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ชื่อโครงการ ไมเซลล์พอลิเมอร์เตรียมจากการดัดแปรหลังพอลิเมอไรเซชันของพอลิเมอร์ที่มีเพนทะฟลูออโรเฟนิลเอสเทอร์

Polymeric micelles prepared from post-polymerization modification of pentafluorophenyl ester- containing polymer

ชื่อนิสิต นางสาวยุวพร ภิญญูกิจ

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ปีการศึกษา 2559

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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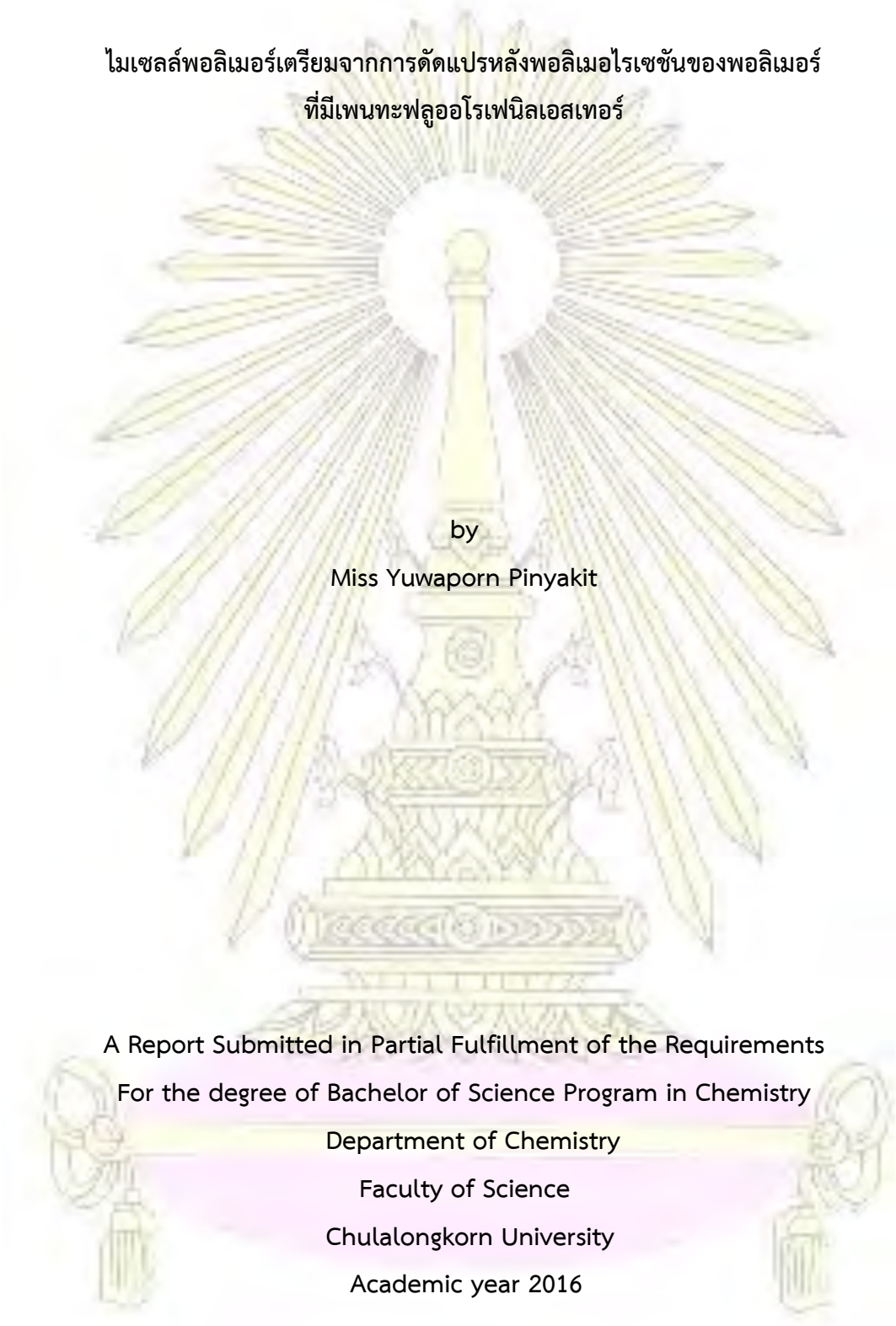

by

Miss Yuwaporn Pinyakit

A Report Submitted in Partial Fulfillment of the Requirements

For the degree of Bachelor of Science Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic year 2016

