mechanical properties were optimized in this chapter. As preliminary biomaterial test, protein adsorption and blood platelet adhesion were also investigated.

In Chapter 3, instead of organo catalyst utilization, organometallic complexes with various classes of structurally ligand such as tris(dimethylsilyl)amido chelating, bis(phenolate) chelating and macrocyclic tetradentate (NNNN)-type cyclen chelating were initiated ROP. Several of metals center catalysts such as rare-earth group Scandium and Lutetium, and metal group such as Magnesium, Tin and Zinc were investigated. Prosperously, PTMCM-MOE3OM could be end up polymerization within 2 hour under neat atmosphere in grove box along simple condition (room temperature) providing PDI below 2. Herein, the preliminary universal screening of inorganic complexes were screened and specific in Zn based and bulky coordinating ligand.

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Chapter 1

Control of Thermoresponsivity of Biocompatible Poly(trimethylene carbonate) with Direct Introduction of Oligo(ethylene glycol) under Various Circumstances

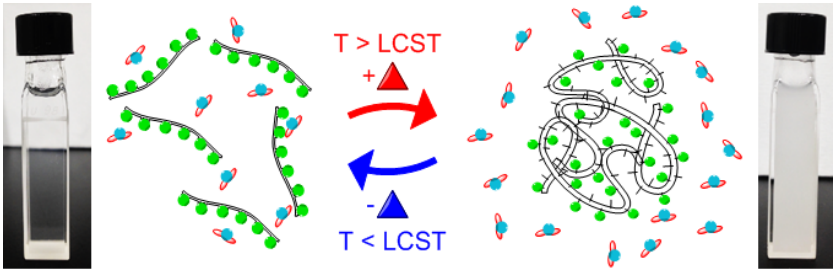
1.1 Introduction

Responsive polymers which respond to environmental stimuli, such as temperature, pH, light, have become an interesting topic in recent years.[1,2] The solubility change of polymers by temperature was recognized as the thermoresponsive polymer, leading to the various application. It is well known that *N*-isopropylacrylamide (NIPAm) uses for the production of various thermoresponsive polymers.[3] Poly(*N*-isopropylacrylamide (PNIPAm) shows a lower critical solution temperature (LCST) around 32˚C which is close to body temperature. Since the LCST changes depending on the concentration, pH or ionic strength, it is essential to clarify the differences and control the temperature for the actual application.[4] Above the LCST, PNIPAm behaves globular structure collapse which shows the cloud point.[5] However, the problematic studies of PNIPAm occurred the strong hysteresis of thermal solubility transition because of intramolecular hydrogen bond, high toxicity and non-biodegradable.[6-9] Another candidate for the switch of thermal property is oxazoline. Poly(2-oxazoline) derivatives with a

substitute molecule at the side chain exhibit LCST transition at a distinct temperature. The LCST of poly(2-oxazaline)s is generally influenced by the molecular weight and the hydrophilic/hydrophobic balancing.[10–16] For example, poly(2-oxazolines)s with *n*-propyl and isopropyl exhibit a LCST at 24 ˚C and 36 ˚C, respectively.[17] At the present, degradable and low-toxic materials with some functions are high demand in medical treatment field. Polycarbonate derivative is one of the candidates to overcome the material problems, because no acidic compounds are generated after degradation, along with their biocompatibility, biodegradability, non-toxicity, and tunable mechanical properties.[18–22] Thus, to design and synthesize biodegradable polymers based on trimethylene carbonate (TMC) considerably required. Poly(trimethylene carbonate) (PTMC) is a well-known biodegradable polymer with an ester-free structure and possessing aliphatic elastomeric polycarbonate as the soft and low T_g segment.[23–26]

Recently, PTMC and its copolymers such as poly(L-lactic acid)-*co*-poly(D,L-lactic acid) (PLDLA) have been investigated along with anionic polymerization for the facile preparation.[27] The influential research to design and develop new polymer with appropriate hydrophilic and hydrophobic balance will show LCST behavior. Therefore, our strategy also focuses on a thermoresponsive biodegradable homopolymer which possesses a cloud point around human body temperature. Due to the hydrophilic moieties, oligo ethylene glycol (OEG) as versatile units are introduced as backbone, grafted onto backbone show the control of cloud

point behavior in aqueous media[28–33] and manipulated the deceleration in lipase solution (pH 7.4).[34] In our previous report, Our laboratory designed a monomer, TMCM-MOE3OM, and its homopolymer, poly(TMCM-MOE3OM) with three repeating units of OEG that exhibited a cloud point range of 31–35 ˚C.[33] It was also observed that a similar design with four units of OEG significantly increased the cloud point up to 72 ˚C. In the next stage for biomedical application, it is necessary to clarify how the OEG units influence thermosensitivity after copolymerization for further functional enhancing. Indeed, there have been a number of important researches focused on the phase transition behavior over the wide temperature range.[10]



Phrase transition change strategy of thermoresponsive polymer

It is important to introduce various stimuli responsivities near the body temperature, to use biomedical application, such as drug delivery.[35] The pH responsivity of deprotonation of carboxylic acid and the charged form[36,37] could be released and accepted, resulting in pH sensitivity. Regarding to electrostatically charge existence, alcohol-included solvent, ions in salt solution, and tuning pH-solution are also important for pharmaceutical system and smart materials.[36,38] In general, pH responsive has been considered in case of cancer or tumor therapy

due to their higher acidity of intramolecular cell at pH 6.2–6.9. Previously, SiO2-PMAA-b-PNIPAM nanoparticles could successfully deliver DOX molecules into the nuclei of Hela cells. The duel responsive nanoparticles approximately 50% of the loaded drugs were released at pH 5 and 25 °C, whereas more than 80% were released at pH 5 and 40 °C.[66] However, it is notable to know the fundamental investigation, our laboratory would like to develop materials in basic study of response and functionality. Therefore, the carboxylate (COO-) has been presented as stimulated bonding of ester group after gelation and further reaction. At the same time with LCST observation, phenomena significantly undergoes the electrostatic interaction, hydrophobic interaction, and solvent interaction participating in the interaction of polymer chain and surrounding water molecules. Recently, it has been reported that the cloud point of a thermoresponsive polymer, PNIPAm, was decreased (fraction of alcohol 0.35 in aqueous) due to the formation of water/methanol clusters via hydrogen bonding.[39–43] Related to control drug dissolving and loading, water/ethanol mixture was reported as useful solvent for well solubility of hydrophobic drugs. Tanaka et al. also suggested that the solvent species contribute to the polymer's hydration, which they called competitive hydration.[44] The effect of competitive hydrogen bonds between polymer-water and polymer-methanol on the downshift of the cloud point has already been reported.[45] Furthermore, there are a variety of UV-light induced reactions such as [2+2] cycloaddition of coumarin, 1,3-dipolar cycloaddition of azobenzene, and [4+4] cycloadditions with anthracenes.[46–48] Light responsive molecules possess the π-π

stacking interaction, coumarin molecules was employed to form physical bonding under irradiation.[49–52] Thus, the composition of copolymer chains are ordinarily considered to control the cloud points for the best values for the application. Based on thermal responsive copolymer or block copolymer, almost previous studies were relate to OEGs response under the external stimuli by introducing large functional composition along the polymer main chain.[36,53–55] Regarding to the previous studies, TMC/PEG di-block copolymer were synthesized by using hydroxyl species such as *m*PEG or cholesterol as initiator.[21,56] Similar temperature-responsive block copolymer were reported to use as drug carrier, micelles,[54] film,[56] and sol-gel.[57] However, all prior studies do not exactly be identical structure to our structures. The backbone of copolymers were distinct when comparing with homo segment of backbone structure. These previously reported functionalized copolymer resulted in the wide range transition of LCST, which are difficult to employ as biomedical materials close to human body temperature range. For that reason, the original sensitivity of copolymer are obliterated. Thus, our noteworthy studies is to synthesize and investigate the stimuli responsive of biodegradable polymer based on TMCM backbone grafting OEGs.

In this study, TMCM-MOE3OM was used as main composition along copolymer chain. Thermal responsive homopolymer, poly(TMCM-MOE3OM) appeared a cloud point[58,59] at around body temperature were studied upon various environment. Herein, homopolymer, as well as copolymer with trivial segment of functional comononers such as carboxylic acid

derivative and coumarin derivatives, were examined under solvent-, pH-, salt stimuli using the homopolymer and copolymers (Figure 1-1).

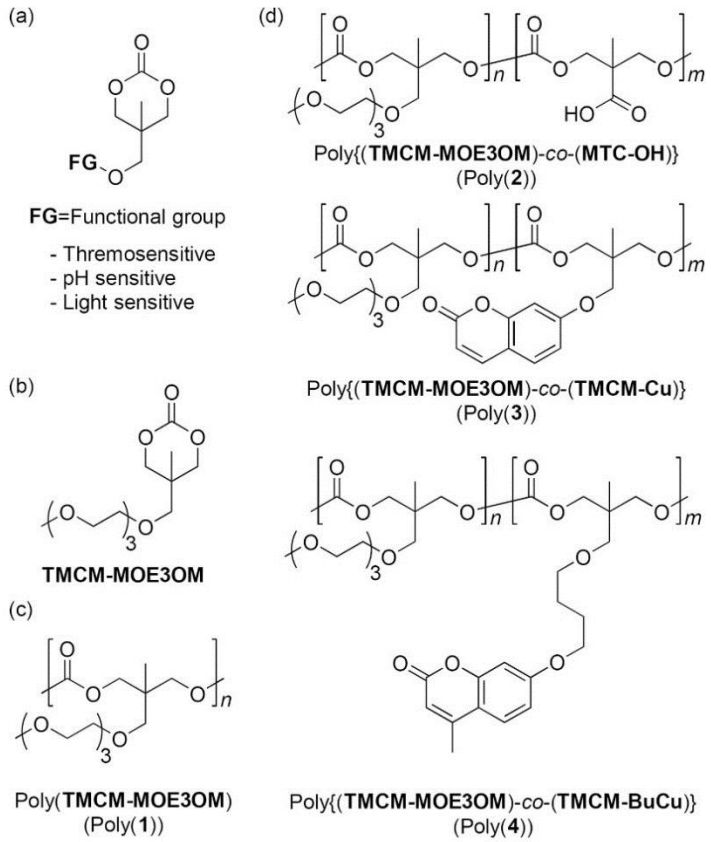


Figure 1-1. Chemical structure of monomer design of TMC derivatives for stimuli-sensitive polymers (a) and TMCM-MOE3OM (b).Chemical structures of thermosensitive poly (**1**) (c) and its copolymers with functional groups (d).

1.2 Experimental Section

1.2.1 Materials

Triethylene glycol monomethyl ether, *p*-toluenesulfonyl chloride, *p*-toluenesulfonic acid, trimethylolethane, 2-methyoxyethyl p-toluenesulfonate, 1,10-carbonyldiimidazole (CDI), acetic acid, potassium carbonate, 1,4 dibromobutane, 4-methylumbeliferone, benzyl alcohol, and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) were purchased from Tokyo Chemical Industry (TCI), Japan. Benzaldehyde and sodium hydride in oil (20%w/w) were purchased from Wako, Japan. Benzyl alcohol and DBU were distilled before used. Anhydrous tetrahydrofuran (THF) and dichloromethane for monomer synthesis, purification, were used and polymerization were distilled with calcium hydride (CaH_2) before use. Unless mentioned, otherwise, all materials were used as received without further purification.

1.2.2 Synthesis of Poly (5-[2-{2-(2-methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one): Poly(TMCMMOE3OM) [Poly(1)]

TMCM-MOE3OM was synthesized same procedure as reported.[33] Polymer was synthesized via anionic polymerization (Figure 1-S1). TMCM-MOE3OM 500 mg (2.45 mmol) was dissolved in 5 mL of anhydrous CH_2Cl_2 and stirred with CaH_2 overnight. Using a cannula with glass filter to remove CaH_2, the monomer solution was transferred to the other flask with three way cock and the solvent CH_2Cl_2 was evaporated under reduced pressure. The monomer solution was added 0.6 mL (0.049 mmol) of benzyl alcohol and 0.6 mL (0.049 mmol) of 1,8-

diazabicyclo[5.4.0]undec-7-ene (DBU) as organic catalyst were added into the flask and started the reaction at room temperature for 8 h. Small amount of acetic acid was added and then the mixture was precipitated into poor solvent of hexane/2-propanol (9/1, v/v). Finally, poly(TMCM-MOE3OM) was recovered by decantation and centrifugation and dried under vacuum (73% yield).

1.2.3 Synthesis of Copolymer TMCM-MOE3OM and 5-Methyl-5-benzyloxycarbonyl-1,3-dioxane-2-one (MTC-OBn)

MTC-OBn was prepared as reported elsewhere.[60] Poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} was synthesized via anionic polymerization (Figure 1-1). TMCM-MOE3OM 500 mg (1.71 mmol) and MTC-OBn 21.4 mg (86.5 μmol) were dissolved in 10 mL of anhydrous CH_2Cl_2 and dehydration with CaH_2 for overnight. The monomer mixture were transferred to the other flask via cannula with glass filter and then, the solvent CH_2Cl_2 was evaporated under reduced pressure atmosphere. Next, 1.78 μL (14.2 μmol) of benzyl alcohol and 2.55 μL (17.2 μmol) of DBU as organic catalyst were added into the flask and started the reaction at room temperature. The polymerization was carried out at 6 days and stopped by adding small amount of CH_2Cl_2, then the mixture was precipitated into poor solvent of hexane/2-propanol (9/1, v/v)

1.2.4 Synthesis of Copolymer of TMCM-MOE3OM and 5-Methyl-5-carboxylic-1,3-

dioxane-2-one (MTC-OH): Poly{(TMCMMOE3OM)-*co*-(MTC-OH)} [Poly(2)]

Poly(**2**) was obtained by removing the benzyl group of poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} (Fig. 1). Poly{(TMCMMOE3OM)-*co*-(MTC-OBn)} 53.6 mg was dissolved in 6 mL of THF and mixed with 10% Pd/C 9.0 mg in 20% $Pd(OH)_2$/C 9.0 mg. The reaction was carried out at room temperature for 24 h to obtain poly(**2**) 40.0 mg (yield 76%).

1.2.5 Synthesis of 7-(5-Methyl-2-phenyl-1,3-dioxane)-hydroxy Coumarin (MPD-Coumarin)

MPD-coumarin was synthesized as similar procedure as MPD-OEG in TMCM-MOE3OM route, [33] except for the tosylation of precursor using 7-hydroxycoumairn. The typical procedure was described as follows: 15 g of 7-hydroxycoumarin (umbeliferone; 92 mmol) was dissolved in anhydrous pyridine (5 mol/L, 18 mL) and kept at 0˚C. A *p*-toluene sulfonyl chloride (17 g, 92 mmol) solution in anhydrous THF (92 mL) was slightly added into the reaction flask and stirred for 23 h. Then, the mixture was extracted with $CHCl_3$ and 0.01 M NaOHaq to recover the MPD-coumarin (21.7 g, yield 75%). The 60% sodium hydride in oil (2.08 g, 52.2 mmol) was placed in a three-necked flask under nitrogen atmosphere and washed by anhydrous THF (10 mL) three times. Anhydrous DMF (20 mL) and anhydrous THF (40 mL) were combined in the flask. As the previous report, [3] 5-hydroxymethyl-5-methyl-2-phenyl-1,3-dioxane (9.88 g, 47 mmol) was introduced with stirring at 0˚C for few hour. After precursor preparation, 7-hydroxy-coumain-*p*-toluenesulfonate (15 g, 47 mmol) was introduced under a

nitrogen atmosphere with stirring at 50 ˚C overnight. The reaction mixture was then extracted by hexane and ethyl acetate (4:1) with water three times to collect the organic layer. The crude mixture was purified by silica gel column chromatography and recrystallized solid [ethyl acetate: hexane (1:6)] to obtain 0.335 g, 20% yield of MPD-coumarin. ^{1}H NMR ($CDCl_3$, 400 MHz) d: 0.96, 1.40 (s, 3H, CH_3C), 3.77 (t, 2H, CH_2OAr), 3.95 (d, 2H, J57.5 Hz, O-$COCH_2$), 4.21 (d, 2H, J57.9 Hz, O-$COCH_2$). 5.51 (s, 1H, CHPh), 6.26–6.28 (d, 1H, aromatic), 6.8–6.9 (m, 2H, aromatic) 7.36–7.41 (m, 5H, Ph), 7.46 (d, 1H, aromatic), 7.5–7.6 (d, 1H, aromatic). FT-IR (cm^{-1}): 3008, 2989, 1747, 1558, 1261, 1027 ESI-MS: $C_{21}H_{20}O_5$ $[M+Na]^+$ m/z observed 375.12.

1.2.6 Synthesis of 7-(2-Methylpropan-diol)-hydroxy Coumarin (Diol-Cu)

MPD-coumarin (0.145 g, 4 mmol) was achieved by refluxing in MeOH (30 mL) and 5 M HCl(aq) (100 mL) at room temperature for 8 hr. The reaction mixture was neutralized by CH_2Cl_2 and ultrapure water to collect the organic layer. The crude mixture was then purified by distillation and then washed with hexane five times to obtain 0.0871 g (80%) of diol-coumarin. ^{1}H NMR ($CDCl_3$, 400 MHz) d: 0.99 (s, 3H, CH_3C), 4.05 (t, 2H, CH_2O), 3.73–3.80 (dd, 4H, J57.2 and 20.4 Hz, O-$COCH_2$), 6.26 (d, 1H, aromatic), 6.87 (m, 2H, aromatic), 7.37(d, 1H, aromatic), 7.64 (d, 1H, aromatic). FT-IR (cm^{-1}): 3448, 3392, 2964, 1701, 1598, 1240, 1012, ESI-MS: $C_{14}H_{16}O_5$ $[M+Na]^{+1}$ m/z observed 287.09.

1.2.7 Synthesis of 7-[(5-(5-Methyl-1,3-dioxa-2-one)methoxy)]-methoxy Coumarin (TMCM-Cu)

Under neat nitrogen atmosphere, an activated molecular sieve (MS4A) and 0.0871 g (3 mmol) of Diol-coumarin was placed in a flask and dissolved in anhydrous CH_2Cl_2 (30 mL). Then, 0.0694 g of CDI (0.4 mmol) was rapidly introduced into the mixture, and stirring was maintained at room temperature for 23 h. After filtration and evaporation, the crude mixture was purified by silica gel chromatography to obtain 0.1067 g of the novel monomer TMCM-coumarin (3.9 mmol, yield 98%).^{1}H NMR (Figure 1-S2) ($CDCl_3$, 400 MHz) d:1.24 (s, 3H, CH_3C), 4.05 (m, 2H, O-$COCH_2$), 4.26 (d, 2H, J57.6Hz, CH_2OCO), 4.46 (d, 2H, J57.2Hz, CH_2OCO), 6.30 (d, 1H, aromatic), 6.84 (m, 2H, aromatic), 7.41(d, 1H, aromatic), 7.64 (d, 1H, aromatic. FT-IR (cm^{-1}): 3082, 2978, 1743, 1714, 1614, 1402, 1234, 1184, 1110. ESI-MS: $C_{15}H_{14}O_6$ $[M+Na]^+$ m/z observed 313.07

1.2.8 Synthesis of 4-Methyl-7-[(5-(5-methyl-1,3-dioxa-2-one)methoxy)butoxy)]-methoxy Coumarin (TMCM-BuCu)

The almost procedure as per the TMCM-coumarin synthesis was repeated, except more one step addition before tosylation of 4-methyl-7 hydroxy coumarin and each steps were purified by silica gel chromatography to obtain each target product. The initial modification of 4-methyl-7-hydroxy coumarin, 1, 4-bromobutane 5.9 mL (27mmol) and 4-methyl-7-hydroxy

coumarin 3.3 g (19 mmol) were dissolved in the acetone. Then potassium carbonate slightly added into the eaction.61 A total of 1.29 g of the novel monomer TMCM-BuCu as obtained 0.0106 g (0.03 mmol, yield 0.7%). ^{1}H NMR (Figure 1-S3) ($CDCl_3$, 400 MHz) d:1.08 (s, 3H, CH_3C), 1.75 (m, 1H, CH_2CH_2O), 1.88 (m, 1H, CH_2CH_2O), 2.39 (s, 3H, CH_3-aromatic), 3.31 (t, 6H, J 0.8 and 6.8 Hz, CH_2OCO), 3.51 (t, 2H, J 5.6 and 5.8 Hz, CCH_2O), 4.31 (s, 2H, CH_2OCO), 6.13 (s, 1H, aromatic), 6.79 (d, 1H, aromatic), 7.50 (d, 1H, aromatic). FT-IR (cm^{-1}): 2927, 1732, 1651, 1558, 1458, 1010, ESI-MS: calculated for $C_{20}H_{24}O_7$ $[M+H]^+$ m/z observed 377.17, $[M+Na]^+$ m/z observed 399.14.

1.2.9 Synthesis of Copolymers of TMCM-MOE3OM and TMCMCu or TMCM-BuCu (Poly(3) and Poly(4))

The copolymer of TMCM-MOE3OM and TMCM-coumarin derivatives were proceeded via ring-opening polymerization using organo catalyst DBU as a catalyst and benzyl alcohol as an initiator. After the polymerization, the reaction mixture was poured into hexane/isopropyl alcohol (9/1, v/v). The insoluble part was collected as a copolymer of TMCM-MOE3OM and TMCM-coumarin derivatives in various component depending on the coumarin composition along the polymer chain. The coumarin moiety of copolymerization were label 1% TMCM-Cu, 10% TMCM-Cu, and 2% TMCM-BuCu.

1.2.10 Characterization

^{1}H NMR spectra were recorded on JEOL JNM-ECX400 operating at 400 MHz. $CDCl_3$ and DMSO-d6 were used as solvents and the chemical shifts were calibrated against residual solvent signals. The molecular weight and polydispersity of the copolymers were determined by ChromNAV system (JASCO Corporation, Japan). Gel permeation chromatography instrument equipped with two linear PL gel columns following a guard column (AS-2055) and a differential refractive-index detector (RI-2031). The measurements were performed using $CHCl_3$ as the eluent for poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} and poly(**2**) at a flow rate of 0.5 mL/min at 30 ˚C and THF as the eluent under PMMA standard. Poly(**3**) and poly(**4**) at flow rate of 0.5 mL/min at 40 ˚C under series of narrow polystyrene standards for the conventional calibration were investigated. The turbidity of polymer were observed by UV-2600 spectrophotometer (Shimadzu S-1700) at 500 nm wavelength. Cloud points were determined by transmission changes by UV–Vis spectrometer at 50% transmission.

1.3 Results and Discussion

All thermoresponsive polymer were successfully synthesized and randomly copolymerized under ring opening polymerization by organo catalyst. The characteristics of all carbonates backbone derivatives were shown in Table 1-1. To observe chain length of the copolymer with benzyl group and carboxylic side group were determined with PMMA standard in $CHCl_3$. PDI of poly(**2**) was occurred the larger PDI (wide distribution) around 1.9 and trivial M_n decreasing. Due to charge interaction in column compared with another polymers. Moreover, the degree of polymerization (DP) may be defined as the average number of repeat units per polymer chain. The molecules are composed of regularly repeating units, or as the average number of co-monomer per molecule where the identical monomers could be determined in specific region in ^1H NMR spectroscopy.

The aim of this study was to control the thermosensitive properties of poly(TMCM-MOE3OM) and its copolymers for the possible medical applications. The author investigated the solvent effects, pH effects, and the light irradiation effects, which include stimuli-responsive OEG moieties. The components were described the result as follow.