Title Polymeric micelles prepared from post-polymerization modification of pentafluorophenyl ester- containing polymer

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Accepted by the Faculty of Science, Chulalongkorn University

In Partial Fulfillment of the Requirements for the Bachelor's Degree

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Date.....May, 2017

Quality of report ☑Very Good ☐Good ☐Fair

ชื่อโครงการ ไมเซลล์พอลิเมอร์เตรียมจากการดัดแปรหลังพอลิเมอไรเซชันของพอลิเมอร์ที่มีเพนทะฟลูออโรเฟนิลเอสเทอร์
ชื่อนิสิตในโครงการ นางสาวยุวพร ภิญญกิจ เลขประจำตัว 5633132623
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บทคัดย่อ

ไมเซลล์พอลิเมอร์เตรียมจากแอมฟิฟิลิกโคพอลิเมอร์ได้รับการยอมรับว่าเป็นพาหะที่มีประสิทธิภาพในการนำส่งแบบควบคุมของสารที่ใช้ในการบำบัดรักษา เช่น ยา, ยีน, โปรตีน งานวิจัยก่อนหน้านี้ได้ประสบความสำเร็จในการเตรียมนาโนเจลที่ตอบสนองต่อการเกิดปฏิกิริยารีดอกซ์ด้วยการดัดแปรหลังพอลิเมอไรเซชันของโฮโมพอลิเมอร์ตั้งต้นเพียงชนิดเดียวที่มีหมู่เพนทะฟลูออโรเฟนิลเอสเทอร์ ดังนั้นในงานวิจัยนี้ผู้วิจัยสนใจที่จะศึกษาต่อถึงความเป็นไปได้ในการใช้แนวทางเดียวกันในการเตรียมไมเซลล์ที่สามารถตอบสนองต่อการเปลี่ยนแปลงพีเอช เริ่มจากการสังเคราะห์พอลิเพนทะฟลูออโรเฟนิลแอคริเลต (PPFPA) ผ่านปฏิกิริยาพอลิเมอไรเซชันด้วยกลไกแบบ reversible addition-fragmentation chain-transfer (RAFT) จากนั้นทำปฏิกิริยากับ 1-amino-2-propanol เพื่อเตรียมเป็นแอมฟิฟิลิกโคพอลิเมอร์แบบสุ่มของ PPFPA-*r*-PHPA ซึ่งสามารถประกอบตัวเป็นไมเซลล์ได้เองในน้ำ มีขนาดน้อยกว่า 200 นาโนเมตร จากการทำปฏิกิริยาของหมู่เพนทะฟลูออโรเฟนิล (PFP) ที่อยู่ในไมเซลล์กับ 1-(3-aminopropyl) imidazole (API) ทำให้ได้ไมเซลล์ที่ตอบสนองต่อการเปลี่ยนแปลงพีเอช ซึ่งสามารถยืนยันได้จากการแตกออกของอนุภาคหลังจากการลดลงของพีเอชจาก 7.4 เป็น 5 ทั้งนี้เป็นผลจากการเปลี่ยนแปลงประจุของวง imidazole จากเป็นกลางเป็นบวกเมื่อมีการลดพีเอช จากผลการทดลองทั้งหมดแสดงให้เห็นว่าไมเซลล์พอลิเมอร์ที่เตรียมได้ในงานวิจัยนี้มีศักยภาพในการพัฒนาเป็นระบบนำส่งสารที่ใช้ในการบำบัดรักษาแบบมีเป้าหมาย ซึ่งการปลดปล่อยจะถูกกระตุ้นให้เกิดขึ้นได้ภายใต้สภาวะที่เป็นกรด

คำสำคัญ: การดัดแปรหลังพอลิเมอไรเซชัน, แอคทีฟเอสเทอร์,การตอบสนองต่อพีเอช, พอลิเมอร์ไมเซลล์