Table 1-1 PTMC derivatives in this study.

Run	Polymer	Comonomer Feed (mol %)	Yield (%)	M_n[a] (g/mol)	PDI[a]	DP[b] of TMCM-MOE3OM	DP[b] of comonomer
1	Poly(**1**)	0	97	11,000	1.36	38	ND
2	Poly(**1**)	0	67	5000	1.25	17	ND
3	Poly(**2**)	5	76	1900[c]	1.90	11	0.5
4	Poly(**3**)	1	96	6200	1.31	21	0.6
5	Poly(**3**)	10	50	6500	1.26	16	0.5
6	Poly(**4**)	2	83	2800	1.50	1.3	7.7

[a] Determined by SEC in THF with polystyrene standard at 40 °C at 0.6 mL/min.
[b] Monomer unit number in copolymer determined by ^{1}H NMR.
[c] Determined by SEC in $CHCl_3$ with poly(methyl methacrylate) standard at 40 °C at 0.6 mL/min.

1.3.1 Solvent Effect

At first, the solvent effects on thermosensitive property was investigated using homopolymer [poly(**1**)] (Figure 1-1). Taking the biomedical application into account, the author selected phosphate buffer solution (PBS) and ethanol as organic solvent initially. The polymer was evaluated by the cloud point of the solutions, as shown in the transmittance against temperature (Figure 1-2). The cloud point in PBS of poly(**1**) (M_n 7400 g/mol) showed at 30 ˚C (Figure 1- 2(a)), while it was previously reported that it was at 33 ˚C in ultrapure water (Figure 1-2(b)).[33] The low temperature of the cloud point in the ionic solution is well known phenomena of salts in PBS are salting-out ions.[62] In case of alcohol, both ethanol and methanol were good solvents for poly(**1**), I anticipated that the hydroxyl group also increased more solubility of the polymer at ratio water/ethanol (9/1, v/v). Hence, the cloud point of polymer in mixture of alcohol was examined at 38 ˚C as shown in Figure 1-2(c), although it shows no cloud point in both methanol and ethanol, maintaining soluble state. Due to the OEG as hydrophilic group, OEG practically surrounded the water molecules to occur the phenomena. While, the PBS

solution with plenty of ions, the solubility slight decreased by the effect of ionic interference. In previous report, cloud point also depends on concentrations, molecular weight, and polymer end group.[33] The results clarified the cloud points under the possible biomaterial applications are available around body temperature. Moreover, the result of the salt environment, it was shown that the increased concentration of aqueous NaCl affected to decrease cloud point tendency.[62] The evidence of ionic effect was shown customarily in Figure 1-3.

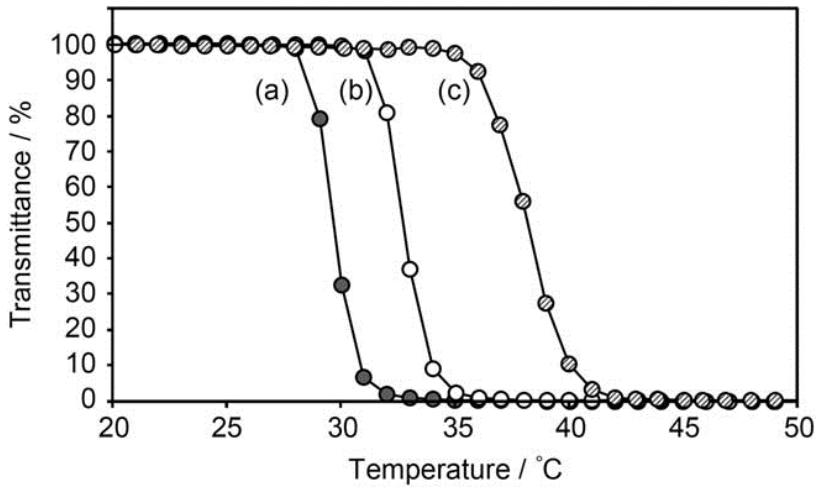


Figure 1-2. Transmittance *versus* temperature plots of poly(**1**) solutions at 2.0 mg/mL in PBS (a), water (b), and water/ethanol 9/1 (v/v).

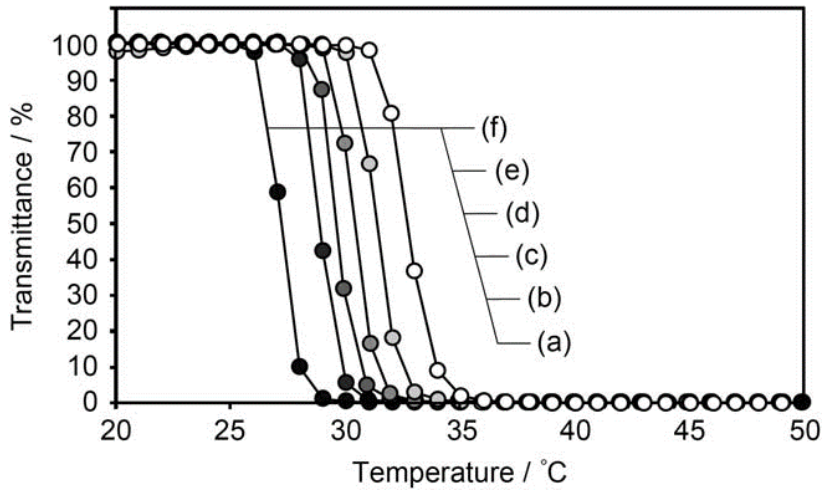


Figure 1-3. Transmittance versus temperature plots of poly(**1**) solutions at 2.0 mg/mL in various concentration of NaCl solution with 0 M (a), 0.05 M (b), 0.10 M (c), 0.15 M (d), 0.20 M (e), and 0.30 M (f).

When increasing the concentration of salt solution, which were 0.05 M (Figure 1-3(b)), 0.10 M (Figure 1-3(c)), 0.15 M (Figure 1-3(d)), 0.20 M (Figure 1-3(e)), and 0.30 M (Figure 1-3(f)), the cloud point trendily were sharp and decreased to 32 ˚C (Figure 1-3(b)), 30.5 ˚C (Figure 1-3(c)), 29.5 ˚C (Figure 1-3(d)), 29 ˚C (Figure 1-3(e)), 27 ˚C (Figure 1-3(f)), respectively. Regarding to the diminishing of cloud point, the effect of ionic strength could be confirmed by the change of solubility of polymers with PEGs. The same ionic strength at 0.15 M in PBS (Figure 1-2(a)) and NaCl aqueous solution (Figure 1-3(d)) resulted in the same cloud points, showing that the change of the cloud points was not influenced by ionic species.

The purpose of this research was investigation of thermosensitivity of ester free PTMC derivatives for the possible biomedical application. The cloud point of ethanol also indicated the good circumstance when body uptake the alcohol, whereas polymer still not be dramatically changed. All determination of various environment, showed clearly solubility around room

temperature and body temperature. Our laboratory highly recommended and attempt to develop poly(**1**), poly(TMCM-MOE3OM), as biomaterials in the future.

1.3.2 pH Effect

When the poly(**1**) would be used as biomaterials, additional functionality might be important, such as crosslinking moiety. When the reacting moiety was introduced into poly(**1**), the original cloud point should move due to the change of hydrophilic and hydrophobic balance. Thus, at first the author has a motivation to examine the pH effect against thermosensitive behavior of poly(**1**), because of the carboxylic acid group is one of the options as reacting moiety to achieve the crosslinked network. Therefore, our laboratory selected the copolymerization between TMCM-MOE3OM and MTC-OBn as shown in Figure 1-4. Then, poly(TMCM-MOE3OM-*co*-MTC-OBn) was removed benzyl group and immobilized acid to obtain poly(TMCM-MOE3OM-*co*-MTC-OH) [poly(**2**)] in ambient condition and THF as shown in Figure 1-1. Yield of poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} and poly(**2**) were 76 and 46%, respectively, indicating almost successful modification acidic group from the benzyl group. To confirm the structure, ^{1}H NMR were indicated the position of proton as the Figure 1-4. It was confirmed the successful preparation of poly(**2**) by the disappearance of the singlet at 5.2 ppm and multiple peaks of phenyl around 7.3 ppm (Figure 1-4(c)) compared with that of the precursor, poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} (Figure 1-4(a) and Figure 1-4(b)).

To introduce the function at the end of side part obviously appeared the sensitivity, copolymer, which are based on TMCM chain containing OEG. Previously, poly(**1**) exhibits cloud point at about 33˚C,[33] the hydrophobicity of the copolymer is enhanced by poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} by the side chain benzyl group. Then, the cloud point shifted to 27˚C at heating process (Figure 1-5(a)). In the case of poly(**2**), carboxylic function is presented and affected to the polymer solubility in each condition. The hydrophilicity of copolymer was increased because of acid group, cloud point moved to higher at 42˚C (Figure 1-5(c)). In addition, measurement of poly(**2**) under the acidic environmental at pH 2 showed cloud point shifted to the lower temperature side and showed about 33˚C (Figure 1-5(b)). The reason could explained in term protonation and deprotonation of COOH. The dissociated constant of carboxylic group is normally form carboxylate ion (COO^-) around pH 3 - pH 6. For example, acetic acid and lactic acid, which contained COOH group, the COO^- ion express when pH more than 4.8 and 3.8, respectively. Therefore, the synthetic copolymer, poly(**2**) at pH 7 formed the deprotonate state (COO^-). Then, the hydrophilicity was high and influenced to the solubility (cloud point at 42˚C) as shown in the (Figure 1-5(c)). However, the protonation form (COOH at pH 2) increased the hydrophobicity, which depicted the cloud point around 33 ˚C (Figure 1-5(b)). As the result, it was found that changing the kind of comonomer units provides a broad range of LCST distribution (Figure 1-5(a) and 5(c)) both above and below the original characteristic (Figure 1-5(b)). Depending on the interference, hydrophobic/hydrophilic

moieties interact with the hydrogen bonding of main polymer chain or induce the possibility of coacervate molecule and extend broaden range of thermoresponsive property.[35] Therefore, the fundamental studies of copolymer based on TMCM-MOE3OM and containing carboxylic group exhibited the pH sensitivity as the effect of charge[62] and anticipated the future reactions and the application. However, since it was possible to introduce a highly reactive carboxyl group into the side chain, I also expected to further functional modification. Moreover, the copolymer carboxylate form at pH 7 was proposed to be use as the ester model for the further gelation in the future.

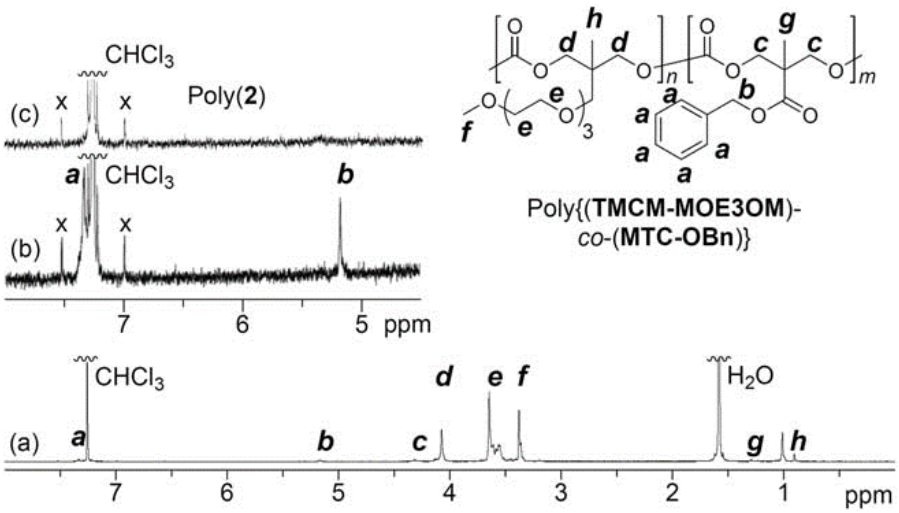


Figure 1-4. ^{1}H NMR spectrum of poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} (a). The expanded spectra of poly{(TMCM-MOE3OM)-*co*-(MTC-OBn)} (b) and poly(**2**) (c) (400MHz, r.t., in $CDCl_3$).

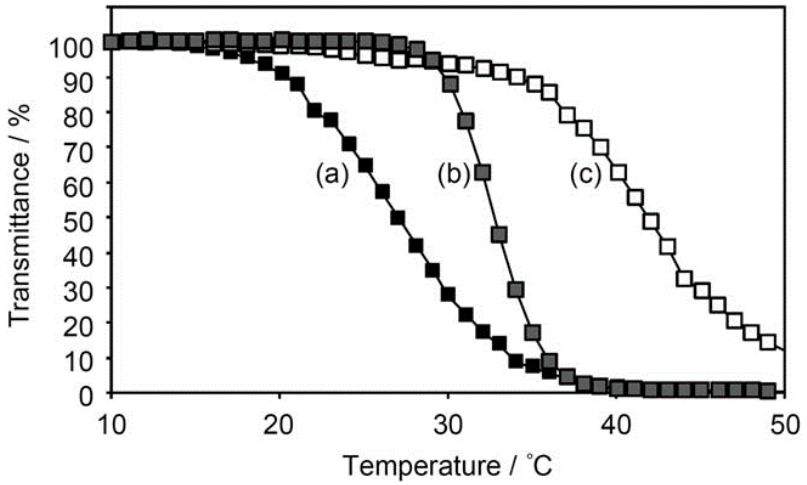


Figure 1-5. Transmittance versus temperature plots of poly(TMCM-MOE3OM-*co*-MTC-OBn) (a), poly(**2**) in 0.01 M HCl (b), and poly(**2**) in ultrapure water (c) at 2.0 mg/mL in heating processes.

1.3.3 Light Effects

To observe the interaction of functional group along the polymer chain, the poly(**3**), which include photo responsive unit with two different introduction by 1 and 10% by weight of TMCM-Cu were shown in Figure 1-1. The average molecular weight of poly(**3**) with (coumarin unit) ratio at 1 and 10% were determined, M_n 6200 g/mol (PDI 1.31) and M_n 6500 g/mol (PDI 1.26), which were analyzed by ^{1}H NMR spectra (Figure 1-S2), respectively. The different amount of photoresponsive moiety indicated the different cloud point because of the difference of hydrophobicity of 7-hydroxycoumarin moieties as shown in (Figure 1-6). According to the balance of the hydrophobic and hydrophilic moiety, coumarin derivative was classified in the hydrophobic part when comparing with homopolymer, poly(**1**) (Figure 1-6(a)). Introduction of 1% of coumarin moiety, the cloud point of poly(**3**) was decreased a degree to 32 ˚C (Figure 1-6(a)), while 10% introduction shifted to 24 ˚C (Figure 1-6(c)). Furthermore, the copolymer, poly(**3**) was measured about the cloud point after irradiating with UV (>280 nm) as shown in

Figure 1-6(d). The cloud point exhibited at 31 ˚C, which occurred because of the dimerization of copolymer (Figure 1-6(e)), evidenced by its UV spectra (Figure 1-S5). The author implied one possibility to obtain trivial hydrophilic by-product of coumarin moiety which affect to LCST after UV irradiation. The first hypothesis of synthesis is introducing more hydrophobic coumarin as poly(**3**). In addition, the percentage of coumarin along the polymer chains were almost similar 1% and 2% for poly(**3**) and poly(**4**), respectively. The result was perhaps anticipated to show the resemble cloud points due to adjacent value. However, the previous studies significantly reported the cloud point could be readily affected by end group and copolymerization. Therefore the molecular weight of poly(**3**) and (**4**) were quite different and the author agreed with the hydrophilic terminated chain end also play the role in thermoresponsibility over the structure and quantitatives.[53,58]

TMCM derivatives show a reversible hysteresis of thermal responsive (miscible-to-immiscible phase transition) in water media with 4 cycles in Figure 1-S4. Furthermore, to confirm the dimerization of coumarin moiety properties, the time dependence of UV absorbance at 500 nm of poly(**3**) were monitored after irradiation (>280 nm) (Figure 1-S5). The gel formation was avoided due to the low percentages in the polymer main chain, and the soluble polymer was successfully analyzed in this study.

Interestingly, the most closest to body temperature of cloud point was achieved at 36˚C as shown in Figure 1-6(e), when the coumarin moiety ratio at 2% was selected for poly(**4**) with

M_n 2800 g/mol (PDI 1.55), which was also analyzed by ^{1}H NMR spectra (Figure 1-S3). The buthyleneoxy spacer obviously increased the LCST values that including more hydrophilic part similar to the previous report.[65] This means the introducing unit ratio and molecular weight is effective to control the cloud point for ester free PTMC derivatives. It is noteworthy that the result also showed as the preliminary report for the tunable and controlled balance unit under light trigger, although the cloud point of dimer close to 1% coumarin and homopolymer poly(**1**). The low molecular weight of each polymer were clarified via ring open polymerization of TMCM-MOE*m*OM derivatives due to the sensitive anionic polymerization. Anion species occurred during the polymerization as Amsden group reported.[63,64] Especially, autopolymerization by DBU or polymer itself indicated the back biting reaction, which induced the low molecular weight of homopolymer.

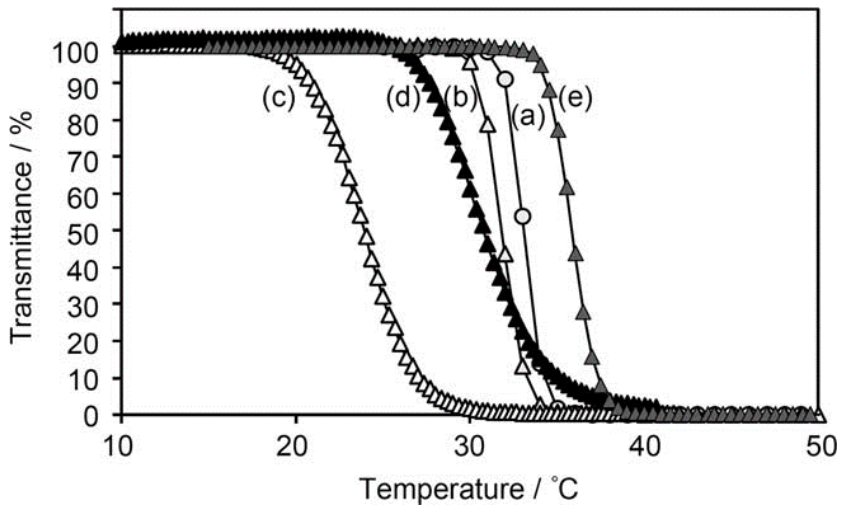


Figure 1-6. Turbidity plots in water upon heating solution 2.0 mg/mL for poly(**1**) (a), poly(**3**) with 1% (b) and 10% (c) coumarin moieties, poly(**3**) with 10% coumarin moiety after UV irradiation (d), and poly(**4**) with 2% coumarin moiety (e).

Poly(**1**) shows its thermosensitive property at body temperature with ester-free structure, strongly suggesting the possible usage for biomedical application. In order to design the actual polymer materials, the author demonstrate the sustainable key property of poly(**1**) after copolymerization with functional groups (Figure 1-7). Our group are currently designing the possible material application using poly(**1**) as a core material.

1.4 Conclusions

We designed thermo-, solvent-, pH-, and photoresponsive copolymers based on TMCM-MOE3OM unit, which produce ester free biodegradable thermosensitive polymers, in order to use the possible biomedical application. We revealed the conditions which the cloud points shows near to body temperature, using poly(**1**) and its copolymers, indicating that the various possible functionalization in the range of the room temperature and body temperature. Cloud points of poly(**1**) ranged between 27˚C and 38˚C, when PBS, alcohol, and NaCl were employed. Poly(**2**) process the pH responsibility under acidic (pH 2) and neutral (pH 7) condition which is 29-32˚C of cloud point range. Poly(**3**) and poly(**4**) exhibit the photoresponsibility due to the photo-induced dimerization (cyclobutane) under UV irradiation. All result are determined the measurement of the turbidity as cloud point temperature. This demonstration is expected to profit the further synthesis and application for biomedical application.

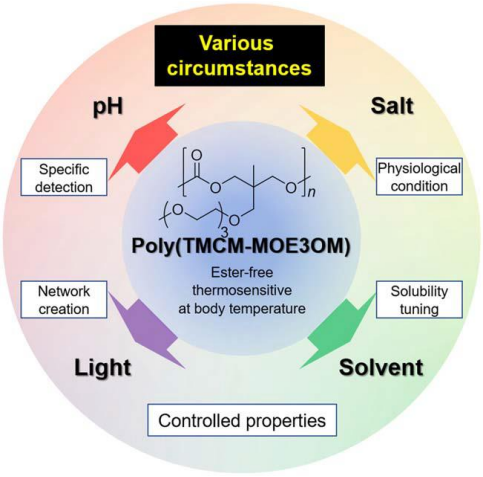


Figure 1-7. Schematic illustration of controlled properties of poly(TMCM-MOE3OM) [poly(**1**)] under various conditions.

1.5 Supplementary Materials

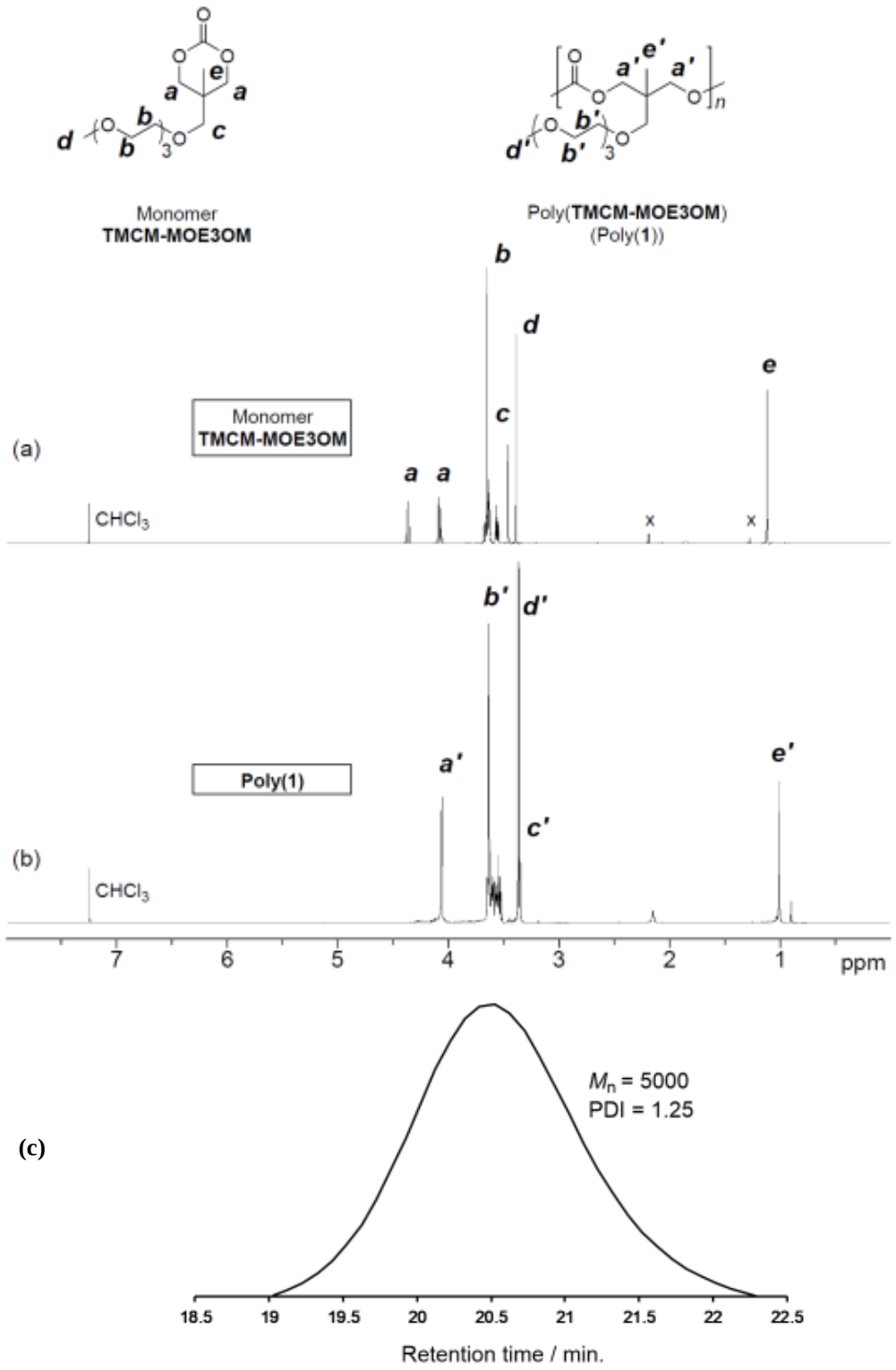


Figure 1-S1. ^{1}H NMR spectra of monomer **1**(a) and poly(**1**) (b) in this study (400MHz, r.t.). M_n = 5,000 g/mol, PDI = 1.25. The molecular weight is determined by GPC using THF as solvent with polystyrene standard at 40˚C flow rate 0.6 ml//min (c).