Project Title Polymeric micelles prepared from post-polymerization modification of pentafluorophenyl ester- containing polymer

Student name Yuwaporn Pinyakit Student ID 5633132623

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Academic Year 2016

Abstract

Polymeric micelles assembled from amphiphilic copolymer have been recognized as effective vehicle for controlled delivery of therapeutic agents such as drug, gene, and protein. We have recently demonstrated that stepwise post-polymerization modification of a single pentafluorophenyl ester-bearing homopolymer can be used as a facile route to redox-responsive nanogels. Here in this research, we would like to explore further the versatility of this similar approach to fabricate pH responsive micelles. Poly(pentafluorophenyl acrylate) (PPFPA) was first synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization. Post-functionalization of PPFPA with varied equivalent of 1-amino-2-propanol yielded amphiphilic random copolymers of PPFPA-*r*-PHPA having different compositions. The copolymers can self-assemble to form micelles in aqueous with sizes of less than 200 nm. By reacting the pentafluorophenyl (PFP) groups remaining in the nanoparticles with 1-(3-aminopropyl) imidazole (API), pH responsive micelles were generated as evidenced by the disintegration of the nanoparticles upon decreasing pH from 7.4 to 5.0. This may be explained as a result of charge transition of the imidazole rings from neutral to positively charged upon pH reduction. These developed nanoparticles possess a strong potential to be used as carriers for targeted delivery of therapeutic agent of which the release can be triggered under an acidic condition.

Keywords: post-polymerization modification, active ester, pH responsive, polymeric micelles

Acknowledgments

First of all, I would like to express my deepest gratitude to my advisor, Associated Professor Dr. Voravee P. Hoven for her precious instruction and sincere encouragement throughout this research. I am really appreciate for all inestimable advice, not only the research course but also livelihood. Moreover, I am grateful to all my committee members, Assistant Professor Dr. Varawut Tangpasuthadol and Dr. Junjuda Unruangsri for their kindness in reviewing my report.

I am also to express my sincere thanks to Miss Susita Noree as well as all members of VH and VT research groups for their invaluable advice, considerable assistance, and cheerful encouragement during my research course.

Financial support for this research work was provide by Faculty of Science, Department of Chemistry.

Finally, my special thanks go to my beloved family and friends for their support and encouragement throughout my entire study.

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List of Schemes

List of Abbreviation

ACVA	: 4,4'-Azobis(4-cyanovaleric acid)
API	: 1-(3-Aminopropyl) imidazole
CMC	: Critical micelle concentration
CPADB	: 4-Cayano-4-(thiobenzoylthiol) pentanoic acid
CTA	: Chain transfer agent
DCC	: Dicyclohexylcarbodiimide
DLS	: Dynamic Light Scattering
FTIR	: Fourier-Transform Infrared Spectroscopy
PBS	: Phosphate buffered saline
PDI	: Polydispesity index
PFPA	: Pentafluorophenyl acrylate
PHPA	: Poly(*N*-(2-hydroxypropyl) acrylamide)
PPFPA	: Poly(pentafluorophenyl acrylate)
RAFT	: Reversible addition-fragmentation chain transfer
TEM	: Transmission Electron Microscope

Chapter 1

Introduction

1.1 Motivation

Polymeric micelles prepared from amphiphilic copolymer have been widely used in drug delivery systems, especially for water insoluble drugs. In polar environment, the amphiphilic copolymer can assemble into micelles. As driven by hydrophobic interactions, the hydrophobic segments of the copolymer would aggregate to form a core and have the hydrophilic entities situate exteriorly as a shell. Not only the polymeric micelles can encapsulate the insoluble drug in the core but also reduce toxicity [1,2] and maintain the stability of the drugs by the hydrophilic shell. Moreover, the modification of polymeric micelles with specific functions such as pH sensitive segments, targeting ligand or thermoresponsive groups etc. can enhance their specificity to target cells [3-7].

Normally, the copolymer is classified into two types. The first one is a block copolymer, monomers which its are arranged in a form of block and each block repeats systematically with analogous species. For this reason, the block copolymer can patently exhibit characteristic properties of each species. However, preparation of the block copolymer often requires tedious process and is challengeable when functional monomers are sequentially added onto polymer chains. The second type is a random copolymer, by which different monomer species are randomly arranged onto the copolymer chain. Synthetic routes to the random copolymers are less rigorous and simpler to be performed as opposed to those of the block copolymers. Applications of the amphiphilic random copolymers in many fields such as drug delivery, diagnosis, sensing have been so far reported [8].

Because amphiphilic copolymer consists of two monomeric repeating units having different solubility, to attain the copolymer with controllable composition via direct copolymerization of the two comonomers is not always successful. Post-polymerization modification has been recognized as an alternative method for the synthesis of such a functional amphiphilic copolymer that has some limitations toward polymerization conditions, incompatibility with solvents, or cannot be prepared by direct polymerization. The post-polymerization modification can be used to provide a wide range of side-chain functionalities. The general principle is a chemical conversion of functional polymer precursors to new functional polymers. That is why the post-polymerization modification is an interesting method for synthesizing amphiphilic copolymer or copolymer combined with multifunctional components [9]. Moreover, the post-polymerization modification can modify polymer chain to obtain various functional moieties in one pot synthesis. Active ester is one of the functional groups often used for the preparation of polymer precursors, *N*-hydroxysuccinimide (NHS) derivative in the form of *N*-acryloxysuccinimide (NAS) and *N*-methacryloxysuccinimide (NMAS), are common examples. Besides the NHS, pentafluorophenyl ester (PFP) derivative, pentafluorophenyl acrylate (PFPA) and pentafluorophenyl methacrylate (PFMA), have recently emerged as more favorable alternatives. Having five fluorine atoms in the phenyl ring, PFP group possesses higher reactivity towards nucleophilic substitution by when compared to the NHS derivatives. Furthermore, PFP derivatives are soluble in a broad range of solvents, whereas NHS derivatives are only soluble in DMF and DMSO [26]. Selective post-polymerization modification by nucleophilic species, especially primary amines are widely used because the reaction can be accomplished under a mild condition.

In 2009, Gibson and coworkers [10] studied on the post-polymerization modification of poly(pentafluorophenyl methacrylate) (PPFMA) with different molecular weights and diverse primary amine nucleophiles. The result indicated that PPFMA with different molecular weights showed good conversions with various nucleophilic amines and the substitution is molecular weight independent [**Figure 1.1**]. Furthermore, the degree of post-polymerization modification can be controlled by verifying the relative amount of desired amines to the ester group. The cytotoxicity investigation of the obtained polymethacrylamides against EaHy 926 huan endothelial cells was carried out and found that the polymethacrylamides showed no toxicity toward the cells. The study also expanded to compare the cytotoxicity of the polymethacrylamides with the poy(*N*-(2-hydroxypropyl) methacrylamide)0 (PHPMA) derived from a direct polymerization.

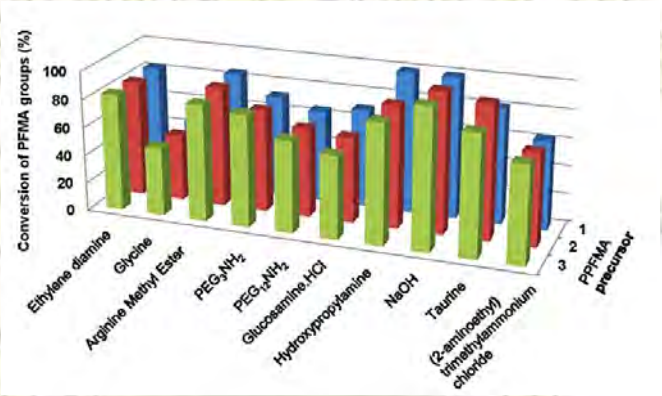


Figure 1.1 Degree of conversion of PPFMA precursors of different molecular weight after post-polymerization modification with a range of different functionalized amines [10].