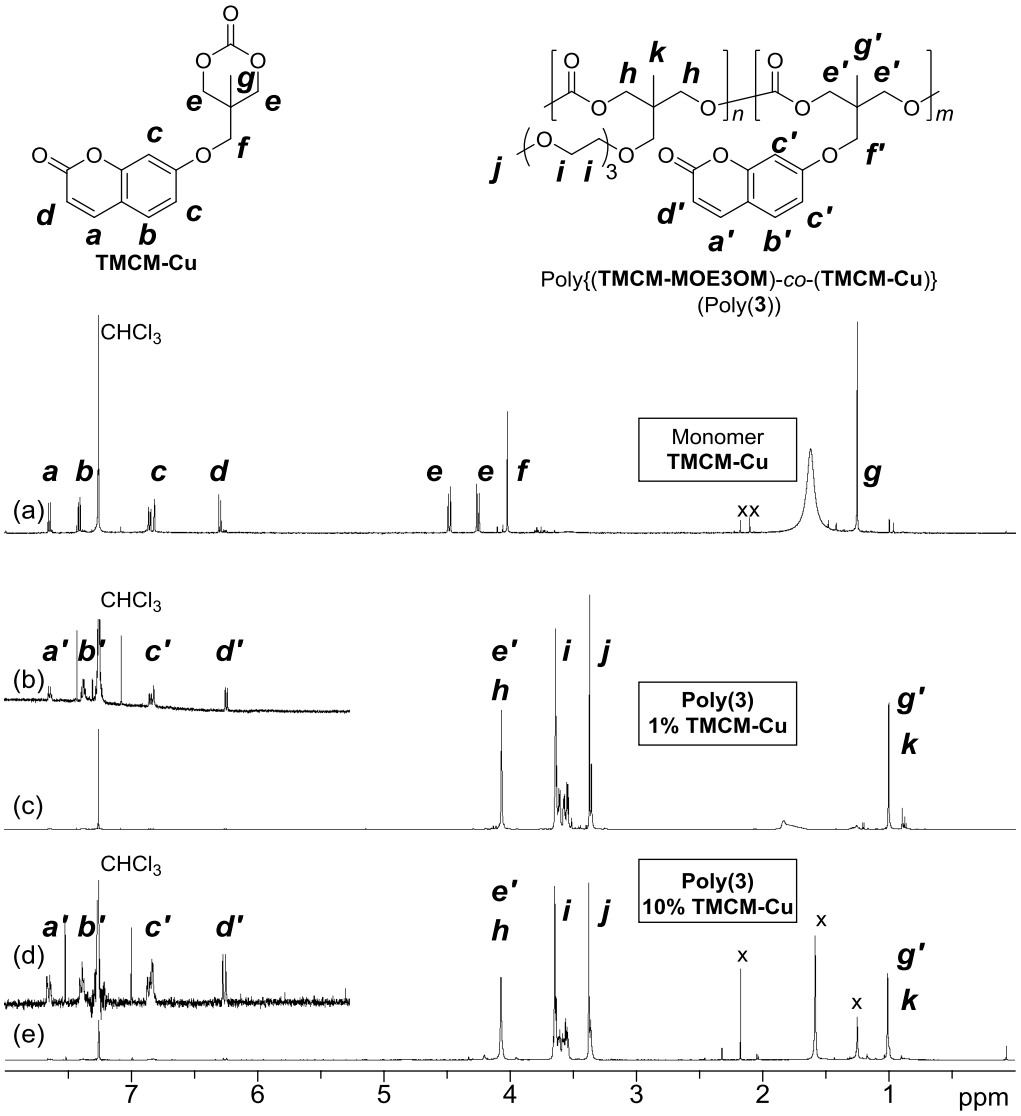


Figure 1-S2. ^{1}H NMR spectra of monomer TMCM-Cu (a), the expanded poly(**3**) with 1% coumarin moiety (b), poly(**3**) with 1% coumarin moiety (c), the expanded poly(**3**) with 10% coumarin moiety (d), and poly(**3**) with 10% coumarin moiety (e) in $CDCl_3$ (40MHz, r.t.)

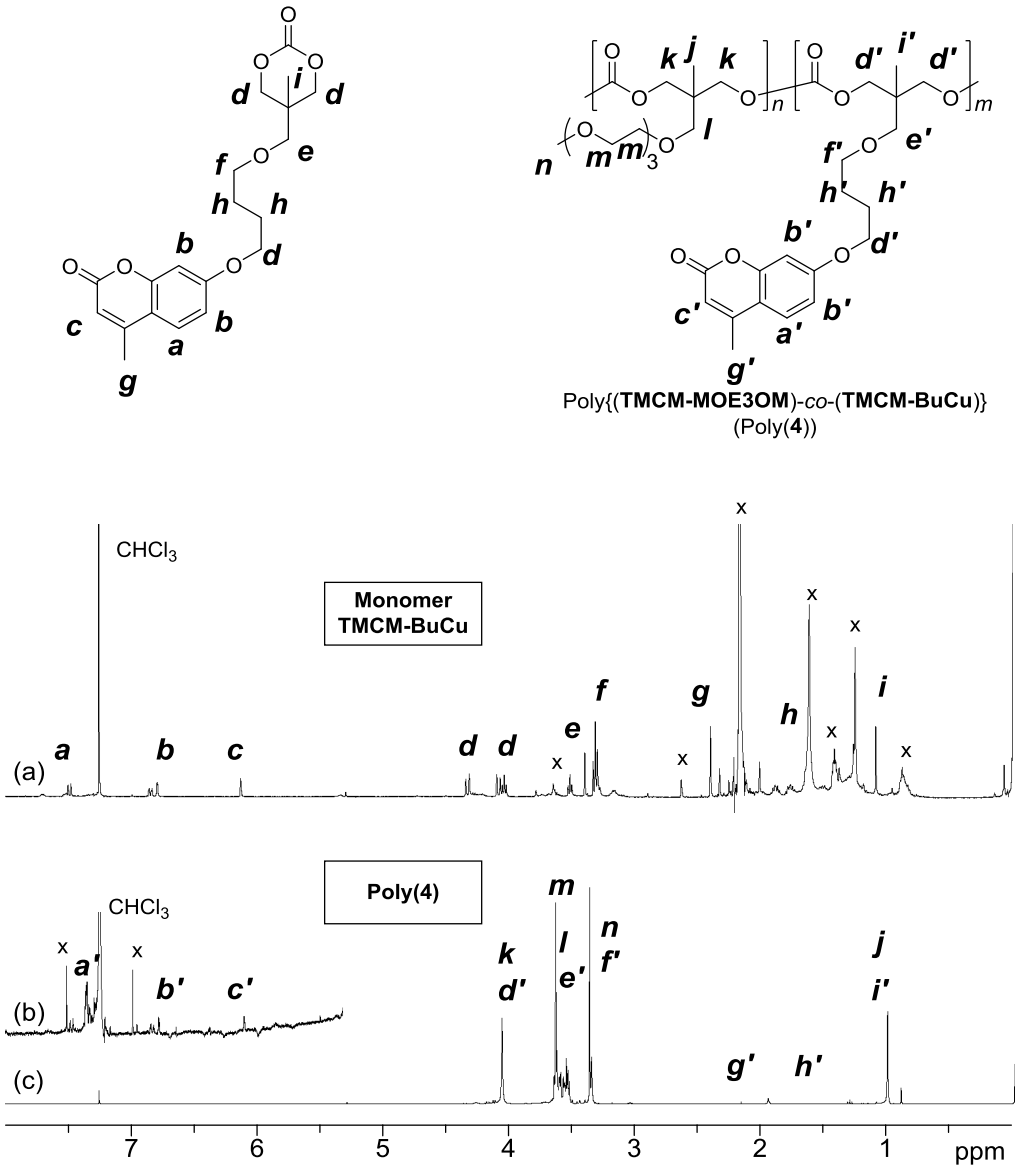


Figure 1-S3. ^{1}H NMR spectra of monomer TMCM-BuCu (a), the expanded poly(**4**) and the poly(**4**)(c) in $CDCl_3$ (400MHz, r.t.)

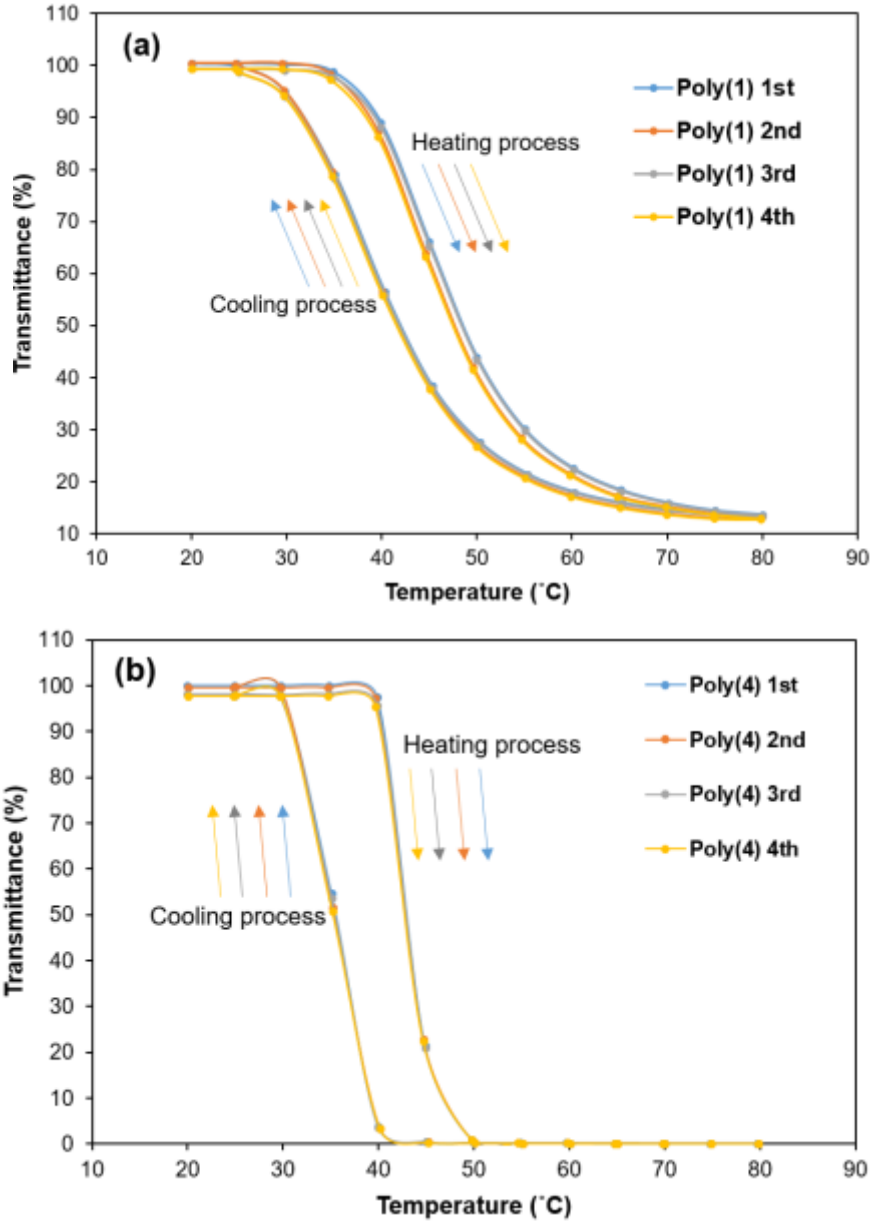


Figure 1-S4. Turbidity measurement of aqueous solutions upon heating and cooling processes at 5˚C/min. (4 reversible cycles) of poly(**1**) (0.5 mg/mL) (a) and poly(**4**) (1.0 mg/mL) (b).

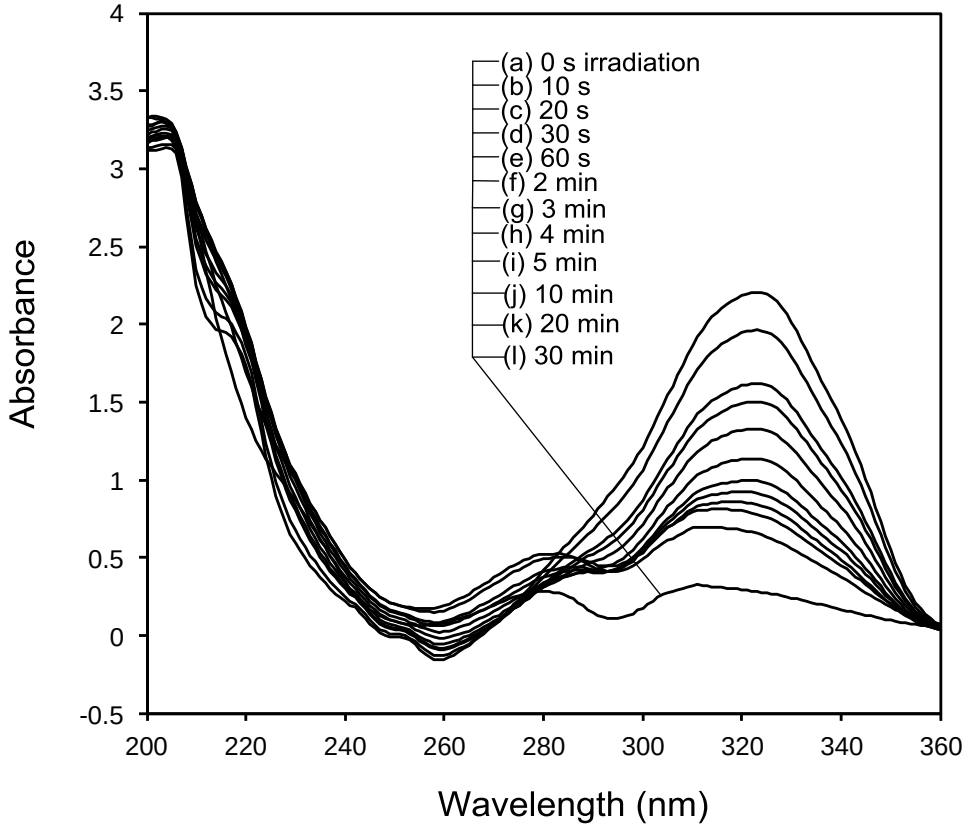


Figure 1-S5. UV spectra of poly(**3**) after UV irradiation.

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Chapter 2

Preparation of Thermosensitive Biodegradable Hydrogel Using Poly(5-[2-{2-(2-methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one) Derivatives

2.1 Introduction

Annually, number of patients who suffer with cardiovascular increase especially blood vessel disorder and was the most underlying cause of death.[1] For potential diagnosis, stent treatment and long implantation therapy,[2-4] the materials with compatible contact between artificial materials and blood platelet are produced.[2] Three-dimensional crosslinked structure as hydrogel with large amount water as soft materials are widely developing for clinical application.[5] Natural source benefit in biological function such as collagen-binding integrin study[6] and cell immigration on various collagen[7], or synthetic hydrogels such as copolymer injectable hydrogel of hyaluronic acid-*g*-poly(*N*-isopropylacrylamide) (AHA-*g*-PNIPAm),[8] chitosan crosslinked glutaraldehyde hydrogel[9], and poly(ethylene glycol)/1,3-dioxan-2-one derivative (PEG/PTMC) hydrogels with bis-carbonate poly(ethylene glycol) macromonomers[10] are possible fabricated under the key requirements of clinical hydrogels which own biocompatibility, biodegradability, mechanical strength, water consumption and stimuli

responsibility.[11] However, due to the limitation of the weakness of natural hydrogel such as collagen[12] or silk[13], the well-known stimuli responsive polymers as synthetic homopolymer, such as poly(vinylcaprolactame) (PNVCL),[14] poly[(oligo(ethylene glycol) methacrylate)] (POEGMA),[15] poly(methyl vinyl ether) (PMVE),[16] and copolymer of poly(*N*-acryloyl-*N*-propylpiperazine) (PAcrNPP),[17] and poly(*N*-isopropylacrylamide) (PNIPAM) have been studied.[18] PNIPAM is one of the most particularly investigated to form hydrogel including thermal responsive at around body temperature.[18] Notwithstanding to that external stimuli response, the drawbacks of biomedical application are toxic and non-degradable based-polymer.

Another well-known aliphatic type of degradable polymers are poly(glycolic acid) (PGA),[19] poly(carprolactone) (PCL),[20] poly(lactic acid) (PLA),[20,21] poly(trimethylene carbonate) (PTMC),[21] and so on. PLA are the most dominant candidate employed for biomaterials as its stiffness, and biocompatibility. Furthermore, the copolymer of lactide with carpolactone[22] and PTMC elastomeric[23] comonomer were investigated on their mechanical control and biodegradable polymer of corresponding structure. However, it is still unclear that the degradation residue from PLA influenced as possible inflammatory. That could be cause the acidic compounds generation due to the ester groups after degradation. While PTMC degradation has been reported under enzymatic acceleration of hydrolysis,[24] PLA was hydrolyzed under both acidic and enzymatic conditions. The copolymerization of trimethylene carbonate (TMC) and caprolactone (CL) were studied in case of mechanical properties,

biological and degradation as novel biomaterials.[25]

Recently, stimuli-responsive synthetic polymers, have been also attracted diversely in actuators,[26] drug delivery,[27] and bio-mimic application.[28] Oligo (ethylene glycol) (OEG) is hydrophilic molecule with its proven high water-solubility, non-cytotoxicity, and biocompatible group. Therefore, it was occasionally reported that the attempting to improve ductility of PLA modified with PEG unit as a pendent group[29] and grafted on material surface[51] as inhibitor function of proteins as good biomaterials. In order to control biomaterial properties, OEG group is important for the new synthetic polymer.

On the other hand, PTMC is a well-known as ester-free based biodegradable polymer as soft segment and facile preparation via anionic ring opening polymerization.[21,24] Due to the significant limits in low mechanical performance of the homopolymer, PTMC, its applications and consequently, several copolymers were developed with the other cyclic lactones.[20] Our group has already reported the modified TMC derivative incorporating of OEG as 5-[2-{2-(2-methoxyethoxy)ethoxy}ethoxymethyl]-5-methyl-[1,3]-dioxa-2-one (TMCM-MOE3OM).[30] Poly(TMCM-MOE3OM) provided light-yellowish viscous liquid with insufficient mechanical strength,[30] while itself providing slow lipase degradation.[31] Aiming to use the polymer as biomaterials, it is essential to enlarge the mechanical strength. So, both of photo crosslinker and chemical crosslinker have been considered. The copolymerization by photo crosslinkers[32,33] was assessed in the previous work with insufficient dimerization linkage,[34] but the addition of

chemical cross-linked unit, the deformation and defect could be better gel formation approach. Upon the chemical crosslinkers, six-membered bis- and tris-(cyclic carbonate)s have been reported.[35] Therefore, I move to select interestingly bis(cyclic carbonate) crosslinker which has similar to TMC precursor[36] for expectation less fluctuated property of gel.

In this study, poly(TMCM-MOE3OM) gel was prepared with the biodegradable crosslinked network for the biomedical application in which volume phase transition temperature (VPTT) at around body temperature would be effective, such as blood diesease.[37,38] For the gel formation, TMCM-MOE3OM and 5-(2-[2-{2-(2-methoxyethoxy)ethyoxy}ethoxy]-ethyoxymethyl)-5-methyl-1,3-dioxa-2-one (TMCM-MOE4OM) as monomers with two kind of crosslinker, such as 2,2'-bis(trimethylene carbonate-5-yl)-butylether (BTB)[36] and 2,2'-bis(trimethylene carbonate-5-yl)-pentaethylene glycol dimethyl ether (BTP-GDE), were used for anionic ring opening polymerization as illustrated in Figure 2-1. Typical characterization has been achieved with swelling ratio, thermal resistance, mechanical properties, and so on. Especially, preliminary protein adsorption and blood platelet adhesion were investigated.

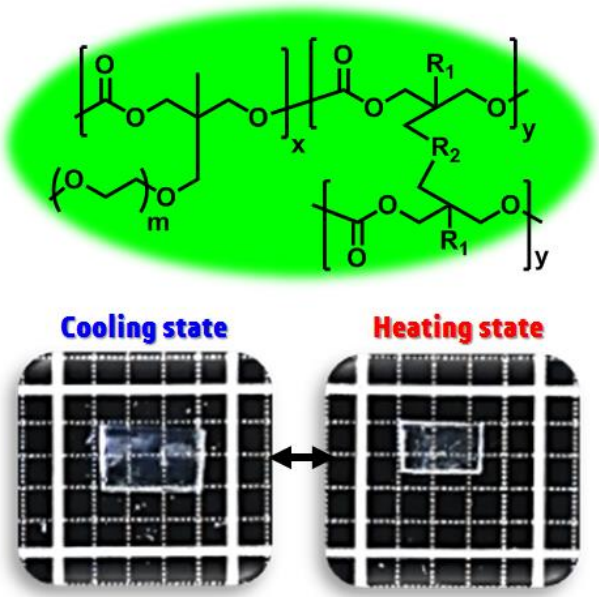

Figure 2-1. Thermoresponsive poly(TMCM-MOE3OM) hydrogel possessing temperature responsive.

2.2 Experimental Section

2.2.1 Materials

Triethylene glycol monomethyl ether, tetraethylene glycol monomethyl ether, pentaethylene glycol, *p*-toluenesulfonyl chloride, *p*-toluenesulfonic acid, trimethylolethane, 2-methyoxyethyl *p*-toluenesulfonate, ethyl chloroformade, acetic acid, potassium carbonate, di(trimethylolpropane), benzyl alcohol, 1,8-diazabicyclo[5.4.0]-7-undecene (DBU), *N*,*N*'-dicyclohexylcarbodiimide (DCC), benzyl bromide (BnBr) were purchased from Tokyo Chemical Industry (TCI), Japan. 2,2-Bis(hydroxymethyl)propionic acid (HMPA), palladium on carbon were purchased from Sigma-Aldrich. Benzaldehyde and sodium hydride in oil (20%w/w) were purchased from Wako, Japan. Benzyl alcohol and DBU were distilled before used. Anhydrous tetrahydrofuran (THF) and dichloromethane for monomer synthesis,

purification, were used. Pierce™ BCA Protein Assay Kit was purchased by Thermo Fisher scientific. Bovine serum albumins (BSA), bovine gramma globulin (BGG) and bovine plasma fibrinogen (BPF) were purchased from Sigma-Aldrich. Unless mentioned, otherwise, all materials were used as received without further purification.