In 2012, Gunay and coworkers [11] used the reversible addition-fragmentation chain transfer polymerization (RAFT) of pentafluorophenyl methacrylate (PFMA) to prepare active ester polymer brushes and studied the feasibility of this thin polymer film for post-polymerization modification with various amines [**Figure 1.2**]. This method can be used to prepare polymer brushes that cannot be prepared by direct surface–initiated controlled radical polymerization (SI-CRP), for example, functional monomers that can form complexes with catalyst or react with the propagating radical species in solution. The reaction was monitored by FT-IR spectroscopy and x-ray photoelectron spectroscopy (XPS). The result showed that post-polymerization modification of PPFMA polymer brushes with amine can be completed. Furthermore, the conversion of post-modified PPFMA depends on steric hindrance and nucleophilicity of amines.

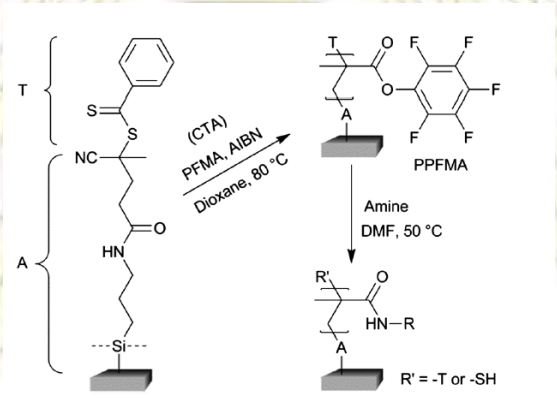


Figure 1.2 Synthesis and post-polymerization modification of PPFMA brushes [11].

In 2015, He and coworkers [12] studied on dual stimuli-responsive block copolymers. Poly(methyl salicylate acrylate)-*b*-poly(pentafluorophenyl acrylate) was used as polymer precursor and reacted with different amines via a post-polymerization modification to prepare responsive functional block copolymers. Moreover, the reactivity of different active ester, methyl salicylate acrylate (MSA) and PFPA, was investigated by using hexylamine as a reference amine and monitored by FT-IR spectrometry and ^{1}H NMR spectroscopy. The result showed that conversion of PFPA reached a 100% conversion after 1 h, whereas methyl salicylate ester group showed low conversion after 1 h [**Figure 1.3**]. This emphasized the higher reactivity of PFPA ester than corresponding MSA. Preparation of dual stimuli-responsive block copolymers by post-polymerization modification of double reactive block copolymer with various amines, benzylamine, *N,N*-diethylethylenediamine, isopropylamine and cyclopropylamine, was monitored by FT-IR spectrometry and ^{1}H NMR spectroscopy and assured the success of this method.

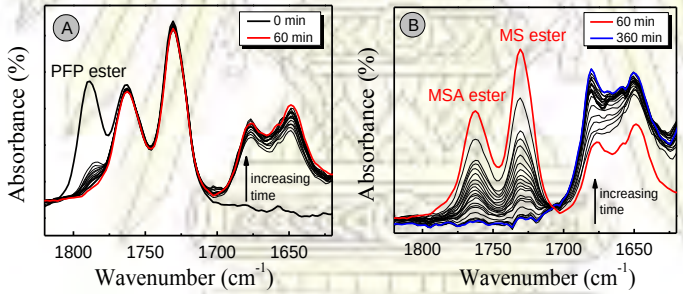


Figure 1.3 IR spectra of PPFPA and poly(methyl salicylate acrylate) in the presence of hexylamine [12].

In 2014, Singha and coworkers [13] synthesized poly(pentafluorophenyl methacrylate) by atom transfer radical polymerization (ATRP) to prepare polymer precursor. The polymer precursor reacted with allylamine via a post-polymerization modification to generate alkene group on the side chain. After that, alkene-functionalized polymer was reacted with the peptide CVPGVG by thiol-ene radical addition for preparing peptide-based polymer applied in the area of biomedical and biomimetic material [**Figure 1.4**]. The result showed that preparation of peptide-based polymer from polymer precursor bearing active ester functional group was accomplished. Although this method can modify the desired pendant on the side chain, degree of the second step post-polymerization modification was comparatively low as a result of steric hindrance of the relatively large peptide chains.