2.2.2 Synthesis of TMCM-MOE3OM and TMCM-MOE4OM

TMCM-MOE3OM and TMCM-MOE4OM were synthesized following our group procedure as previously reported.[30]

2.2.3 Synthesis of 2, 2'-bis(trimethylene carbonate-5-yl)-butylether (BTB)

Crosslinker BTB was synthesized according to the reference by David J. et al. in 2015.[36] Ditrimethylopropane (10.2 g, 41 mmol) and anhydrous THF (250 mL) were added to round-bottom flask under nitrogen atmosphere. Then stirring for an hour, ethyl chloroformate (28.5 g, 263 mmol) was added to the mixture. Next, suspension was added dropwise of trimethylamine (24.1 g, 238 mmol) under ice-bath. The reaction was left at room temperature and continuous stirring for 4 hours. To purify compound, heterogeneous mixture was filtrated and solution was evaporate to yield white solid. Finally, pure flake of crosslinker A (8.1g, 67.3%) was recrystallized in THF. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 (d, J = 11.2 Hz, 4H, -$CH_2OCOOCH_2$-), 4.18 (d, J = 11.2 Hz, 4H, -$CH_2OCOOCH_2$-), 3.50 (s, 4H, -CH_2OCH_2-), 1.50

(q, J = 7.8 Hz, 4H, -CH_3CH_2C-), 0.91 (t, J = 5.7 Hz, 6H, CH_3CH_2C-). IR (solid, ATR) 2972, 2881, 1734 (C=O stretch), 1463, 1412, 1165, 1104, 762 cm^{-1}.

2.2.4 Synthesis of 2,2'-bis(trimethylene carbonate-5-yl)-butaethylene glycol dimethyl ether (BTB-GDE)

Crosslinker BTB-GDE was synthesized with 4 steps resemblance to the TMC monomer. Initially, pentaethylene glycol (10 mL, 47.3 mmol) was dissolved in 5M NaOH (44 ml, 141.8 mmol) under 0˚C. *p*-Toluene sulfonyl chloride in anhydrous THF(100 mL) were added in to flask under 0˚C, respectively. The reaction was stopped after 24 hours and extracted with chloroform and water. Organic layer was reduced pressure and yielded as 3,6,9,12-tetraoxatetradecane-1,14-diylditosylate (ODT) (26 g, 50 %yield).

Sodium hydroxide in oil (9 g, 382 mmol) was washed three time by anhydrous THF (30 ml) under dried flask and nitrogen atmosphere. HMPD (23.6 g, 114 mmol) was inserted under nitrogen atmosphere and stirred 24 hr. Then, ODT (25 g, 46 mmol) was mixed under nitrogen and kept stirring for 48 hr. The mixture was extracted by dichloromethane (DCM) and NaCl (aq) solution in which target compound dissolving in organic layer. Additionally, crude mixture was purified by silica gel column chromatography to acquire 1,18-bis(2-phenyl-1,3-dioxan-5-yl)-2,5,8,11,14,17-hexaoxaoctadecane (bis-PDO) (43 g, 90%yield).

Then, deprotecting group removal was done by refluxing of bis-PDO in MeOH (140

mL) and 5 M HCl (70 mL) at 90˚C overnight. The mixture was extracted by DCM and water to obtain the aqueous layer and then evaporated to obtain 2,21-bis(hydroxymethyl)-4,7,10,13, 16,19-hexaoxadocosane-1,22-diol (bis-HOD) (5.1mL, 17% yield).

Regarding to carbonate cyclization step, bis-HOD diol (5.2 mL, 12 mmol) has been dissolved in anhydrous THF (60 mL) with ethyl chloroformate (10 mL, 68 mmol) under nitrogen atmosphere. The mixture was kept at 0˚C and triehtylamine was added dropwise. The reaction was stirred 24 hr and then worked up by filtration and extraction by 1M HCl and DCM, respectively. Next, the crude mixture was distilled and purified by column chromatography (hexane: EtOAc) (10:7 v/v). Finally, BTP-GDE (5.6g, 95%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (d, J = 10.4 Hz, 4H, -$CH_2OCOOCH_2$-), 4.05 (d, J = 11.2 Hz, 4H, (-$CH_2OCOOCH_2$-), 3.63 (s, 20H, -CH_2OCH_2-), 1.08 (s, 6H, CH_3C-). IR (solid, ATR) 2981, 2868, 1744 (C=O stretch), 1472, 1402, 1254, 1175, 1101, 768 cm^{-1}

2.2.5 Preparation TMC hydrogel: TMCM-MOE3OM and TMCM-MOE4OM

TMC hydrogel was prepared as same as polymer procedure. Due to cyclic carbonate molecule of synthetic crosslinked agent, DBU and benzyl alcohol were used as catalyst and initiator via ring-opening polymerization (Figure 2-2). Dichloromethane was used as solvent and silicone sheet was cut in size 1cm × 5 cm as sandwich template of gel planar and hollowed tube shape.

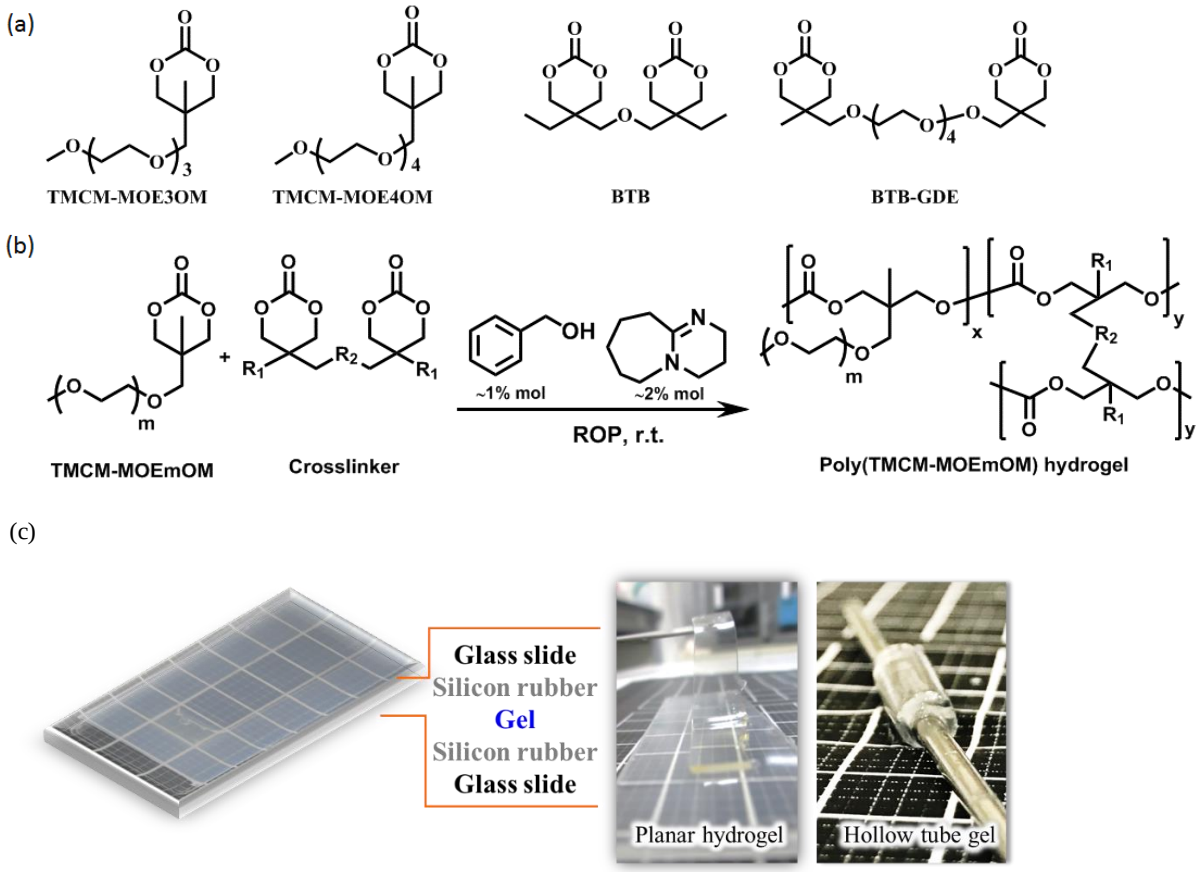

Figure 2-2. Chemical structure of monomer and cross linker (a), scheme of TMC hydrogel preparation (b) and silicone sandwich template of gel planar and hollowed tube shape (c).

2.2.6 Synthesis of 2-(2-(2-methoxyethoxy)ethoxy)ethyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate (MTC-MOE3OM)

The synthesis of MTC-MOE3OM was followed the previous route by starting at 20 g (149 mmol). Beside, this compound was prepared by the same procedure as reference[40], using triethylene glycol monomethyl ether (7.75 g, 47mmol) as the alcohol, and was purified by precipitation in acetonitrile to remove by product. The rest solution was evaporated and give the product as a clear oil. Yield: 1.6 g (33%). ^{1}H NMR:δ 4.72 (d, J = 5.4 Hz, 2H, CH_2), 4.38 (m, 2H,$COOCH_2CH_2$), 4.21 (d, J = 5.6 Hz, 2H, CH_2), 3.72 (m, 2H, $COOCH_2CH_2$), 3.68 (m, 2H,

OCH_2CH_2O), 3.57 (m, 2H, OCH_2CH_2O), 3.38 (s, 3H, OCH_3), 1.36 (s, 3H, CCH_3). ESI-MS: m/z calculated for $C_{13}H_{22}O_8$ 306.13; found 306.13.

2.2.7 Characterization

^{1}H NMR spectra were recorded on JEOL JNM-ECX400 operating at 400 MHz. $CDCl_3$ were used as solvent and the chemical shifts were calibrated against residual solvent signals. Attenuated total reflection (ATR) Fourier transform infrared spectrometer (FT-IR) spectra were obtained with IR Affinity-1S Shimadzu.

2.2.8 Swelling ratio and surface property

The degree of swelling of the hydrogel network was determined gravimetrically at each temperature due to their thermal responsive-based monomer. The swelling ratio (S.R.) was calculated according to follow equation.[39]

$$\text{S. R.} = \frac{W_s - W_d}{W_d}$$

Where W_s : final weight of swollen sample
W_d : weight of dried sample

A piece of gel which were size of 4 mm × 5 mm × 1mm (width × length × thickness), was weighted after soaking in deionized water in 10 ˚C, 25 ˚C, 35 ˚C and 37 ˚C. Generally, the three replicated samples were taken out and excess water was removed by tissue paper on gel

surface. Then, hydrogel was weighed and returned to incubated temperature. Next, all of sample were heated up to 150 ˚C and collected as dried weight.

Water contact angle (WCA) measurement, contact angles were measured by Flow Design CAM-004 (Tokai Hit TPX-S). Images were taken after DI water 2 μL was dropped on gel surface at various temperature with three replicates.

2.2.9 Thermal resistance measurement

Thermal properties and decomposition was observed in order to evaluate thermal stability of hydrogel and S.R. confirmation. The principle is weight change against to elevating temperature under nitrogen atmosphere. Sample was investigated by thermogravimetric analyzer TGA-50 (Shimazu) with heating temperature from room temperature to 500°C at rate 10°C/min and 10-20 mg of samples loading amount.

2.2.10 Mechanical property measurement

To understand the mechanical properties of the hydrogels, rheological experiments have been achieved by Kinexus pro^{+} rheometer (Malvern Panalytic) using a parallel plate geometry on a pettier plate. The diameter of the plate is 20 mm, the plate gap is 150 μm. Gel samples were measured in oscillated mode (Toolkit_O002, CP 4/20) at room temperature with 0.1m gap resolution.

As mechanical strength was also measured with compressive strength and tensile strength using EZ-SX texture analyzer (Shimadzu). Compressive test specimen was prepared in 2 mm × 2 mm × 1 mm thickness with three replications. Tensile test specimen with flat dog-bone shape was prepared by template stamp with gauge length 15 mm and 1 mm thickness, loaded between movable grip crosshead.

2.2.11 Hydrogel degradation experiment

The hydrogel samples (5 mm × 2 mm × 1 mm thickness) with 3 replicates were soaked in 10 mL of 5mg/mL lipase in phosphate buffered saline (PBS). Additionally, alkaline and acid circumstances were also investigated under 10 mL of 0.01M NaOH and 0.01M HCl and incubated at 37 ˚C in bottle screw cap. Each 1 mL of solution was taken to observe residue and degraded weight after 1, 3, 7, 20, and 40 days.

2.2.12 Surface properties

Biocompatibility could defined as the surface properties of the hydrogel to prolife or adhere by protein or blood platelet cell. Protein adsorption were tested along major protein in blood which are BSA, BGG, and BPF, detecting by bicinchoninic acid (BCA) protein assay reagent. At beginning, all hydrogel samples and reference sample were soaked in phosphate-buffered saline (PBS, pH 7.3-7.4) at 37 ˚C overnight. The test was performed as following.

Each sample was immersed into 900 μL of each protein solution, BSA/PBS solution (4.5 mg/mL) for 4 h at 37 ˚C. After incubation, gels were washed three times with PBS at 37˚C. Then, the sample were continuously soaked into 1 mL of sodium dodecylsulfate (SDS) in PBS solution (10 mg/mL) for 4 h at 37°C in order to detach protein on sample surface samples. The mixture of 400 μL of BCA solution and 400 μL of previous detached protein solution was incubated for 2 h at 37°C. Finally, UV absorbance intensity (562 nm) based on the adsorbed amount of protein on gel surface was interpreted by absorbance microplate reader (MTP-310lab, Corona Electric) referring to standard control and calibration curve of the same protein.

Regarding to possible application, platelet adhesion test is essential for preliminary test. The procedure was following reference.[28] Fresh blood sample was drawn from healthy volunteer. The mixture of 0.1% sodium citrate and blood was centrifuged at 1000 rpm for 7 min to obtain heterogeneous supernatant, platelet-rich plasma (PRP) and platelet poor plasma (PPP). PRP layer was diluted 3-fold with PBS and then platelet concentration (3.0×10^5 cells μL^{-1}) in diluted PRP was determined using fluorescence microscope. The hydrogel (1mm × 1mm × 1mm) were placed at the center of glass petri dish and washed by deionized water 3 times and soaked in PBS for 24 hr at 37°C. The PRP solution was added onto sample and incubated at 37°C 60 min. Gel was washed by PBS solution 3 times and then 2 ml of 2% glutaraldehyde in PBS at 4°C 2 hr. The samples were finally cleansed with 2 ml PBS (3 times) and water, and dried under vacuum overnight. Number of adhered platelets on gel surface was

counted and made average by SEM image. Low Vacuum Scanning Electron Microscope (SU6600, Hitachi) was used in condition, accelerating voltage: 1 kV magnification: 1,000x without metal coating of SE signal. Five different area of sample were captured and counted as average value with standard deviation.

2.2.13 Ester-free VS Ester- TMCM-MOE3OM polymer degradation test

The polymer based on ester and ester-free monomer were prepared by organocatalyst ring opening polymerization (ROP) which 20 mg of each sample with 3 replicates were investigated during enzymatic and alkaline condition soaking in 13 mL of deionized water, 0.005 mM Lipase and 0.003 mM NaOH. The color comparison by naked eye were determined via universal pH test paper litmus strip and chronic degradation during 30 days by digital pen pH meter.

2.3 Results and Discussion

2.3.1 TMC hydrogel preparation.

The previously reported PTMC derivatives are expected to be the potential candidate as biomaterials. Due to their high water-soluble ability and biodegradable property, polymers are crucial to improve mechanical strength for the several functional medical application. Therefore, our group strongly endeavored to develop viscous liquid based thermoresponsive[30] poly(TMCM-MOE3OM) and poly(TMCM-MOE4OM) to hydrogel. According to avoid the cross linker-incorporated effect,[41] BTB and BTB-GDE cross linkers based on TMC backbone were synthesized (Figure 2-2). These hydrogels were proudly to present as the novel robust network as shown in Figure 2-2. Subsequent amount of cross-linkers were introduced and performed via ring-opening polymerization with DBU and benzyl alcohol as ordinary homo- or copolymer method. All gel preparation has been done with 1%mol initiator and 2%mol catalyst in both bulk and dichloromethane solvent (CH_2Cl_2). Hydrogels with BTB crosslinked agent were labeled as A series as well as BTB-GDE as B series (Table 2-1).

Regarding to the experiment, at least amount of BTB crosslinker was 10% for complete gelation. However, A0 could not provide gel (Table 2-1, entry 1) with lower amount of BTB (9%) as the same concentration as A1 (Table 2-1, entry 2). In addition, 7M (Table 2-1, entry 3) and bulk concentration (Table 2-1, entry 4) with same amount of precursor were successfully formed gels, respectively. In case of BTP-GDE, which had more hydrophilic ethylene glycol

chain of bridge (Figure 2-2), 5% of cross linker could not achieve gelation, significantly indicating the minimum amount of cross linkers in this experiment is necessary extended (Table 2-1, entry 5). The amount of BTB-GDE were subsequently achieved with 10%, 20%, and 40% for TMCM-MOE3OM monomer for the variety of gels (Table 2-1 entries 6, 7 and 8). According to more hydrophilic monomer with 4 units OEG of TMCM-MOE4OM, the high ratio BTB-GDE cross linker (50%) was introduced and for gel preparation (Table 2-1, entry 9). Furthermore, the series of hydrogels were fabricated to investigate the effect parameters and characterization of TMC hydrogel in advance.

Table 2-1 TMC hydrogel preparation.

Entry	Sample[a]	Monomer	Cross-linked type	Crosslinker (%mol)	Solvent	Concentration
1	**A0**	TMCM-MOE3OM	BTB	9%	CH_2Cl_2	3M
2	**A1**	TMCM-MOE3OM	BTB	10 %	CH_2Cl_2	3M
3	**A2**	TMCM-MOE3OM	BTB	10 %	CH_2Cl_2	7M
4	**A3**	TMCM-MOE3OM	BTB	10 %	-	-
5	**B0**	TMCM-MOE3OM	BTB-GDE	5 %	CH_2Cl_2	1M
6	**B1**	TMCM-MOE3OM	BTB-GDE	10 %	CH_2Cl_2	3M
7	**B2**	TMCM-MOE3OM	BTB-GDE	20 %	-	-
8	**B3**	TMCM-MOE3OM	BTB-GDE	40 %	CH_2Cl_2	5M
9	**B4**	TMCM-MOE4OM	BTB-GDE	50 %	-	-

[a][monomer]:[initiator]:[catalyst] = 100:1:2

2.3.2 Thermoresponsive and swelling abilities

Since the series of the present monomers produced thermosensitive linear polymers, the stimuli responsive hydrogels were also prepared with cross linkers, indicated by the change of S.R. at various temperature. The homopolymer of TMCM-MOE3OM derivatives as itself behave initial aggregation above LCST around 33˚C, whereas the polymer of TMCM-

MOE4OM showed at 72˚C. The thermoresponsive hydrogel exhibited slightly volume changes according to the external temperature. The S.R. of hydrogels at cooling state, room temperature and body temperature was measured (Table 2-2). The typical example of volume change such as A3 and B2 gels were shown in Figure 2-3. A1 gel with low cross linker content obviously absorbed a large amount of water both 10˚C (S.R.= 7) and at 37˚C (S.R.=2) (Table 2-2, entry 1), comparing to the others (Table 2-2). For example, A3 with bulk reaction provided S.R. value as 1.3 at 10 ˚C while it was 0.2 at 37 ˚C (Table 2-2, entry 3). In parallel, B1 and B2 hydrogel behaved less of swollen ratio when increasing cross linker amount as 2.1 and 1.1, respectively (Table 2-2, entries 4 and 5). Furthermore, 50% cross linker addition was prepared in B4 sample and showed the small S.R. ratio with 0.9 (Table 2-2, entry 7). At around body temperature, the S.R. ratio of A3, B1, B2, B4 were investigated below than 0.5 (Table 2-2, entries 3, 4, 5, and 7). It could be considered that concentration and crosslinked amount affected to the density of hydrogel network which related to water content possess. Due to the structure balance hypothesis, two candidate of different hydrophilic cross linker were synthesized and merged. As presuming, hydrogel with long chain of ethylene glycol as bridge may help to increase ability of swollen ratio due to more long length molecule. The results of the experiment concerned that our expectation to improve loosen structure with more hydrophilic BTB-GDE was unclear.. At the same amount of crosslinked agent, sample A1 (Table 2-2, entry 1) and B1 (Table 2-2, entry 4) revealed that containing of BTB induced more water absorption, compared

gel with BTB-GDE crosslinker both at 10˚C and 37˚C. However, A1 and A3 with different polymerization concentration of BTB crosslinked agent showed loosen sample species. Herein, SEM image in cross section topology presented the more dense structure of BTB-GDE containing and bulk polymerization (Figure 2-S1). So, the concentration of polymerization condition was main important to control S.R. in this experiment.

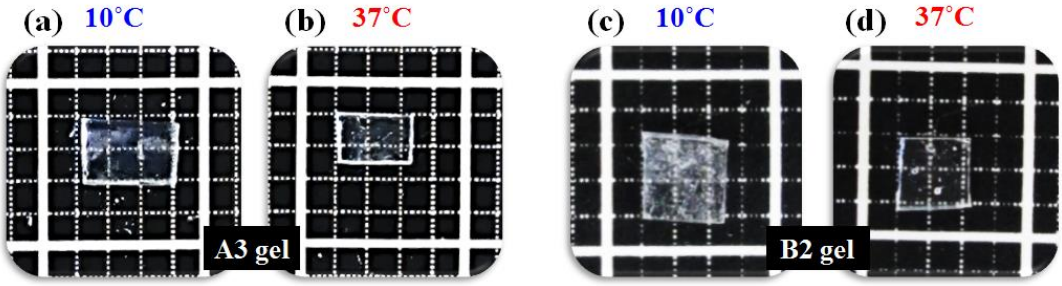

Figure 2-3. Swollen gel of A3 during cooling (a) and heating (b) and B2 gel during cooling (c) and heating (d).