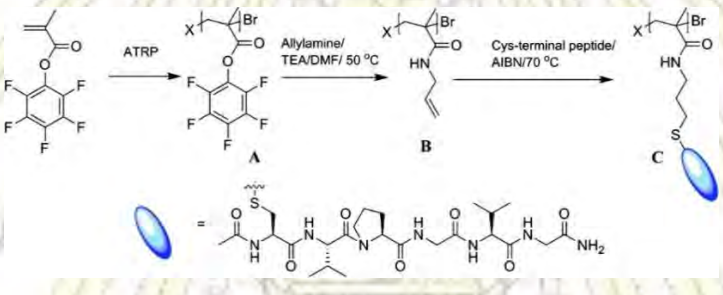


Figure1.4 Biofunctionalized polymethacrylate via post-polymerization modification [13].

In 2016, Gunay and coworkers [14] synthesized cyclic peptide disulfide-poly(*N*-(2-hydroxypropyl) methacrylamide) (PHPMA) conjugates by post-polymerization modification of poly(pentafluorophenyl methacrylate) (PPFMA). This approach used *N*-terminal amine of cyclic peptide disulfides (CXC) to conjugate active ester functional polymer. The post-polymerization modification is an alternative method to prepare CXC-polymer conjugates instead of thiol-based conjugation strategies, which can cause side reactions between free thiols and disulfides. The PPFMA precursor reacted with propargylamine and dansyl cadaverine in the primary post-polymerization modification step for preparing dual functional conjugates. Subsequently, the remaining pentafluorophenyl groups reacted with 1-amino-2-propanaol to result in PHPMA copolymer. Finally, the CXC conjugated to copolymer scaffold via CuAAC coupling.

In 2011, Zhuang and coworkers [15] prepared nanogels from amphiphilic random copolymer containing poly(ethylene glycol methacrylate) (PEGMA) as the hydrophilic unit and poly(pentafluorophenyl acrylate) (PPFPA) as the hydrophobic unit. The active ester of PPFPA was used to react with cross-linker, cystamine and hexamethylenediamine, and isopropylamine via a post-polymerization modification to improve the stability of the nanogels and eliminate the residual PFP moieties respectively [**Figure 1.5**]. The results showed that the post-polymerization modification can yield multifunctional nanogels. Furthermore, the nanogels were proven to be potential drug delivery system for encapsulation of lipophilic guest molecules as a drug model.

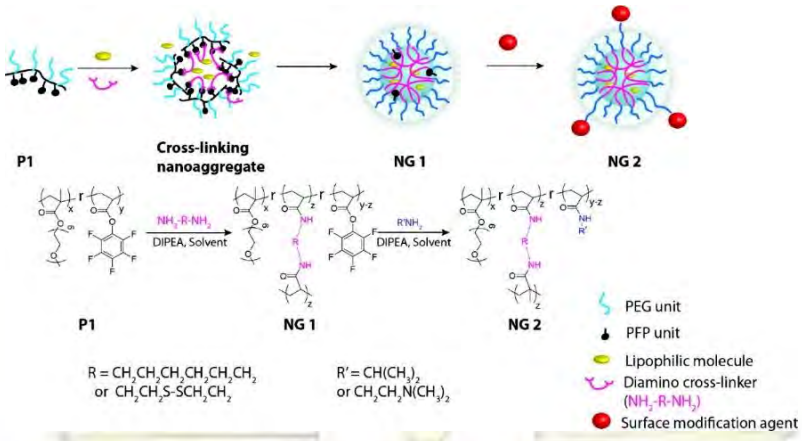


Figure 1.5 Schematic route of design and synthesis of the cross-linked polymer nanogels [15].

In 2010, Jochum and coworkers [16] prepared double thermoresponsive block copolymer containing biotin end group. RAFT polymerization is used to synthesize poly(oligo(ethylene glycol) methyl ether methacrylate-*block*-poly(pentafluorophenyl mathacrylate) (POEGMA-*b*-PPFPMA) followed by the post-polymerization modification with isopropylamine and *N*-biotinyl aminoethyl methanethiosulfonate to give poly(oligo(ethylene glycol) methyl ether mathacrylate -*block*-poly(*N*-isopropyl methacrylamide) (POEGMA-*b*-PNIPMAM) containing biotin end group within one step. PPFPA was converted to PNIPMAM via aminolysis and biotin end group was obtained by direct conjugation of dithioester end group. The results showed that post-polymerization modification is a facile and efficient approach to modify multifunctional components simultaneously. Subsequently, block copolymer formed micelles at 50 ^{o}C, because of the thermoresponsive behavior of PNIPMAM [**Figure 1.6**].