Table 2-2 Characteristics of gels

Entry	Sample	S.R. in water [a]		Contact angle [a] (˚)		VPTT (˚C)	Protein adsorption [a]		
		10˚C	37˚C	10˚C	37˚C		BSA	BGG	BPF
1	A1	7.2 ± 0.04	1.7 ± 0.33	52 ± 1.5	106 ± 1.6	26	11 ± 2.1	8 ± 0.3	16 ± 1.7
2	A2						10 ± 0.5	4 ± 0.7	2 ± 0.9
3	A3	1.3 ± 0.04	0.2 ± 0.02	55 ± 2.5	100 ± 0.3	33	5 ± 0.01	3 ± 0.1	1 ± 0.5
4	B1	2.1 ± 0.04	0.4 ± 0.04	69 ± 2	95 ± 1.2	28	7 ± 1.2	3 ± 0.3	4 ± 1.8
5	B2	1.1 ± 0.44	0.3 ± 0.50	70 ± 6.9	88 ± 2.2	33	6 ± 0.4	3 ± 0.5	2 ± 0.4
6	B3						9 ± 1.2	5 ± 0.7	4 ± 0.4
7	B4	0.9 ± 0.23	0.5 ± 0.03	53 ± 4.7	76 ± 1.7	27	6 ± 0.5	4 ± 0.5	3 ± 1.4

[a] Experiment with 3 replicates = 3.
Bovine Serum Albumin (BSA), Bovine Gamma-Globulin (BGG) and Bovine Plasma Fibrinogen (BPF)

Thermoresponsive property of hydrogels was shown in range of body temperature between 26˚C and 33˚C in Table 2-2, determining by the degree of swelling changes drastically during hysteresis (VPTT).[42] Another finding was the VPTT behaviors of the hydrogels in pure

water at appropriate temperature, which would be convenient for the possible biomedical applications like PNIPAM.[42]

2.3.3 Contact angles of thermoresponsive hydrogel

According to the key problem for medical application, the surface contact angles, and protein adsorption of the hydrogels were investigated. Surface wettability was examined by WCA measurement at 10˚C and 37˚C. For more comprehension, the images of wettability were presented which corresponding to the Table 2-2. Commercial glass slide (Figure 2-4b) and silicone rubber (Figure 2-4c) which used as template mold, were measured as control for 40˚ and 107˚ respectively. A series gels (Figure 2-4d, 4e, 4h, and 4i) were determined 52˚-55˚ at 10˚C and as 100˚-106˚ at 37˚C with thermosensitive gels (Table 2-2, entries 1 and 3). In case of B series (Figure 2-4f, 4g, 4j, and 4k) including more hydrophilic cross linker, they were 69˚-70˚ (at 10˚C) and 88˚- 95˚ (at 37˚C) (Table 2-2, entries 4, 5, and 7). When introducing more hydrophilic TMCM-MOE4OM, hydrophilic surface increased as shown with decreasing of contact angle to 53˚ at 10˚C and 76˚ at 37˚C (Table 2-2, entry 7). Many previous coating research were reported the film or gel preparation with OEG which is hydrophilic, nontoxic and non-immunogenic. Biomaterials including OEG characteristically provides inhibition of protein or lipid adsorption with steric hydrophilic chain. However, our hydrogel surface exhibited almost hydrophobic surface (sessile drop) at 37˚C which possibly could be induced

contact sides of hydrophobic silicone rubber. During hydrogel preparations, orientation of hydrophilic/ hydrophobic groups of monomers was randomly rearranged to the material phase and could not control. According to confirm surface hydrophilicity, the captive bubble experiment has been measured on A3 gel at 37˚C (Figure 2-S2). Thus, poly(TMCM-MOE3OM) hydrogels were important to observe surface behavior as itself modified with OEG (Figure 2-4).

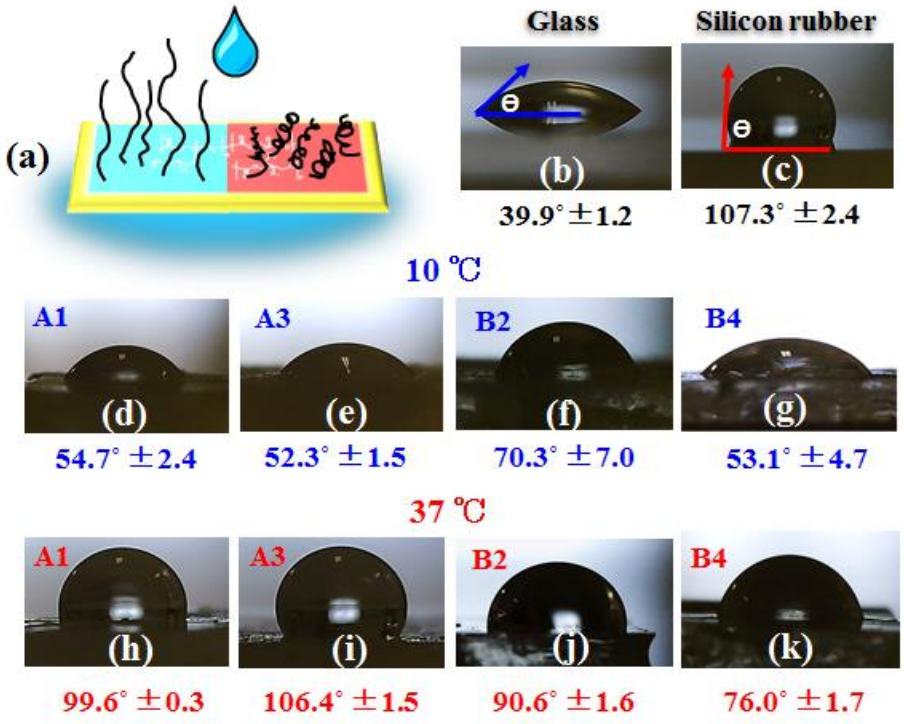

Figure 2-4. Illustration of contact angle on surface of glass (b) and silicone rubber (c) as control and hydrogel surface at 37˚C at 10˚C A1 (d), A3 (e), B2 (f), B4 (g), at 37˚C A1 (h), A3 (i), B2 (j), and B4 (k).

2.3.4 Thermal stability and degradation property

For the future application, thermal resistance was examined to optimize the limit degradation temperature by TGA. The onset of thermal degradation at which 10% weight loss

appearance was determined as T_{10}. Figure 2-5 showed the results of TGA analysis of hydrogels, as well as pure monomer and crosslinkers. As results, hydrogel holding water in structure related to amount of water evaporated by TGA at around 100˚C. All hydrogel samples depicted the first peak of water combustion correspond to mass loss reduction as A1 (40%) > B4 (20%) > B2 (15%) > A3 (10%), which related to tendency of water consumption at 37˚C (Table 2-2, entry 1, 3, 5, and 7). Then, gel structure were easily identified by their different temperatures of combustion depending on composition in second slope. The combustion of A1 and A3 were represented at 273 and 282˚C (Table 2-3, entries 1 and 2) in the range of pure TMCM-MOE3OM and BTB cross linker at 179˚C and 298˚C, respectively (Table 2-3, entries 5 and 6). On the other hand, the T_{10} of B2 and B4 hydrogels showed up to 330˚C (Table 2-3, entries 3 and 4), probably because of the high value of T_{10} BTB-GDE cross linker at 335˚C (Table 2-3, entry 8). It is interesting that the thermal stability properties of those hydrogels might dominantly depend on gel internal structure. By comparing the results from B2 and B4 (Table 2-3, entries 3 and 4) which B4 sample have more double amount of cross linker and higher degraded temperature itself than B2, the T_{10} values were shown close to each other. These results indicates the type of cross linker would influence on the physical properties very much, such as thermal stability.

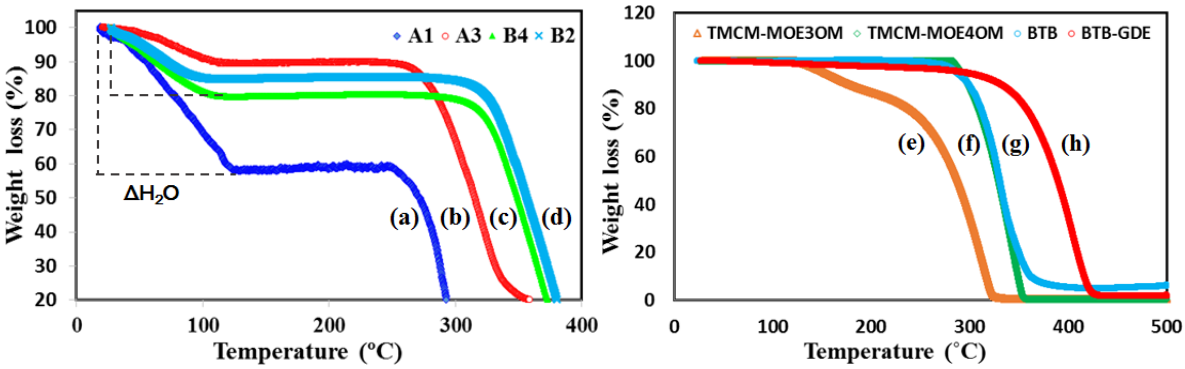

Figure 2-5. TGA curve of hydrogels degradation as A1gel (a), A3 gel (b), B2 gel (c) and B4 gel (d) and precursor degradation as TMCM-MOE3OM (e), TMCM-MOE4OM (f), BTB crosslinker (g), and BTB-GDE (h).

Table 2-3 Thermal data of precursor and hydrogels.

Entry	Sample name	Mass loss (%)	Component	T_{10} (°C)
1	**A1**	39	TMCM-MOE3OM + 10% BTB (3M)	273
2	**A3**	12	TMCM-MOE3OM + 10% BTB (bulk)	282
3	**B2**	14	TMCM-MOE3OM + 20% BTB-GDE (bulk)	330
4	**B4**	20	TMCM-MOE4OM + 50% BTB-GDE (bulk)	327
5	**TMCM-MOE3OM**	10	Monomer	179
6	**TMCM-MOE4OM**	10	Monomer	298
7	**BTB**	10	Cross linker	299
8	**BTB-GDE**	10	Cross linker	335

TGA measurement: heating from 25°C to 500°C under nitrogen (50 ml/min) with rate 10 C/min. T_{10} : temperature for 10% weight loss.

Among the candidates of biodegradable polymers, PTMC has appealed much concern as a bioabsorbable material in the design of clinical materials systems because of the non-acidic residue after degradation.[43-44] In previous, there was report the PTMC decomposition could be accelerated under lipase environment.[24] The author motivated to introduce the functional groups without ester linkage, so we designed the series of TMCM-MOEmOM. Recently, our group has already reported the degradation behaviors of poly(TMCM-MOE3OM) in lipase and alkaline condition with positively against in lipase condition and rapidly degraded in NaOH

solution.[31] To convince as predominant structure design, the degradation comparison with similar pendant group of linear homopolymers was comprised.

In order to clarify the merit of ester free structure, the residue of hydrolysis degradation was investigated using the model compound, MTC-MOE3OM. In short, TMCM-MOE3OM as ester-free structure and MTC-MOE3OM as ester based linkage carbonate cyclic were individually polymerized, then the pH change was monitored of their accelerated hydrolysis conditions (Figure 2-6). The phenomenon effect of alkaline solution of NaOH (0.003 mM) and simulated creature system of lipase (0.005 mM) in PBS were confidently studied with 3 replicates (n=3) in Figure 2-6. By comparing the results from pH shifting, ester-free structure is dominant and deserve to be continuously research as biomaterials. Ester-free homopolymer was closed to the blank reference in lipase representing pH 6.5 (Figure 2-6a and 6b) and trivial some degradation in strong base (Figure 2-6d and 6e) as same as in hydrogel result pH 9.5 and 8, respectively (Figure 2-S3). In contrast, poly(MTC-MOE3OM) indicated major both difference in enzymatic system and acidulous solution as pH 5 and 4 (Figure 2-6c and 6f). The result supported that the acidic residue could be found along ester structure and led to lower pH.

During consequence monitoring (30 days), the increment pH of ester polymer may be affected by the rapid side chain and backbone degradation (Figure 2-6c and 6f). Regarding to the accessibility in the previous report,[31] TMC backbone degradation could be prevented by EG along the ester-free polymer in the lipase environment. On the contrary, the ester-based

molecule indicated very fast side chain degradation and produced acid residue. That can be a reason of low pH at day 0 in Figure 2-6(f). Without EG interference, it might allow the hydrophobic molecule such as lipase attacking to TMC backbone continuously. Typically, TMC backbone was degraded giving carbon dioxide (CO_2) and diol residue. The CO_2 was released to air during measurement then pH slightly increased with time course. The results now present evidence in long term experiment (30 days), our polymer design of poly(TMCM-MOEmOM) could be clearly avoided degradation and produced acidic residue of both linear polymer and gel. This is the novel potential information supporting to our degradation hypothesis and avoid the risk of acidic compound production as inflammable materials in advance.

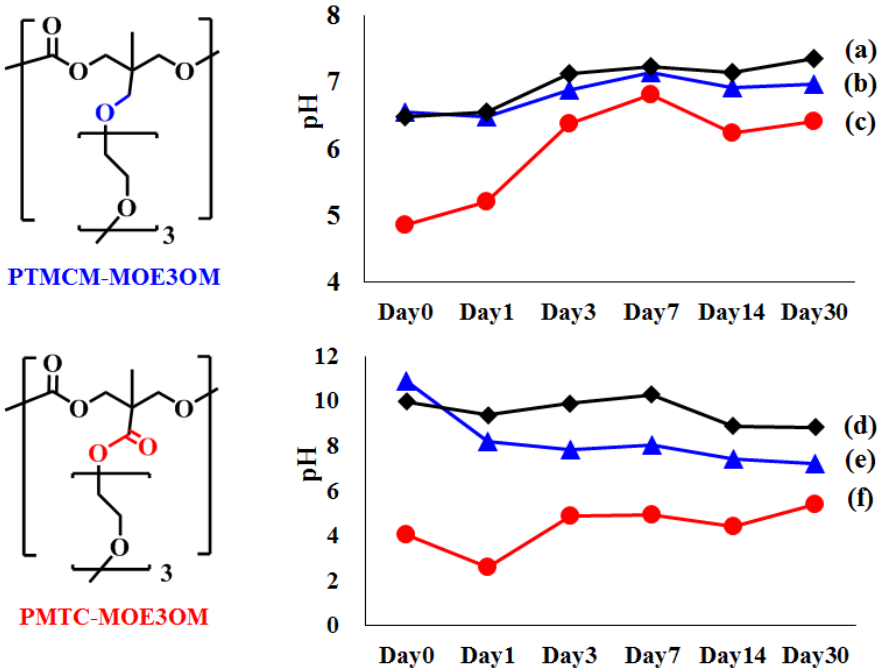

Figure 2-6. The pH change diagrams in 0.003mM lipase of blank (a), ester-free poly(TMCM-MOE3OM) (b), and poly(MTC-MOE3OM) (c), and 0.005mM NaOH of blank (d), ester-free poly(TMCM-MOE3OM) (e), and poly(MTC-MOE3OM) (f) during 30 days.

The hydrogel degradation in lipase (neutral), 0.01MHCl (acidic) and 0.01MNaOH (alkaline) were also clarified in this study during 40 days with three replicates as in Figure 2-7. The denature of gel structure were weighted and recorded at 1, 3, 7, 21, and 40 days in each environments. The residue of soaking solution was extracted and organic layer was confirmed by ^{1}H NMR. Obviously, the hydrogels indicated the strong stability among lipase solution with less than 5% for couple month (Figure 2-7a). In contrast, the degradation of sample proceeded very quickly under alkaline condition and seem to be faster than acidic condition (Figure 2-7b). At last day, denature or decomposed gel was shown in Figure 2-7 and could be observed size change due to the destroying penetrated through gel structure. To summarize the characterization, hydrophilic moieties would possess the resistance against hydrolysis in lipase, as well as assemble to human body state, while the acidic and alkaline accelerated the hydrolysis gradually.

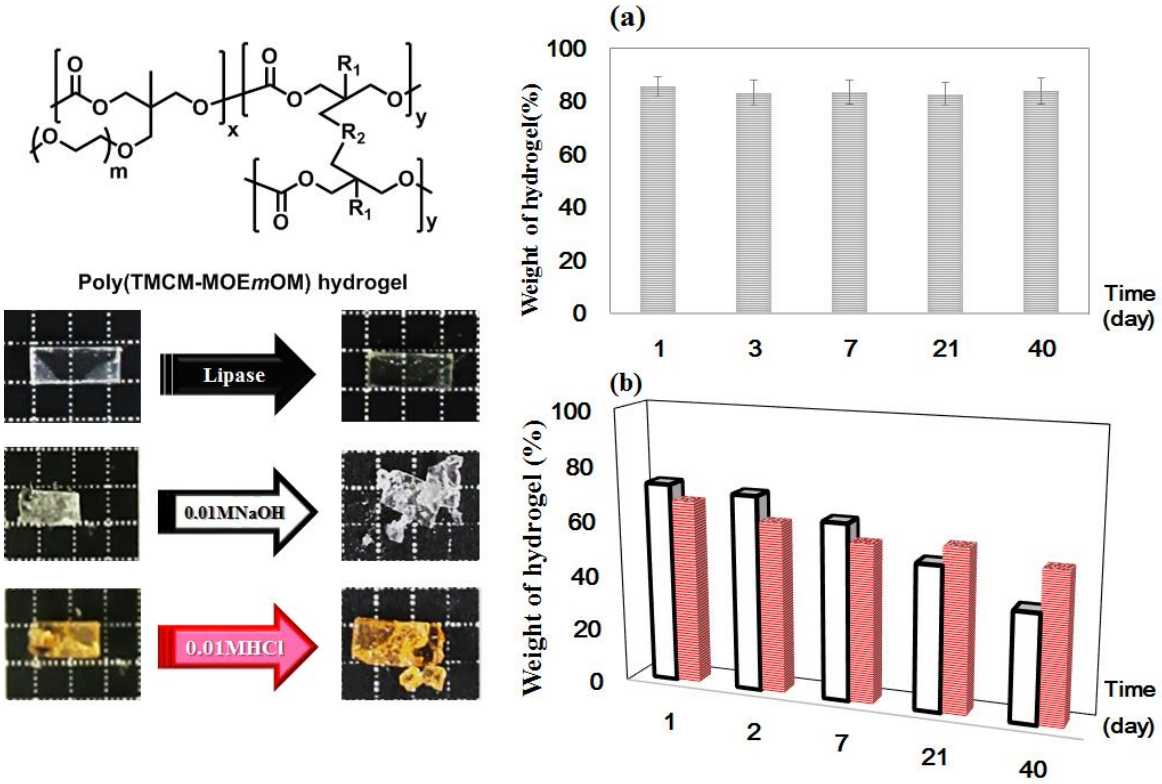

Figure 2-7. Degradation plot of TMCM-MOE3OM A3 gel in lipase (a) 0.01MHCl (filled bar) and alkaline (unfilled bar) circumstances (b).

2.3.5 Mechanical property

In order to investigate the stiffness of the gels, their physical behaviors of the storage moduli and the loss elastic modulus were measured by rheometer. In typically rheological experiment, samples were placed between plate-plate probe type under condition of oscillation (shear) testing mode, constant shear strain and various frequency at 37 ˚C.[45] In Figure 2-8, the limitation of frequencies that will not destructed sample network was pretreat and represented at 0.1-10 Hz. In the result, storage modulus (G') was higher than loss modulus (G") which characterized the gel appearance and elastic materials response. The elastic moduli (G´) of A1 and A3 gels were determined as approximately 1kPa due to the exact monomer proportional

and crosslinked ratio. However, G" values of A1 are slightly larger than those of A3, indicating the more soft matter than A3 gel behavior due to dilute polymerization system.

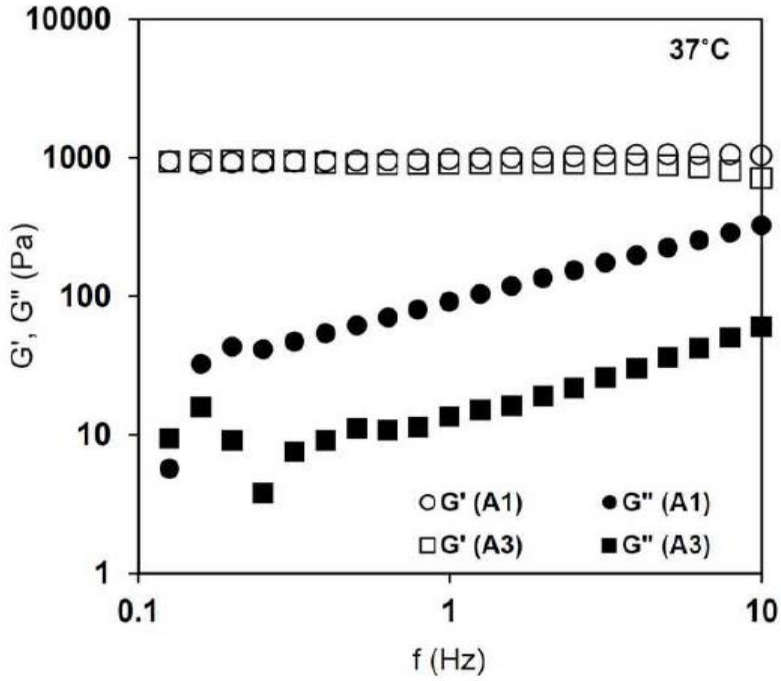

Figure 2-8. Frequency dependence of storage modulus (G') and loss modulus (G'') of A1 (a and c) and A3 (b and d) hydrogel in DI water, respectively.

According to materials durability, their mechanical properties were analyzed preliminarily by tensile and compressive test (Figure 2-9). As the results of tensile tests, A3 gel showed 0.07± 0.010 MPa in Figure 2-9b, as well as A1 gel with 0.022 ± 0.002 MPa in Figure 2-9a. The fracture stress were also estimated for A1 and A3 gels as 2.20MPa ± 0.40 MPa and 0.73MPa ± 0.07 MPa, respectively (Figure 2-9c and d). The author could explain that A1 and A3 hydrogel behaviors as reinforced material which regularly have much higher compressive strengths than it tensile strengths. The evidence convincing flexible and elastomeric materials is large difference between compressive and tensile strength of A1 sample at room temperature

with hydrophilic condition. For mechanical property, the present hydrogels are considered to be a potential candidate with sufficient stiffness much larger than the natural polymers[46] or PEG hydrogels.[47]

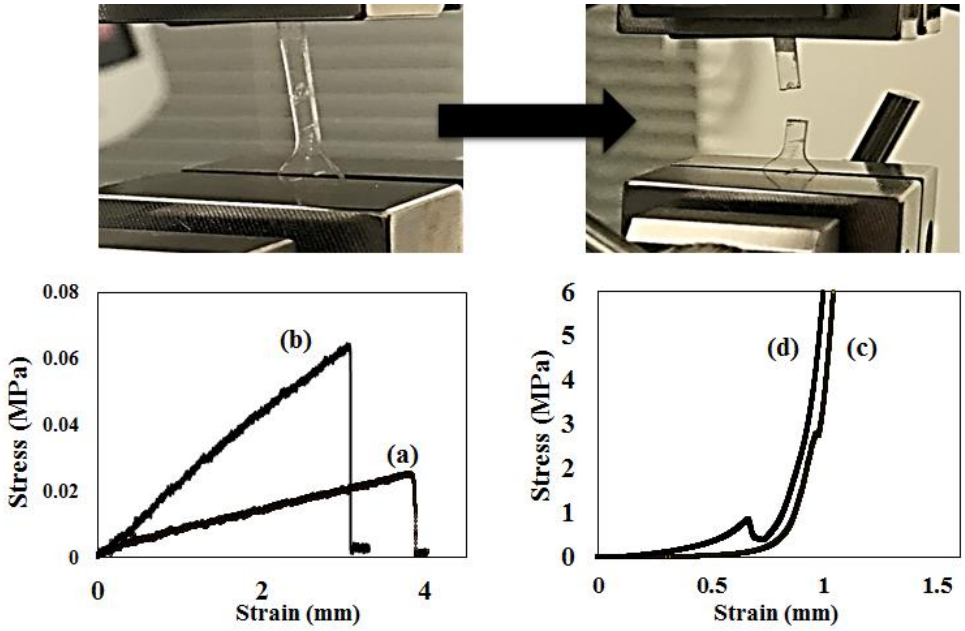

Figure 2-9. Tensile durability test of TMC hydrogel with dumbell shape at fracture point of A1 gel (a) and A3 (b), the compressive modulus of A1 gel (c) and A3 gel (d).