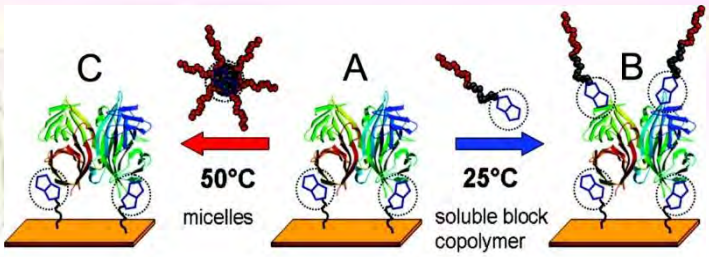


Figure 1.6 Structural change of polymeric micelles at different temperature [16].

In 2016, Chan and coworkers [17] prepared polymeric mixed micelles from two different block copolymer, poly(ethylene glycol)-cathechol (PEG-cathechol) and poly(ethylene glycol)-imidazole (PEG-imidazole) through the post-polymerization modification of mPEG-*b*-polycarbonate bearing pentafluorophenyl (PFP) moieties. Active ester of PFP on the copolymer chain reacted with catechol and imidazole functional group [**Figure 1.7**]. Afterwards, primary amine present in doxorubicin (DOX) was conjugated to catechol side chains via the aza-Michael addition followed by a tautomerization to give acid labile bond.

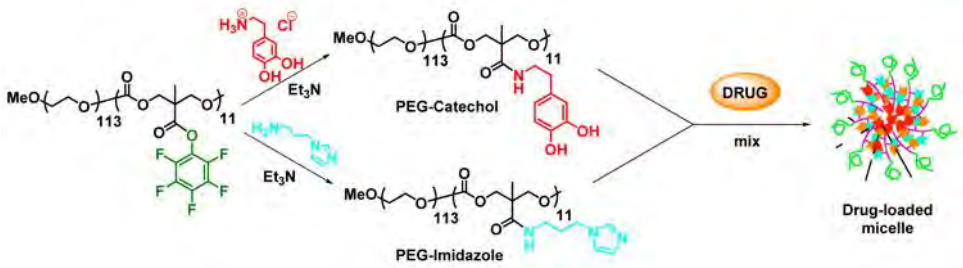


Figure 1.7 Post-polymerization modification of mPEG-*b*-polycarbonate copolymers via pentafluorophenyl active ester and mixed micelles system [17].

In 2017, Noree and coworkers [25] prepared redox-responsive nanogels by using stepwise post-polymerization modification of a single homopolymer. Poly(pentafluorophenyl methacrylate) (PPFPMA) was first synthesized by RAFT polymerization as a polymer precursor followed by the post-polymerization modification with varied equivalent of oligo(ethylene glycol) methyl ether amine (OEG-NH_2). The amphiphilic copolymer, poly(pentafluorophenyl methacrylate) (PPFPMA)-*co*-poly(oligo(ethylene glycol methacrylamide) (POEGMAM), self-assembled to form micellar nanoparticles in aqueous media with size of less than 100 nm. After self-assembly, the micelles were post functionalized with cystamine as a dithiol crosslinker to prepare nanogels. Subsequently, the last step of post-functionalization was performed in suspension with isopropylamine in order to remove the residual pentafluorophenyl (PFP) groups in the nanogels. The results showed that the stepwise post-polymerization of a single homopolymer is successful. Moreover, the release of developed nanogels can be accelerated by glutathione especially at 37 °C. Moreover, cytocompatibility evaluation (MTT assay) showed that the nanogels post functionalized with IPA were non-toxic in the range of 0.016-2.0 mg/mL.