2.3.6 Protein adsorption and platelet adhesion

To comprehend biological expression for biomaterials, it is necessary to understand the protein adsorption on material surface as prescreening process *in vitro*. Prospectively, materials for blood vessel disorder is developed, the existence of PEG group could be able to prevent serum albumin adsorption. Many studies have referred protein repelling activity with the hydrophilicity and non-charge of PEG as surface modifier.[48-50] Generally, fibrinogen is influence the adhesion of macrophages or platelets, and leads to fibrous proliferation. Typically,

there are many serum proteins in creative blood, BSA, BGG, and BPF were determined on TMCM-MOEmOM gel surface as preliminary test. As experimental results, polypropylene (PP), silicone rubbery and polyethylene terephthalate (PET) were selected as references. PET has been significantly as a positive control in many reports due to its high thrombus formation or blood clotting (coagulations activity).[50] As same as our experiment, reference surface expressed high adsorption of protein in Figure 2-10. Three replicates (n=3) of each hydrogel samples was examined with human albumin (4.5 mg/ml), globulins (1 mg/ml) and fibrinogen (0.3 mg/ml). The protein adsorption on TMC hydrogel surface were suppressed comparing with PET substrate as 6 ng/mm^2 BSA, 15 ng/mm^2 BGG and 23 ng/mm^2 BPF (Figure 2-10c). In case of gel samples, B4 was indicated the amount of BSA, BGG and BPF as 6 ng/mm^2, 15 ng/mm^2 and 22 ng/mm^2 (Figure 2-10d) which slightly smaller than PET. B2 samples adsorbed amount were 6 ng/mm^2, 12 ng/mm^2 and 20 ng/mm^2 (Figure 2-10e) which orderly smaller than B4. Furthermore, the adsorption of albumin, globulin and fibrinogen of A1 were 11 ng/mm^2, 8 ng/mm^2 and 16 ng/mm^2 (Figure 2-10g). In case of A3 sample, it is notable that A3 were dominantly diminished to 3 ng/mm^2, 3 ng/mm^2 and 2 ng/mm^2 (Figure 2-10f). The surprising results indicate that A3 gel surface exhibits excellent inhibition of protein adsorption, while it is still unclear even if similar component to A1 gel. Once, it could be good discussion in high hydrophilic surface at 37°C with agreement of captive bubble WCA (Figure 2-S2) and assorted parameters.

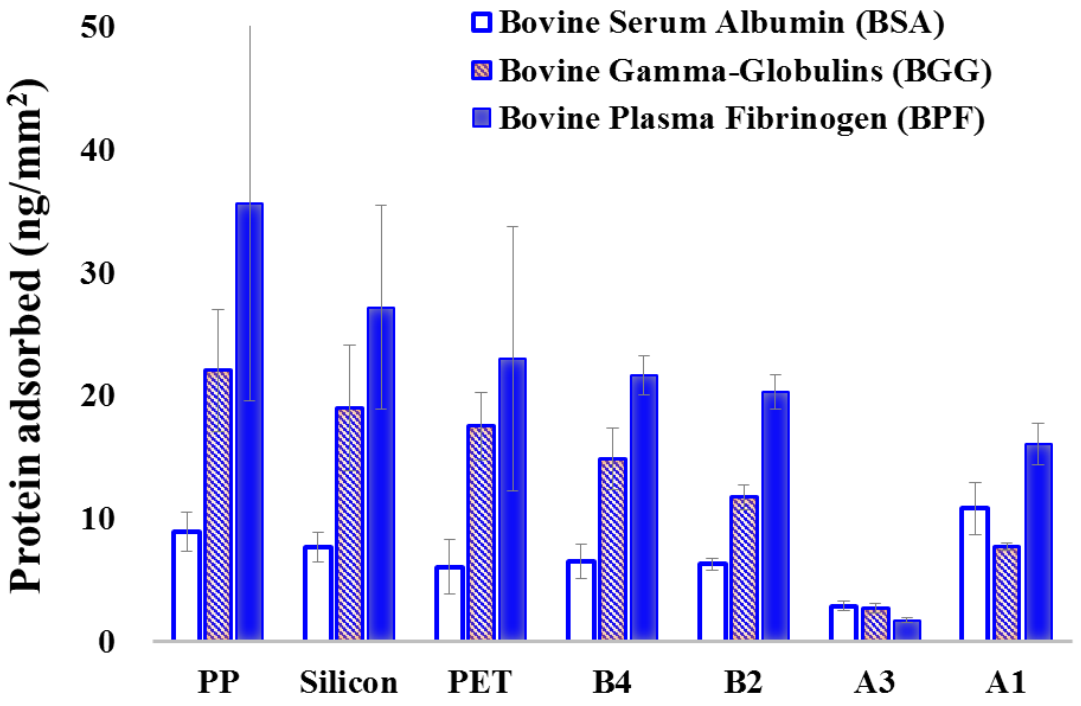

Figure 2-10. Quantitative plot the concentration of proteins per area on gels surface.

Tamada and cowokers was reported the potential of protein, platelet and cell adhesion related to the surface angle.[53] The WCA of PET was 65˚ ± 3˚ with possessing high protein adsorption and cell adhesion at 37 ˚C in 1988.[53] As hydrophobic tendency over PET at 37 ˚C, TMC hydrogels were assumed to induce slightly less protein adsorption due to hydrophilic TMCM-MOEmOM added amount as PET > B4 (50%) > B2 (80%) > A1, A3 (10%). However, the author expected that the hydrophobicity of gel surface could be induced by the silicone as contact covering in hydrogel preparation. Moreover, hydrophobicity was possibly enlarged when increased the crosslinked amount as B4 50% B2 20% and A1 10%. It was also related to the relationship of substrate stiffness, protein and cell behaviors as Wang and coworkers have reported.[57]

Interestingly, the important finding is the amount of adhered platelets paralleling to potential of protein adsorption as expectation, and SEM images are presented in Figure 2-11 and 12. PET and B4 were determined high protein adhesion as similar to contact angle and protein adsorption ability (Figure 2-11b and 11c). In ordering, B2 showed gradually lower than B4 (Figure 2-11d) and closed to silicone rubber (Figure 2-11a). Surprisingly, A1 hydrogel was easily adhered by platelets (Figure 2-11f) with almost surface ability to A3 (Figure 2-11e). The platelet numbers were compared and represented in Figure 2-12. Although the wettability (sessile drop) of A1 and A3 were almost similar but the mechanical behavior also supported the flexible and rubbery surface. However, the captive bubble of A3 was measured to assure the hydrophilicity surface at around body temperature whether it was over VPTT. Hence, the author also speculated that the roughness of surface between A1 and A3 (Figure 2-S4) might be certain influenced to favor platelet adhesion because of increased areas available for adhesion, as well as geometrical niches for adhesion.[56] Combining our results at present and previous literatures,[50,55] it concerned that TMC hydrogel materials may be affected by crosslinked agent and it could be adjust in advance. The main factor is the amount of components on gel itself structure, with plenty of oligo ethylene glycol worked as inhibitor to suppress the adsorption of protein and platelets adhesion (Figure 2-12e). The high moiety of that such as A3 (low amount of cross linker) in bulk, which could provide high density, is significantly force to decrease the adhesion. Due to impurity (crosslinked agent) and random polymerization, it could not compare

ability with brush type surface modification. However, TMC based monomer itself is achieved in design and pre-screen medical property.

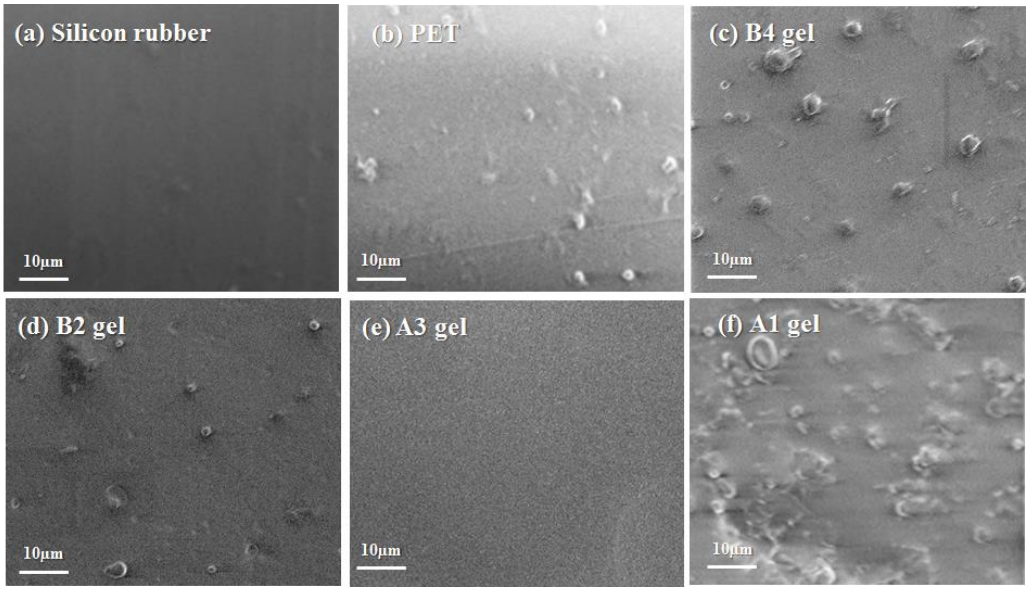

Figure 2-11. SEM images of platelet adhesion on materials surface; silicone rubber (a), PET (b), B4 gel (c), B2 gel (d), A3 gel (e), A1 gel (e) (n=5, 1kV at 1000x magnification, scale bar 10 μm).

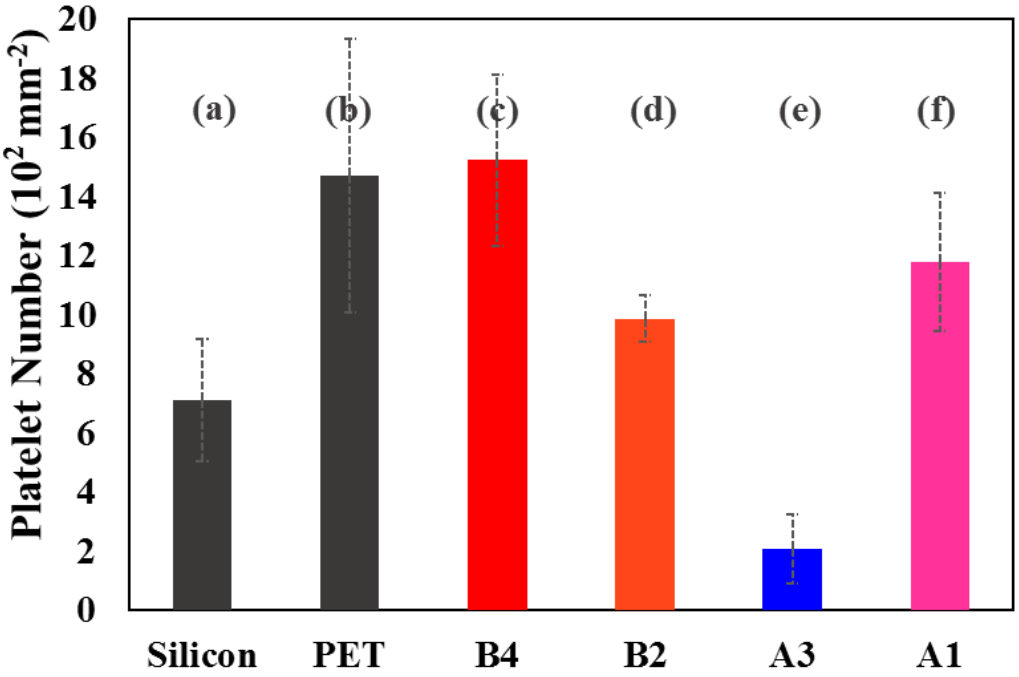

Figure 2-12. Chart plot adhered platelet number on materials surface; silicone rubber (a), PET (b), B4 gel (c), B2 gel (d), A3 gel (e), A1 gel (f) (n=5).

2.4 Conclusions

Various TMC hydrogels were successfully prepared that mainly compose of TMCM-MOE3OM with TMC crosslinked agent via ring-opening polymerization. BTB and BTB-GDE cross linkers were synthesized and introduced varied percentage. The hydrophilicity as WCA degree enlargement when amount of crosslinker was increased at body temperature. At present, a comparison of hydrogel with 10% cross linker was considered. A1 and A3 gel was dominant candidates and showed definitely different in pre-screening *in vitro* test as preliminary protein adsorption and platelet adhesion. Both hydrogels could resist thermal degradation up to 270˚C and enzymatic degradation during 30 days. Furthermore, rheological result and compressive test were concerned more elastic structure of A1 gel (2.1MPa) with three-fold than A3 (0.7MPa) due to diluted polymerization system. The novel TMC hydrogel rely on the interaction of molecular interaction force on the materials surface. Admittedly, the capable resistance in lipase is pointed as non-acid residue production as biomaterial usage in the future. Finally, these results go beyond previous reports, showing that TMCM-MOE3OM polymer could be constructed as hydrogel instead of viscous liquid and our ester free structure hypothesis was potential.

2.5 Supplementary Materials

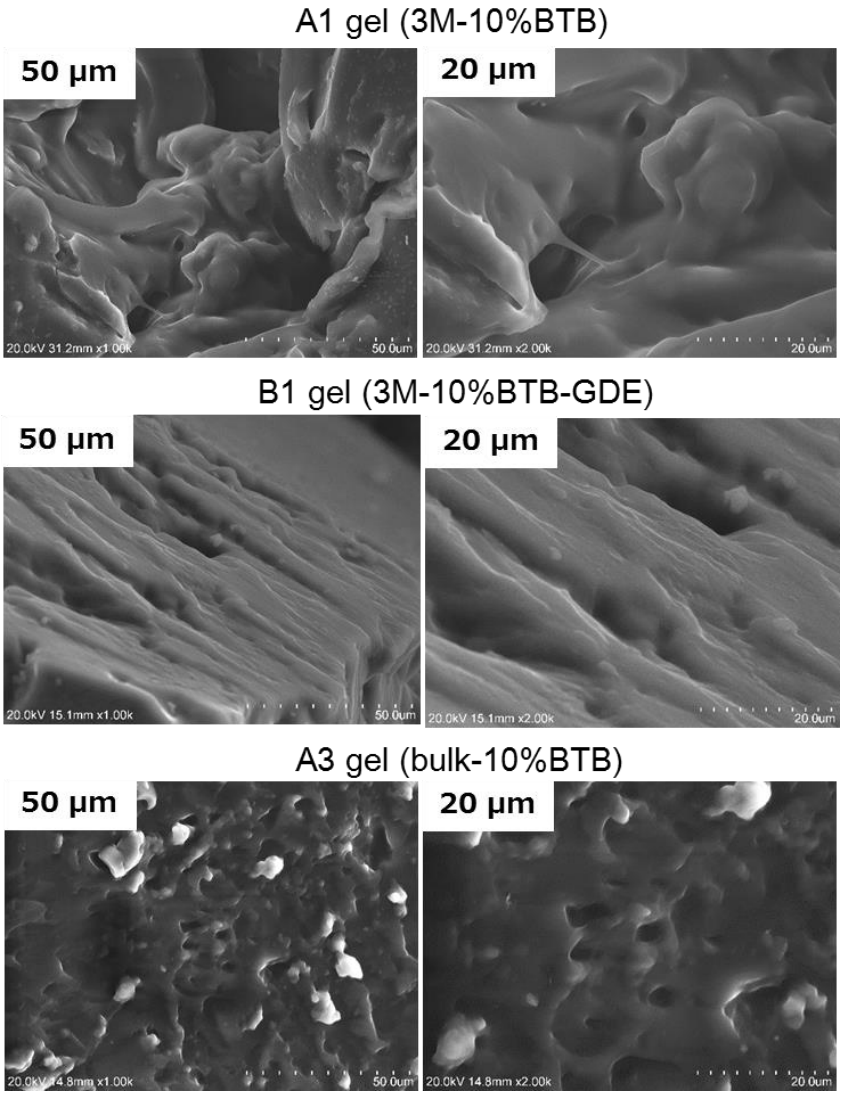

Figure 2-S1. Topography of freeze-dried A1, B1 and A3 hydrogel by SEM images (cross-section) at 20kV 1000X and 2000X.

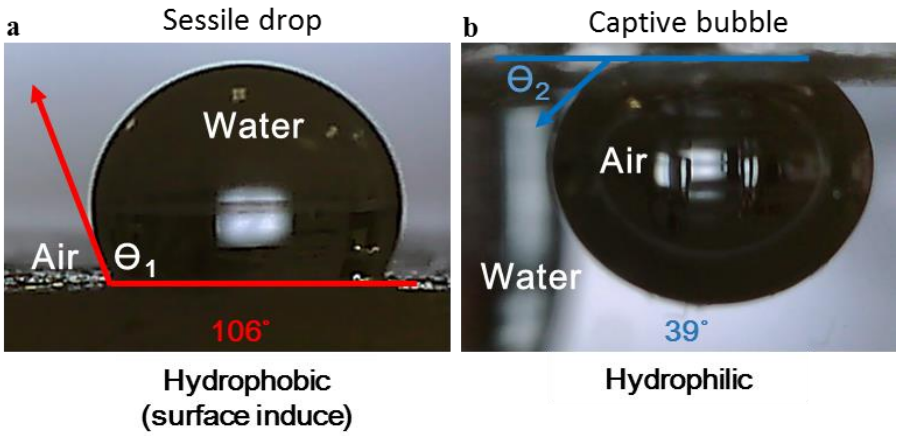

Figure 2-S2. Photos of water droplet (sessile drop) on A3 gel in air (a) and air bubble on A3 gel in water (captive bubble) (b) at 37°C.

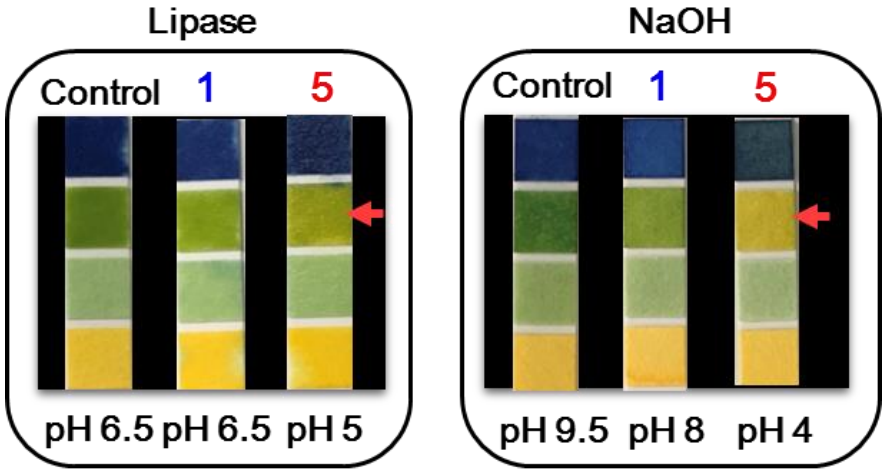

Figure 2-S3. The comparison by litmus pH paper test between ester-free PTMCM-MOE3OM (polymer 1) and PMTC-MOE3OM (polymer 5) in various condition (b).

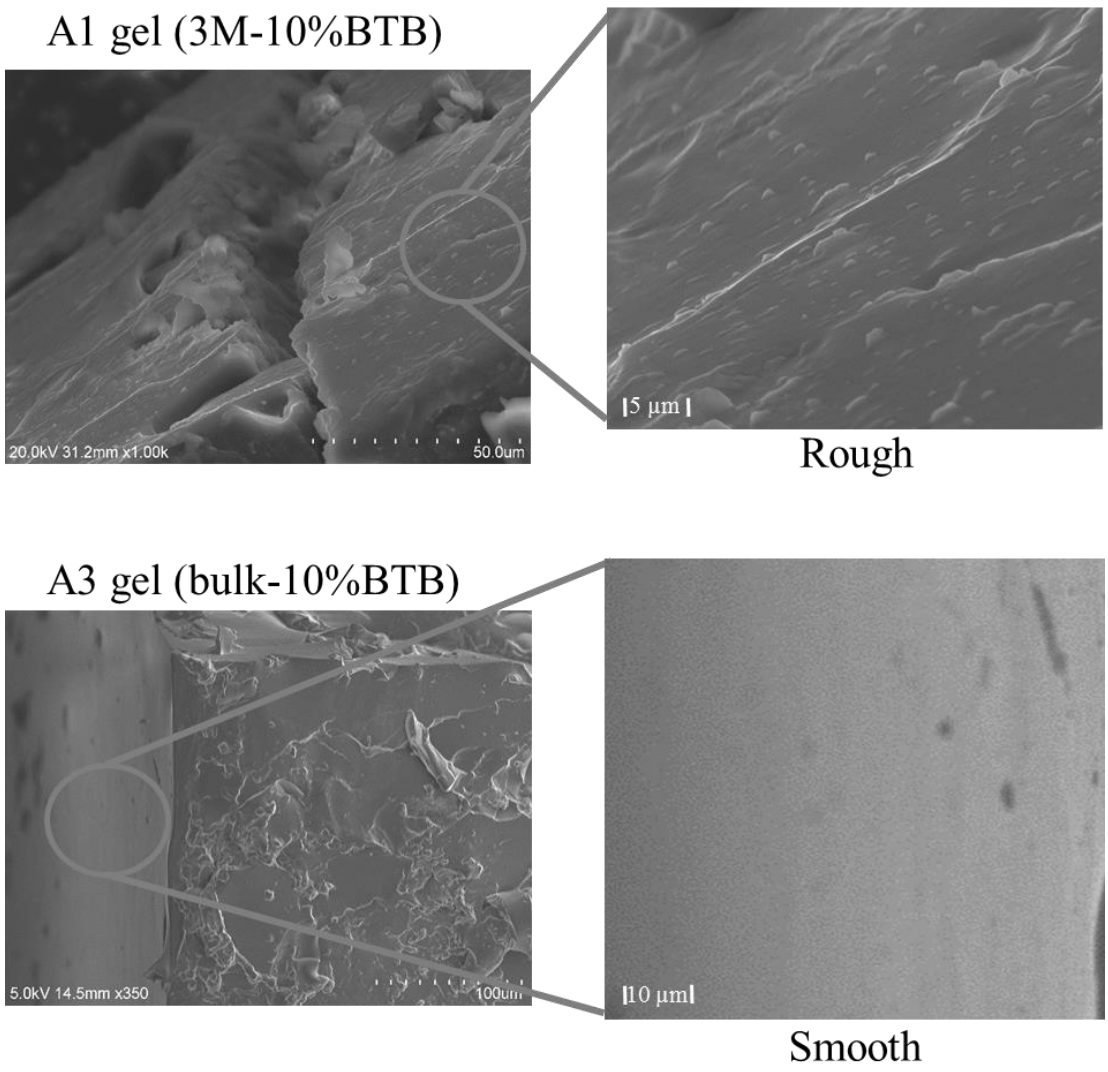

Figure 2-S4. Topography SEM images (surface) of Freeze-dried TMCM-MOE3OM hydrogel network dilute polymerization (A1 gel) and bulk polymerization (A3 gel).

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Chapter 3

Investigation on Polymerization of 5-[2-{2-(2-methoxy ethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one by Organometallic Catalysts

3.1 Introduction

In recent years, lots of investigation concerns potential of organic catalyst reactivity for ring opening polymerization (ROP), especially strong amidine 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) have been reported.[1] The bases behave as nucleophiles and react with electrophile cyclic ring derivatives.[2] The study field of organic catalyst has expanded rapidly and become a powerful alternative to more traditional metal-based catalyst[3] obviously in the past more 10 years. In case of lactide and cyclic ester polymerization, the point of organocatalyst advantage are not only the low cost and easy to use, including mild condition, stable and dominant control over polymerization.[4,5] In addition of merits of organocatalyst design and keys, the precise reaction ability for architecture, highly active and highly selective have been studied.[6] Under the condition with super base DBU, it is possible to obtain high catalytic reactivity with >98% monomer conversion of lactide and trimethylene carbonate (TMC) within 2 hours at ambient temperature with or without alcohol.[7]

Regarding to the increasing demand of polymeric materials in advance application, commercial and functional polymer based on six member ring monomer are obtained by ring opening polymerization.[7-9] TMC could be outstanding candidate[10] which providing thermal responsive along the ester-free structure as very low toxicity structure of hypothesis. Particularly, biocompatible 5,5-dimethyl-trimethylene carbonate monomer with three units of methyoxyethoxy (MOE) pendant group was designed as a thermoresponsive polymer. The poly(5-[2-{2-(2-methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one) (poly(TMCM-MOE3OM)) has been reported using organocatalyst DBU and benzyl alcohol initiator with various number average of molecular weight and narrow polymer distribution (PDI).[11] Interestingly, poly(TMCM-MOE3OM) shows LCST at 33°C near to body temperature. Furthermore, no acidic compounds would be released after hydrolysis that could contribute biomaterials.

However, Amsden and coworkers have particularly reported further TMCM-MOE3OM homopolymerization using Tin(II) 2-ethylhexanoate ($SnOct_2$) as the catalyst and solvent effect due to hydrogen bonding. The in situ formation between DBU and solvent could be obstructed the polymerization.[12] Experimentally, PTMCM-MOE3OM molecular weight were much lower than theoretical pre-calculation which indicated the lack of control of organic catalyst with proceeding number average molecular weight was less than the value of M_n 4000 g/mol. Furthermore, ring-chain equilibrium, backbiting and auto polymerization during

TMCM-MOE3OM polymerization was also reported.[12]

On the other hand, several typical effective agents such as Tin(II) 2-ethylhexanoate ($SnOct_2$) and diethyl zinc ($ZnEt_2$) are widely used as initiator and examined kinetic analysis for excellent production of polylactide, copolymer, and PTMC for decades.[13-14] With decent amount of $SnOct_2$, the preparation of commercial TMC in range 100-160°C temperature reaction was determined with or without alcohols. To add to this, M_n can be controlled and reached to high molecular weight up to 50,000 g/mol in rapid reaction following mechanism pattern with temperature dependence.[15] In case of PTMC with $ZnEt_2$, the molecular weight 4,000-65,000 g/mol with narrow PDI was reported.[16-17] Interestingly, up to the present, several synthetic rare earth metal complexes generally possess good performance and high sensitivity in ring opening polymerization. As efficient selectivity of polylactide preparation, there were historically reports with differences rare-earth core metal and coordinated ligands were studied such as Tin(II) alkoxide complexes[18], Lu or Y metal silyloamido complexes[19], bis(phenolato) Sc complexes (OSSO type)[20] and Y tetradentate complexes[21]. Addition, Zinc-diazadiene complex could give a narrow disperse PCL with high molecular weight.[22] Then, the design and development of Zn catalyst has been reported in wide field with positively catalysis of polymerization of cyclic carbonate[23] or cyclic ester.[24] As controlled tacticity of lactide polymerization, scandium complexes gave positively higher heterotacticity than another along with an [OSSO]-type bis(phenolate) ligand.[25] In addition, those inorgano-catalysts have mainly

initiated an effective ROP of polylactide raising to 100,000 g/mol with more 80% conversion.[25]

In this study, various catalysts were employed to investigate ROP of TMCM-MOE3OM. Recently, the various cationic complexes, transition metal with oxidation number +2, +3 and +4 are chiefly studied and available along bulky ancillary ligand. With the search for catalysis, a coordination-insertion mechanism is mainly speculated and attractive with or without recourse of exogenous protic nucleophile.[26] At the present, our TMCM-MOE3OM ROP was typically performed in bulk at ambient within 8 – 48 hours with organocatalyst reaching M_n 4,000-6,000 g/mol. In this hypothesis study, the prescreening investigation of organometallic complexes to TMCM-MOE3OM polymerization is proceeded. The variety of organometallic compound based on $SnOct_2$, $ZnEt_2$, Tin (IV), Titanium (IV), Scandium (III), Lutetium (III), Zinc (III) complexes with diverse ligands as catalysts. The kinetic reaction were monitored and discussed between commercial $ZnEt_2$ and DBU-catalyst with benzyl alcohol initiating and polymerization mechanism could be proposed.

3.2 Experimental Section

3.2.1 Materials

Triethylene glycol monomethyl ether, *p*-toluenesulfonyl chloride, *p*-toluenesulfonic acid, trimethylolethane, 2-methyoxyethyl p-toluenesulfonate, ethyl chloroformate, acetic acid, potassium carbonate, 1,4 dibromobutane, 4-methylumbeliferone, benzyl alcohol, and 1,8-

diazabicyclo[5.4.0]-7-undecene (DBU) were purchased from Tokyo Chemical Industry (TCI), Japan. Benzaldehyde and sodium hydride in oil (20%w/w) were purchased from Wako, Japan. Benzyl alcohol and DBU were distilled before used. Anhydrous tetrahydrofuran (THF) and dichloromethane for monomer synthesis, purification, were distilled with calcium hydride (CaH_2) before use. Unless mentioned, otherwise, all materials were used as received without further purification.

5-[2-{2-(2-Methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyltrimethylene carbonate (TMCM-MOE3OM) was synthesized via a 5 step processes as protocols of our previous report.[11]

Those compounds **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **13** and **14** were synthesized as previously reported in Figure 3-2.[19,21,25,27,28]

3.2.2 Glovebox procedure

All operations were performed under inert atmosphere (argon, nitrogen; < 2 ppm of O_2) by using standard Schleck-line, vacuum line, small vials and the glovebox (MBRAUN UNIlab Plus) techniques. Solvents were thoroughly dried and deoxygenated by standard methods and distilled before use. $CDCl_3$ was dried over 4A molecular sieves. TMCM-MOE3OM was dried over mixture of molecular sieves and stored under N_2 and confirmed with ^1H NMR before used.

Toluene, *n*-pentane, and THF from Fisher Scientific were distilled under argon prior to use. Benzene (d_6) (Sigma-Aldrich) and $CDCl_3$ (Sigma–Aldrich) were carefully dried and stored in a glovebox. All other chemicals were commercial available and used after purification. Glassware and vials used for polymerization were dried in an oven at 120 °C and vacuum–argon flow cycles three times.

3.2.3 Apparatus

^1H NMR spectra were recorded on a Bruker DRX 400 MHz spectrometer at 25°C. Molecular weights and polydispersity was determined by size exclusion chromatography (SEC) in THF at ambient at a flow rate of 1 mL/min polystyrene as standard (PSS Polymer Standards Service GmbH, Germany).

3.2.4 Synthesis of Compound 6-{1,4,7-trimethyl-1,4,7,10-tetraazacyclododecane}-2,4-di-tert-butylphenolate (12)

$ZnEt_2$ (0.123g, 0.99mmol) were dissolved in 2 mL of dry toluene (using an MBraun solvent purification system (SPS)) a vial in a glovebox under argon. 433 mg of the ligand precursor were dissolved in 3 mL dry toluene under the same conditions. The $ZnEt_2$ solution was transferred to a Schleck tube equipped with a magnetic stirring bar using a glass pipette. 1 mL of toluene was used to take up remaining solution from the vial. The ligand precursor

solution was added dropwise in the same way (cleaning the vial with 2 times 0.5 mL toluene) and the mixture was stirred in the box. After 2 h, all volatiles were removed under vacuum. The crude sample was taken up in 1mL of toluene, 2 mL of dry pentane were added and the sample was kept in the freezer of the box at -30 °C over two days. ^{1}H NMR (C_6D_6): 7.68 (d, 1H ArH); 7.00 (d, 1H, ArH); 3.43 (S, 2H, C-CH_2-N), 1.93 (d, 12H, N-CH_2-CH_2_N), 1.84 (s, 9H, p-Bu^t); 1.77 (t, 9H, o-Bu^t), 1.53 (s, 9H, CH_3-N), 1.42 (m, 2H, Zn-CH_2-CH_3), 0.52 (t, 3H, CH_3-CH_2), ^{13}C NMR (C_6D_6): 56.38, 56.04, 42.56, 35.76, 34.08, 32.35 (CH_2-N), 21 (Zn-CH_2), 65.00, 57.32, 13.55 (CH_3-N), 30.33 (*p*-Bu^t); 2.74 (*o*-Bu^t), 165.94 (C-O), 129.16, 128.43, 125.65, 134.25, 137.66 (ArC).

3.2.5 Polymerization of TMCM-MOE3OM

At ambient polymerization, complexes catalysts were prepared as stock solution and added to start the polymerization (50 mg of monomer, 0.1710mmol) at room temperature in small vial in glove box. The monomer to catalyst ratio was from 100 to 2000 equivalent. At desirable time, some were taken out to analyze the monomer conversion via ^{1}H NMR spectroscopy. For purification, the reaction mixture was poured into mixture of cold hexane/2-propanol (9/1, v/v) to recover insoluble part.

For the elevated temperature conditions, homopolymerization of TMCM-MOE3OM with Tin(II) 2-ethylhexanoate ($SnOct_2$) as the catalyst were prepared in glove box and then heat

up at 100°-160°C in fume hood and monitored monomer conversion within few day.

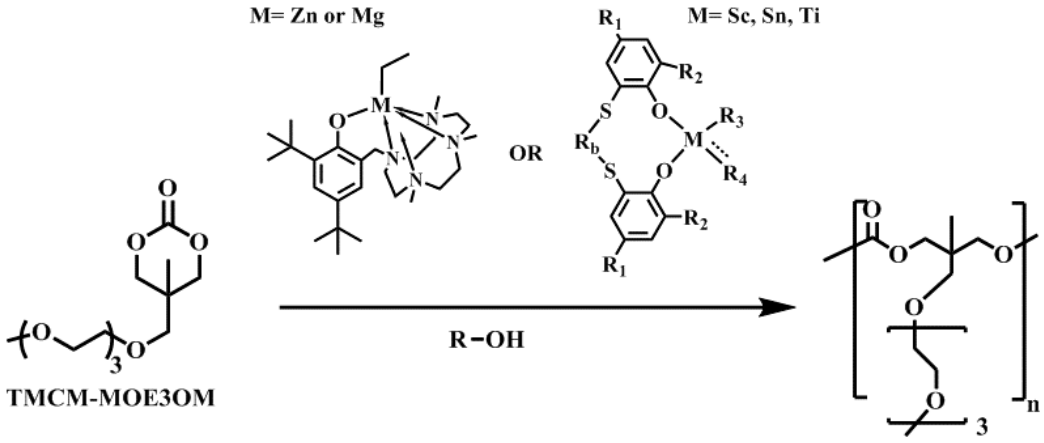

Figure 3-1. Polymerization of TMCM-MOE3OM using various organometallic complexes.

3.3 Results and Discussion

In previous, there were many reports revealed that organometallic complexes such as salen, OSSO-ligand and cyclen could be initiated significantly and control the configuration of polylactide or polystyrene polymerizations.[15-22] As ordinary polymer preparation, TMCM-MOE3OM has been polymerized using organo-based catalysts under mild condition.[11-12] However, it is worthy to explore the efficient catalysts for TMCM-MOE3OM polymerization. Thus, the variety of complexes were challenged as pre-screening in polymerization, in order to investigate polymerization mechanism of TMCM-MOE3OM. Those organometallic complexes were prepared, purified, and structurally characterized as previously described.[19-21,25] As plenty of the rare-earth metal classify and commercial available catalysts were selected, they were compared in three group containing diverse of mainly prolonged ligand such as tris(dimethylsilyl)amido; [M{N(SiHMe$_2$)$_2$}$_3$(thf)], [M{N(SiHMe$_2$)$_2$}$_3$], bis(phenolate) silylamido complexes; [M-(OSSO){N(SiHMe$_2$)$_2$} (thf)] and tetradentate cyclen-derived; [(1,4,7-trimethyl-1,4,7,10-tetraazacyclododecane)MC$_2$H$_5$], alkoxy derivative; 2,4-di-tert-butylphonolate (dbp) and so on. The series of catalysts are represented in metal group in Figure 3-2.

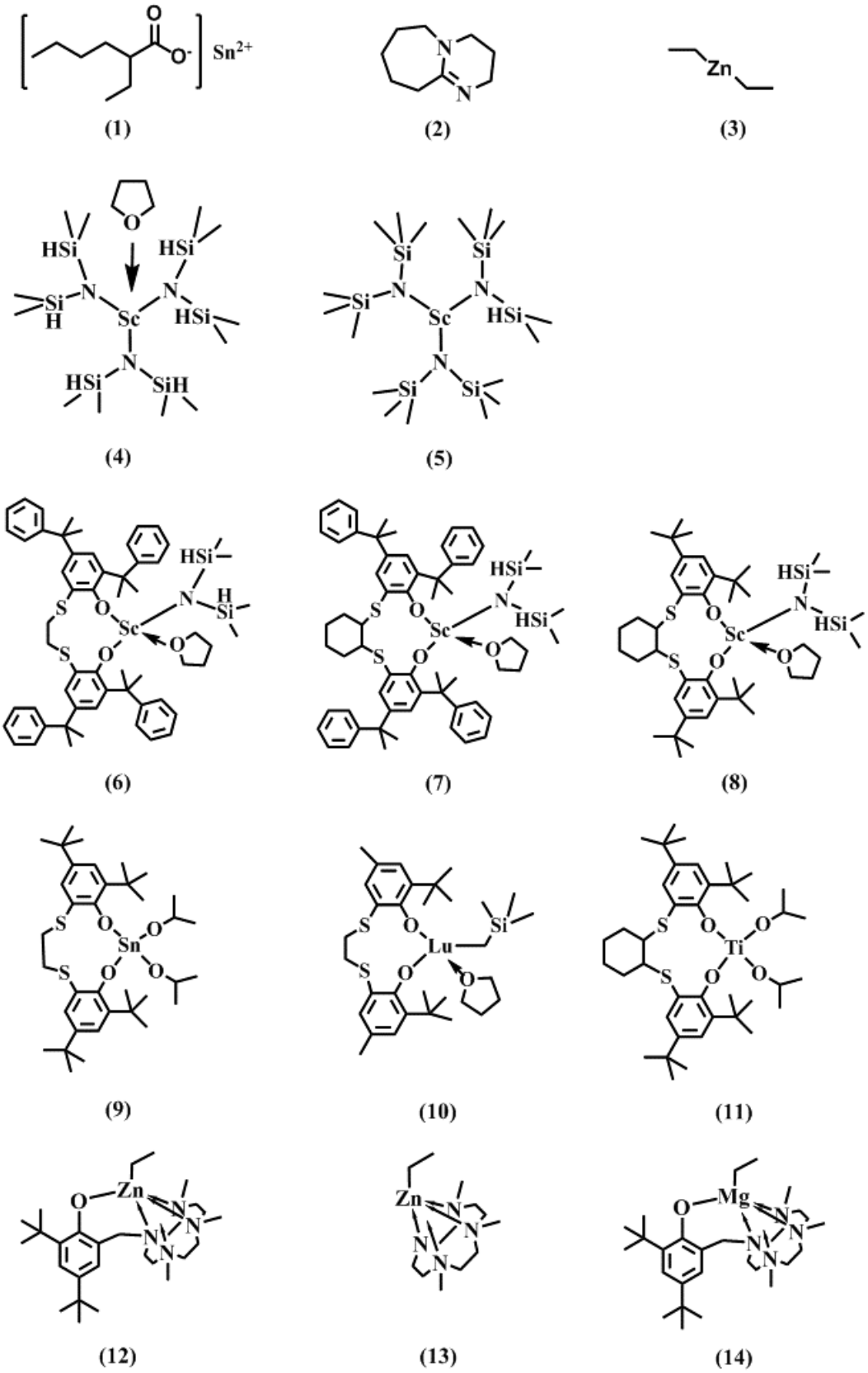

Figure 3-2. Chemical structure of catalysts, (**1**) Stannous octanoate, (**2**) DBU, (**3**) Diethyl zinc, (**4, 5, 6, 7**, and **8**) Scandium metal complexes, (**9**) Tin metal complexes, (**10**) Lutetium metal complexes, (**11**) Titanium metal complexes, (**12** and **13**) Zinc metal complexes, (**14**) Magnesium metal complexes.

Polymerization by commercial catalysts. Recently, Amsden and coworkers reported that kinetic of TMCM-MOE3OM polymerization was slow when commercial Tin(II) 2-ethylhexanoate ($SnOct_2$, **1**) as catalyst and benzyl alcohol (BnOH) as an initiator were employed under low monomer concentration at 130°C.[12] Herein, the bulk polymerization of TMCM-MOE3OM homopolymer have been investigated at the range between 100 and 160 °C with or without the initiator (Table 3-1, entries 1 and 2) within 24hr. As co-initiator involved with proper elevated temperature, the molecular weight 5,000 g/mol and >90% conversion was provided (Table 3-1, entry 2). In contrast to room temperature reaction, there was not observed any polymerization using **1** (Table 3-1, entry 3). This is consistent with what has been found in previous, the moderate temperature is essential for activated mechanism TMC[15] and TMCM-MOE3OM. However, a more broaden distribution and not much enlargement of molecular weight could be reached comparing with typical DBU (**2**) reaction. In order of **2** as organocatalyst over 3 day reaction (Table 3-1, entries 4-8), a reaction without BnOH initiator was launched and indicated the possibility of auto-polymerization[12] by monomer itself (Table 3-1, entry 4). With the presence of the initiator, the monomer conversion grew up to 98% with almost similar M_n and PDI (Table 3-1, entry 5). With the highest concentration, the 2M system molecular was reached to 6,300 g/mol and PDI 1.2 (Table 3-1, entry 8) while 0.4M dilute system could not be found polymer (Table 3-1, entry 6). While the dilute system was considered, 1M and 2M CH_2Cl_2 were prepared with expanded reaction time (Table 3-1, entries 7 and 8).

Additionally, the ring opening polymerization of commercial catalyst was studied as diethyl zinc ($ZnEt_2$, **3**) (Table 3-1, entries 9-16). Along $ZnEt_2$/BnOH could co-initiated TMCM-MOE3OM monomer speedily within 2 hour in bulk polymerization with high conversion >95% of which 6000 g/mol molecular weight. Rather narrow polymer distribution (PDI) were also addressed at room temperature of **3** (Table 3-1, entries 9-13). As catalyst **3** co-initiating, fourfold initiator to catalyst were introduced at room temperature were proceeded in dilute system and bulk (Table 3-1, entries 9 and 10). With different catalyst, the same result of dilute system also appeared low molecular weight as 3,000 g/mol (Table 3-1, entry 9) whereas 6,000 g/mol in bulk system (Table 3-1, entry 10). In order to dilute system (Table 3-1, entry 9), the polymerization proceeded slowly with the decreased M_n to approximately 3400 g/mol. Regarding to the diluted system (Table 3-1, entry 9) and elevated temperature condition in bulk (Table 3-1, entry 11), it seems the inherent reactivity is not related to viscosity in this investigation giving less molecular weight of polymer. The polymerization of twofold initiator to catalyst with less amount were also examined and provided at around 5,000-6,000 g/mol (Table 3-1, entries 12 and 13). At -30°C, the reaction was inhibited (Table 3-1, entry 14), while at 60°C was retarded reaction providing few thousand M_n (Table 3-1, entry 11). However, the limitation of **3** in bulk was apparent at 800:400 of catalyst: initiator ratio with no polymerization (Table 3-1, entry 15). According to bulk system, it is notably that 200:100 ratio was optimize condition comparing with less, twice and triple mole ratio (BnOH : catalyst) were indicated

efficiency reduction (Table 3-1, entries 12,13,15 and 16). Nevertheless, using available $ZnEt_2$ instead of DBU catalyst at ambient reaction, molecular weight could not be increased under comparable condition. This may alter or improve aspects of shorten reaction time within 2 hr for 5,000-6,000 g/mol Poly(TMCM-MOE3OM) of **3**.

Table 3-1. ROP of TMCM-MOE3OM Initiated by stannous octanoate, DBU and diethyl zinc.

Entry	Catalyst	Complexes (metal)	[Monomer] /[Catalyst]	[Monomer]/ [BnOH]	Temp. (˚C)	Time (h)	Concentration	Conversion (%)	M_n^{theo}[a] ×10³ (g/mol)	M_n^{SEC}[b] ×10³ (g/mol)	PDI^{SEC}[b]
1	**1**	**Sn** (II)	200	-	160	24	bulk	83	49	2.6	1.6
2	**1**	**Sn** (II)	200	100	100	24	bulk	91	53	5.1	1.5
3	**1**	**Sn** (II)	200	100	r.t.	18	bulk	n/a	-	-	-
4	**2**	**DBU**	100	-	r.t.	97	bulk	90	26	5.7	1.2
5	**2**	**DBU**	200	100	r.t.	89	bulk	98	57	5.3	1.2
6	**2**	**DBU**	200	100	r.t.	18	0.4MCH_2Cl_2	n/a	-	-	-
7	**2**	**DBU**	200	100	r.t.	186	1MCH_2Cl_2	95	56	5.4	1.2
8	**2**	**DBU**	200	100	r.t.	162	2MCH_2Cl_2	93	54	6.3	1.2
9	**3**	**Zn** (II)	200	50	r.t.	144	0.5M CH_2Cl_2	53	31	3.4	1.2
10	**3**	**Zn** (II)	200	50	r.t.	2	bulk	96	56	6.0	1.2
11	**3**	**Zn** (II)	200	50	60	142	bulk	55	32	1.8	1.4
12	**3**	**Zn** (II)	200	100	r.t.	18	bulk	95	56	6.6	1.1
13	**3**	**Zn** (II)	400	200	r.t.	24	bulk	72	84	5.2	1.1
14	**3**	**Zn** (II)	400	200	-30	24	bulk	n/a	-	-	-
15	**3**	**Zn** (II)	800	400	r.t.	24	bulk	n/a	-	-	-
16	**3**	**Zn** (II)	70	100	r.t.	15	bulk	86	18	5.4	1.3

[a]Theoretical M_n calculated by ([monomer]/[catalyst]) ×292.33×% conversion.
[b]Determined by SEC measurement *vs* polystyrene standards in THF solvent at ambient.

Interestingly, the kinetics comparative relation between DBU and $ZnEt_2$ were demonstrated as plot in Figure 3-3. Bulk polymerization of Zn involved indicated predominant over DBU dealing with high conversion (Figure 3-3a) and molecular weight (Figure 3-3e). While diluted system wasrevealed slow growth of polymerization (Figure 3-3b) as well as molecular weight (Figure 3-3g). From these results it is clear that solvent could possible retarded reaction and affected to the chain growth in this experiment (Figure 3-3d and 3h).

Notably, DBU and Zn catalyst appearance were both provide capable of narrow distribution (Figure 3-4a-4d).This delivers significantly better results due to the perfect linear of **3** (Figure 3-4a) was represented as ideal growth of molecular weight with conversion. Contrary to the findings of slow initiation, the results were clarified using organocatalyst (Figure 3-4c and 4d) and chain transfer on the molecular weight evolution with the presence of solvent (Figure 3-4b). By using readily screening, metal catalysts, such as Zn-based catalyst, could be a potentially candidate to control the reaction of TMCM-MOE3OM. Therefore, we next move to the various metal-based catalysts.

Among the interesting discrete metal complexes as the initiators of ROP, we focused on the results of their PLA stereo selective control. The effort devoted to initiator design were became wide spread out, TMCM-MOE3OM monomer are now confronted with the difficulty in molecular weight control under hypothesis involving transesterification reaction. Therefore, the organometallic complexes were considered to examine with wide potential catalyst in several of substitutes, ligand and metal centers with blind studies.

Polymerization by traditional complexes metal coordinating lanthanoid tris(trimetylsilyl)amide ($M(N(SiMe_3)_2)_3$. With bulky hydrocarbon backbone built-in base, compound could be soluble in wide range of organic solvent. Therefore, the first series, rare earth Scandium (Sc) metal center with silylamide, were studied at moderate reaction

temperature (50 °C). $Sc(NSiMe_3)_3 \cdot thf$ as complexes (**4**) and $Sc(NSiMe_3)_3$ (**5**) were commonly used as catalysts and precursors for synthesis another derivative. This study has shown that **4** catalyzed the reaction with 55% conversion providing broaden PDI around 5 (Table 3-2, entry 1), while **5** was inactive (Table 3-2, entry 2).

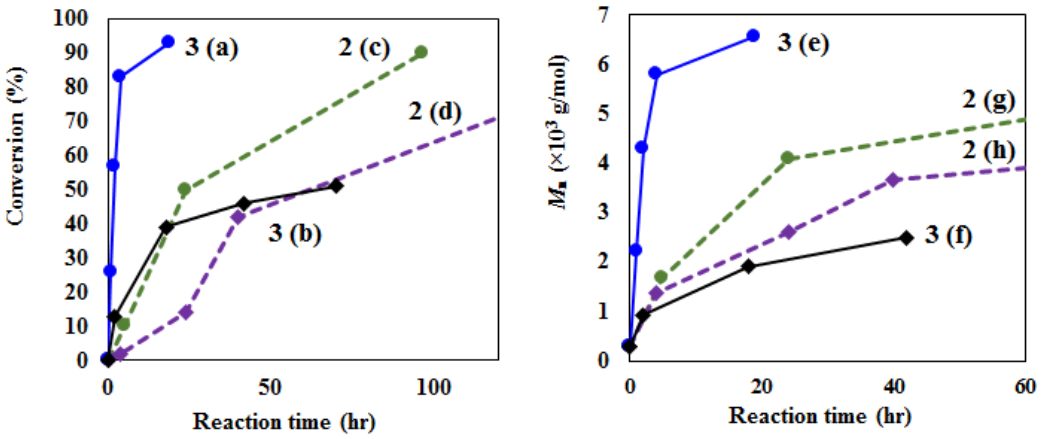

Figure 3-3. Monomer conversion using catalyst **3** in bulk reaction (a) and 0.5M CH_2Cl_2 (b) and using catalyst **2** in bulk reaction (c) and 1M CH_2Cl_2 (d). The growth of molecular weight using catalyst **3** in bulk reaction (e) and 0.5M CH_2Cl_2 (f) and using catalyst **2** in bulk reaction (g) and 1M CH_2Cl_2 (h).

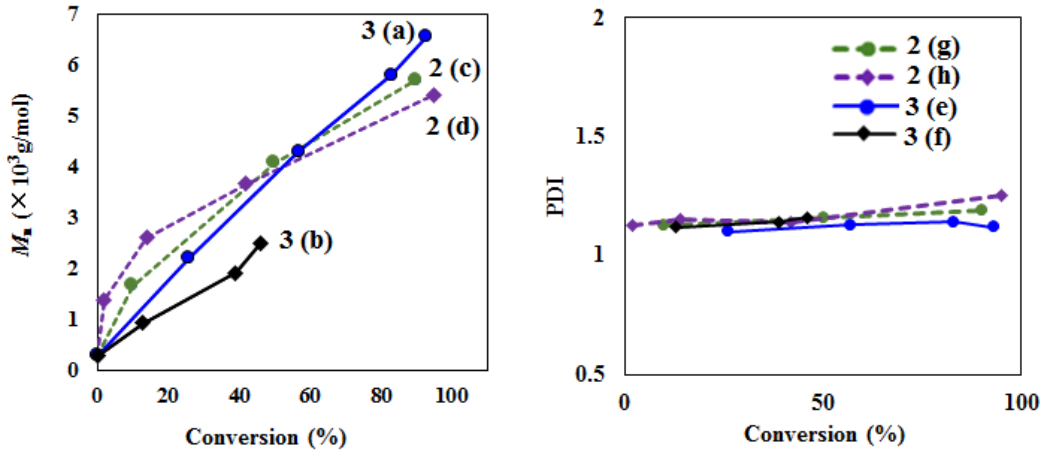

Figure 3-4. Molecular weight corresponding to conversion using catalyst **3** in bulk reaction (a) and 0.5M CH_2Cl_2 (b) and using catalyst **2** in bulk reaction (c) and 1M CH_2Cl_2 (d). Polymer distribution corresponding to conversion using catalyst **3** in bulk reaction (e) and 0.5M CH_2Cl_2 (f) and using catalyst **2** in bulk reaction (g) and 1M CH_2Cl_2 (h).

Although there are no exact data or evidence, the possible reason could be considered in type of the homoleptic or heteroleptic complexes which could not drive initiation step during ROP, while it is with tetrahydrofuran coordinating presented some polymerization. Thus, the coordinated complexes then were investigated.

Polymerization by rare-earth metal coordinating with 1,ω-dithia-alkanedinyl-bridged bisphenolato tetradentate [OSSO]-type. A straightforward synthesis of a new type of tetradentate dianionic [OSSO]-type complexes were determined due to potentially highly selective catalyst for PLA or styrene polymerization. The OSSO ancillary ligand has previously been found to active initiator for PLLA.[27] Among the variation of the steric ortho-substituents, alter bridges and metal were examined with TMCM-MOE3OM.

As compound **6**, **7**, and **8**, Sc based catalysts were almost successfully good performed with trivial heating up (Table 3-2, entries 3-9). The corresponding C_2-bridge which aliphatic binding, methylene as **6** (Table 3-2, entry 3) was not much active ROP of TMCM-MOE3OM comparing with alicyclic bridge of **7** (Table 3-2, entry 4). The polymer catalyzedby **7** was obtained at 50 ◦C within 48 hr at around 80% conversion while **6** was 10%. As similarity coordination ligand, cumyl substitute on OSSO ligand and thf coordinated molecule, the data obtained is broadly consistent with the major trends of bridge effect between sulfur atom, corresponding to bond length and flexibility of backbone. Obviously, **7** and **8** proceeded very

well with themselves initiator without coinitiator of BnOH. According to the degree of polymerization of **7** (Table 3-2, entries 5-8), the relative of monomer to catalyst readily rationalized by consideration of poly(TMCM-MOE3OM) average molecule at final. At 50 °C reaction, M_n of each monomer/catalyst ratio 100, 400 and 600 were raised to 6,900, 7,600 and 8,100 g/mol as theoretical ideal (Table 3-2, entries 5, 6, and 8). At 100 °C, catalyst 7 and 8 have also provided potential reactivity to ester-free cyclic ring close to each other in range of PDI 1.5 and decent molecular weight 6,900 g/mol (Table 3-2, entries 7 and 9). As present data, the finding was quite surprising that **7** and **8** were the trivial differ at substitute on OSSO-type bis(phenolate) ligand, whereas cumyl group for **7** and tert-butyl group for **8**. Hence, it was shown that the substitute was not main role to the catalytic acitivity. Despite to [OSSO]-type bis(phenolate) ligand derivative, catalyst with central metal of Sn (**9**) (Table 3-2, entries 10 and 11), Lu (**10**) (Table 3-2, entries 12 and 13), and Ti (**11**) (Table 3-2, entry 14) showed an inactivate ring-opening polymerization. Probably discussion, our group have also considered the consequences of metal-type coordinate and the presence of bis(alkoxide) ligand instead of silylamide ligand along OSSO-type heteroleptic complexes. Thus, the precise reaction of complexes are influenced by metal size, Lewis acidity and further parameters which are tricky to predict. Therefore, it is essential to be tested, another single active side along macrocyclic N-based type ligand were proceeded in next investigation.

Polymerization by metal coordinating with a cyclic tetra-amine 1,4,7-trimethyl-1,4,7,10-tetraazacyclododecane, (NNNN)-type macrocyclic ligand (Me_3TACD)[29]. As tetradentate cyclen complex derivative was also reported which performed highly active for ambient ROP of PLA within 30 min (100 equiv monomer).[29] In parallel, Metal group II and Zn(II) based complexes were positively achieved, giving similarly TMCM-MOE3OM polymer generating as DBU. Thus, our influential hypothesis in ROP reaction under amido base ligand became apparently a consideration that main aim. From this standpoint, the macrocyclic (NNNN) type ligand coordinating with Zn and Mg depicting structure in **12**, **13** and **14**, was referred in Table 3-3.

Table 3-2. ROP of TMCM-MOE3OM Initiated by diverse rare-earth metal coordinating with [OSSO]-type ligand, bis(dimethylsilyl)amido group and bis(alkoxide).

Entry	Catalyst	Complexes (metal)	[Monomer] /[Catalyst]	[Monomer] /[BnOH]	Temp. (˚C)	Time (h)	Conversion (%)	M_n^{theo}[a] $\times 10^3$ (g/mol)	M_n^{SEC}[b] $\times 10^3$ (g/mol)	PDI^{SEC}[b]
1	**4**	**Sc** (III)	100	100	50	48	55	16	0.581	4.9
2	**5**	**Sc** (III)	100	100	50	48	n/a	-	-	-
3	**6**	**Sc** (III)	100	100	50	48	10	3	1.1	1.1
4	**7**	**Sc** (III)	100	100	50	48	83	24	3.9	1.3
5	**7**	**Sc** (III)	100	-	50	71	96	28	6.9	1.4
6	**7**	**Sc** (III)	400	-	50	71	96	112	7.6	1.3
7	**7**	**Sc** (III)	400	-	100	21	89	104	6.9	1.5
8	**7**	**Sc** (III)	600	-	50	144	95	167	8.1	1.3
9	**8**	**Sc** (III)	400	-	100	164	91	106	6.9	1.5
10	**9**	**Sn** (IV)	100	-	r.t.	71	n/a	-	-	-
11	**9**	**Sn** (IV)	100	-	100	24	n/a	-	-	-
12	**10**	**Lu** (III)	100	100	50	48	n/a	-	-	-
13	**11**	**Ti** (IV)	100	-	r.t.	71	n/a	-	-	-
14	**11**	**Ti** (IV)	100	-	100	24	n/a	-	-	-

[a]**Bulk polymerization. Theoretical M_n calculated by ([monomer]/[catalyst]) × 292.33 × % conversion.**
[b]**Determined by SEC measurement *vs* polystyrene standards in THF solvent at ambient.**

The present study confirmed the findings about the worse and inactivate reactivity were clearly distinguished between bulk and diluted system with differ of organic solvent (Table 3-3, entries 1-3). It performs well, giving good results of polymerization with Zn and Mg complexes have done with 2-4 hour and almost >90% conversion. Besides, the consequence influence of monomer to catalyst and initiator were involved with range 20 to 0.5 mmol equivalent with slightly large PDI under Zn (**12**) (Table 3-3, entries 4-9). Due to single site reactivity compound, one to one feeding ratio of [In]/[Cat] is established, however, four and eightfold of [In] to [Cat] were investigated (Table 3-3, entries 10 and 11) with no significant distinguish and reach to ceiling at around 5,000 g/mol. From these results it is clear that the success of control molecular weight of PTMCM-MOE3OM in range 3,200-11,400 g/mol (Table 3-3, entries 4-9). Most interesting part came up with very tiny amount of catalyst keep affording to polymerization up to 0.5 mmol equiv. The limitations are becoming clear at that very low initiator, it was defined conversion only 39% with multimodal peaks at top of 11,400 (Table 3-3, entry 9). On the other hand, the rapid polymerization with 8,000 g/mol was perfectly overcome with >97% conversion (Table 3-3, entry 8). Additionally, it seems like **13** and **14** attribute to ROP reaction providing narrow PDI and more enlarged M_n comparison in same condition of **12** (Table 3-3, entry 5, 12, and 13). According to unlike structure of ligand between **12** and **13**, 2,4-di-tert-butylphenolate (dbp) was also chelating to the metal. Nevertheless, the low stability of **13** was occurred and dead easily during the stock solution preparation. It could

be possible that the dbp chelating may help more stability of Zn complex. With chemically exhibit on common oxidation state (+2), and the Zn^{2+} and Mg^{2+} ions are of similar size. Superior results are seen for Mg complex **14**, which possessed {Me_3TACDH}-dbp ligand as similar to **12** (Table 3-3, entries 13-15), presented appealing reactivity within 2 hour polymerization obtaining narrow PDI as well as DBU and M_n up to 7,000 g/mol (Table 3-3, entries 13 and 14). The limitation is apparent introducing with low initiator (Table 3-3, entry 15), the polymer could not be obtained. However, less stability occurred again which this does seem to depend on Mg metal center effect.

Table 3-3. ROP of TMCM-MOE3OM Initiated by Zn and Mg metal coordinating with tetradentate (NNNN) cyclen derivatives.

Entry	Catalyst	Complexes (metal)	[Monomer] /[Catalyst]	[Monomer] /[BnOH]	Time (h)	Solvent	Conversion (%)	M_n^{theo} [a] $\times 10^3$ (g/mol)	M_n^{SEC} [b] $\times 10^3$ (g/mol)	PDI^{SEC} [b]
1	**12**	**Zn** (II)	100	100	2	0.3M toluene	90	26	1.7	1.6
2	**12**	**Zn** (II)	100	100	2	0.3M THF	88	26	2.6	1.9
3	**12**	**Zn** (II)	400	400	2	0.3M CH_2Cl_2	n/a	-	-	-
4	**12**	**Zn** (II)	50	50	2	bulk	98	14	3.2	1.8
5	**12**	**Zn** (II)	100	100	4	bulk	97	28	3.7	1.8
6	**12**	**Zn** (II)	200	200	4	bulk	96	56	4.4	1.9
7	**12**	**Zn** (II)	400	400	2	bulk	97	113	5.7	1.8
8	**12**	**Zn** (II)	1000	1000	2	bulk	97	284	8.1	1.8
9	**12**	**Zn** (II)	2000	2000	2	bulk	39	228	11.4	1.4
10	**12**	**Zn** (II)	400	100	4	bulk	96	112	5.0	2
11	**12**	**Zn** (II)	800	100	4	bulk	73	171	4.9	1.4
12	**13**	**Zn** (II)	100	100	4	bulk	91	27	5.6	1.2
13	**14**	**Mg** (II)	100	100	2	bulk	95	28	7.4	1.2
14	**14**	**Mg** (II)	400	400	2	bulk	98	115	6.9	1.4
15	**14**	**Mg** (II)	1000	1000	2	bulk	n/a	26	-	-

[a] **Reaction at room temperature. Theoretical M_n calculated by ([monomer]/[catalyst]) × 292.33 × % conversion.**
[b] **Determined by SEC measurement *vs* polystyrene standards in THF solvent at ambient.**

In addition, the absence of BnOH during ROP with only diehtylzinc and monomer could be operated polymer as well as isolated DBU appearance (Table 3-1, entry 4). Furthermore, the supporting of involved autopolymerization of monomer was revealed with only catalyst presence as well as Amsden group investigation.[12] Therefore, the proposed mechanism for autopolymerization could be possibly occurred. Nonetheless, the final actual molecular weight of PTMCM-MOE3OM were massively far away corresponding to catalyst rational raising. The low ROP efficiency may distribute to the ring-chain equilibrium of TMC itself consistance[12] among available complexes catalysts could accompanied along polymerization. Apparently, the assumption of ROP factor also concerned with decent amount, correlating to metal-type, functional ligand and stability of complexes to overcome with high control polymerization.

It is worth discussing these interesting facts revealed by the results of catalytic comparison among commercial (Table 3-1), rare-earth OSSO-type complexes (Table 3-2) and metal-tetradentate Me_3TACDH (Table 3-3). Overall, our method was the one that obtained the most robust results that we acknowledge the considerable discussions among this demonstration, the elevated temperature and dilute system are obstacles for TMCM-MOE3OM polymerization. According to the bulky ligands, each species have several aspects. However, ligand type generally tends to decrease fluxionality, reaction space and provide well defined coordination. As results, the rare-earth with bulky ligand could be led good polymerization but still suffering

with extended reaction time. Our data indicate that complexes **12**, **13** and **14** in Table 3-3; a result that casts a new light on shorten reaction time, ease system and dealing with the decent M_n as same as commercial DBU with >90% conversion at ambient. As proposed for the *i*ROP of LA [17,30] and TMC[17] by binary catalyst. As previous investigation, the ROP catalysis with Zinc and Magnesium chelating diamino-phenolate as almost similar structure to **12** and **14** were initiated along TMC.[31] Our results of catalyst **12** and **14** concerned that the phenol group might not actually be necessary. To our knowledge and data, this is the first screening report of organometallic catalysis on TMCM-MOE3OM, the author expected similar pattern of ROP by coordination-insertion.[26] The initial work hypothesis of ROP mechanism for TMCM-MOE3OM significantly Zn-center simple activated monomer and alcohol may attack the monomer, although the discrete detail is still unclear as well as the tacticity control.

3.4 Conclusions

The polymerization of TMCM-MOE3OM by rare earth metal coordinating tris(dimethylsilyl)amido complexes, bis(phenolate) complexes and macrocyclic tetradentate (NNNN)-type cyclen were employed as screening initiation using single-site metal initiators. Collectively, our results appear consistent with Sc-OSSO type and the dilute system of Zn-(NNNN) ligand presented few thousand M_n, regarding to catalyst type and kinetic of polymerization. In these dominant results, only bulk polymerization reached the effective of catalytic polymerization, whereas slightly M_n enlargement and accelerated time procedure under neat argon environment in glove box along simple condition (room temperature) providing PDI below 2 were concluded. Especially, the presence of Zn (II) metal chelating Me_3TACD-dbp and Sc (III) catalyst with bulky ligand OSSO were provided consequence as well as DBU typical candidate with shorten polymerization time and slightly molecular weight increment, respectively. More generally, these basic findings are consistent with research showing that Sc (II)-tris(trimetylsilyl)amide could not activate ROP of TMCM-MOE3OM.

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Concluding Remark

The objective of the thesis in topic of “Preparation of Thermosensitive Biodegradable Poly(trimethylene carbonate) Derivatives with Oligo(ethylene glycol)” are presented historical development of biodegradable materials along TMCM-MOE3OM monomer. I was wholly endeavor to investigate and discuss in scientific comprehension including the stimuli effect examination, hydrogel characterization and alternative inorganic complexes candidating along ring opening polymerization (ROP).

In chapter 1, “Control of Thermoresponsivity of Biocompatible Poly(trimethylene carbonate) with Direct Introduction of Oligo(ethylene glycol) under Various Circumstances”, the multifunctional monomers were designed and copolymerization. In this content modifications, all functional monomers were studied thermal- pH- and photo- responsive via external triggers after polymer modification. Meaningfully, the cloud point or thermal responsive were examined and discussed LCST change effects in details in this partition. Therefore the balance of hydrophilic and hydrophobic molecules is point out under core monomer of TMCM-MOE3OM unit. However, the mechanical properties of homopolymer and copolymer were possibly insufficient to apply as biomaterials in advance. Therefore, the chapter 2 was created and investigated to approach more close to the clinical application.

“Creation of Thermosensitive Biodegradable Hydrogel Using Poly(5-[2-{2-(2-

methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one) Derivatives with cross linkers" as title of chapter 2, it is valuable and preferable to fabricate polymeric three-dimensional network materials based on ester-free TMC derivative as gel form. The presence of thermal response along biocompatible TMCM-MOE3OM hydrogels is challenged and successfully overcome by adding resemble chemical crosslinked agents. After fabrication, the surface wettability and *in vitro* test, which deal with different ratio of crosslinker-type and TMC precursors were also defined and made discussion. Especially, mechanical property as one of the most essential property were demonstrated in advance of application. Interestingly, the resistance of hydrogel along lipase condition (as internal body state) resistance was notably premised. As demanding of polymeric materials in further application was origin to chapter 3.

Finally, to look into the detailed of ROP comparison, "Polymerization control of 5-[2-{2-(2-methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one by Various Catalysts and Initiators" was addressed in chapter 3. Various catalysts were examined to initiate polymerization underneath perfect argon atmosphere (glove box) with alternative catalysts and initiators. Instead of using general organocatalyst, rare earth metal complexes were investigated and expressed as comparative choices for TMC polymerization. Prosperously, PTMCM-MOE3OM could be end up polymerization within 2 h and high monomer conversion which involved of some speedily ROP catalyst. However, the molecular weight by SEC spectroscopy is not much increased comparing with DBU/BnOH condition. Herein, the preliminary universal

screening of inorganic complexes were studied in screening and specific in Zn based and bulky coordinating ligand.

To date the novel design molecular is key of basic knowledge in order to occupy in clinical biomaterials field. The main aim of graduation has been reached. The author suggested that our potential report and publications expectantly keep valuable less or more in the future and next generation. With the establishment of first TMC hydrogel fabrication and polymerization property by our group, all investigations were categorized and summarized as valuable research and interesting materials as elementary for the future biomaterials application.

List of Publications

Chapter 1:

Nalinthip Chanthaset, Yoshikazu Takahashi, Yoshiaki Haramiishi, Mitsuru Akashi, Hiroharu Ajiro,

"Control of Thermoresponsivity of Biocompatible Poly(trimethylene varbonate) with Direct Introduction of Oligo(ethylene glycol) under Various Circumstances",

J. Polym. Sci. Part A: Polym. Chem **2017**, *55*(20), 3466-3474.

Chapter 2:

Nalinthip Chanthaset, Hiroharu Ajiro,

"Preparation of Thermosensitive Biodegradable Hydrogel Using Poly(5-[2-{2-(2-methoxyethoxy)ethyoxy}-ethoxy methyl]-5-methyl-1,3-dioxa-2-one) Derivatives,"

Materialia, submitted on 29th June **2018**.

Chapter 3:

Nalinthip Chanthaset, Klaus Beckerle, Jun Okuda, Hiroharu Ajiro,

"Investigation on Polymerization of 5-[2-{2-(2-methoxyethoxy)ethyoxy}-ethoxymethyl]-5-methyl-1,3-dioxa-2-one by Organometallic Catalysts",

Polymer Chemistry submitted on 5th July **2018.**

Other Publications

(1) **Nalinthip Chanthaset,** Hiroharu Ajiro, Mitsuru Akashi, and Chantiga Choochottiros, "A novel comb-shaped polymethacrylate-based copolymers with immobilized 2,4-dihydroxybenzaldehyde for antifungal activity", *Polym. Bull*. **2018**, *75(4)*, 1349-1363.

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Nalinthip CHANTHASET