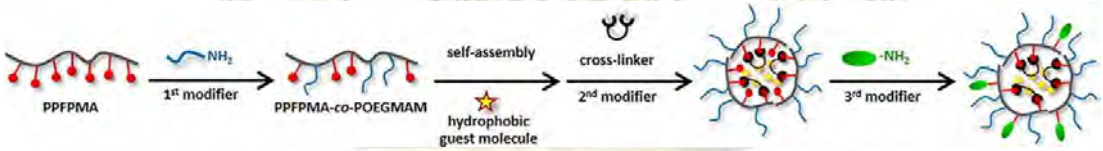


Figure 1.8 Cascade post-polymerization modification of PPFPMA with amine modifiers to generate nanogels as redox-responsive carriers for hydrophobic guest molecules. [25].

To build up specific function of polymeric micelles, pH responsive micelles are attractive systems especially for anti-cancer drug carrier. Normally, pH responsive polymeric micelles can be prepared using acid labile bond, i.e. hydrazone, benzoic-imine, or pH responsive monomer, i.e. histidine, 1-(3-aminopropyl) imidazole.

In 2015, Yang and coworkers [18] prepared pH responsive polymeric mixed micelles from amphihilic block copolymer, cRGD-PEO-*b*-PCL, and PEG conjugated drug with benzoic imine bond. The block copolymer was synthesized using a sequential ring opening polymerization followed by self-assembled in aqueous media to form polymeric micelles. Although the preparation of the pH responsive amphiphilic copolymer can be accomplished, the experimental procedure is rather complicated.

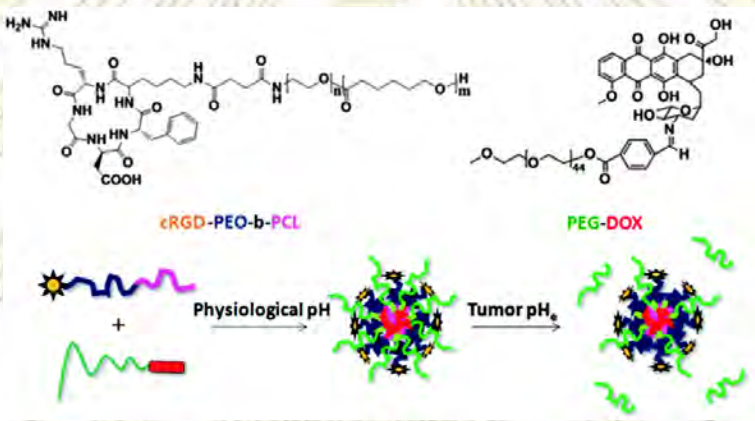


Figure 1.9 Illustration of mixed micelle formation by cRGD-PEO-b-PCL and PEG-DOX, and the pH induced structural change [18].

In 2015, Guan and coworkers [19] prepared pH responsive amphiphilic block copolymer containing imidazole ring. The synthetic method was performed for 72 h using the ring opening polymerization of *N*-carboxyanhydride of β-benzyl-L-glutamate. mPEG-NH_2 was used as a macroinitiator. Then, 1-(3-aminopropyl) imidazole (API) was conjugated by a coupling reaction. However, the experimental procedure is time-consuming and complicated

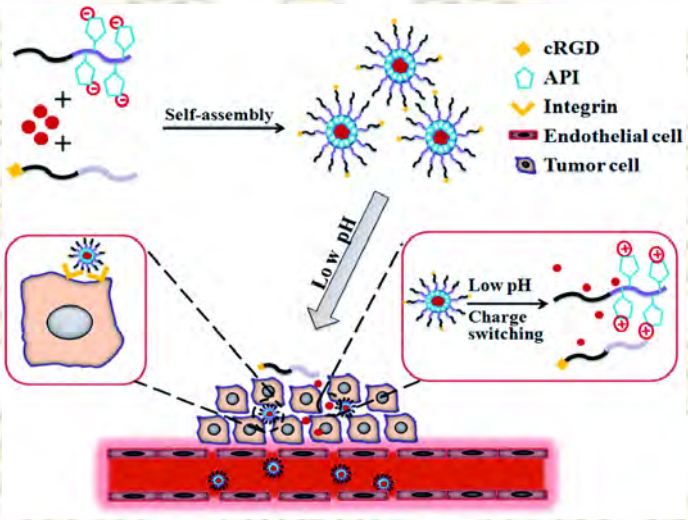
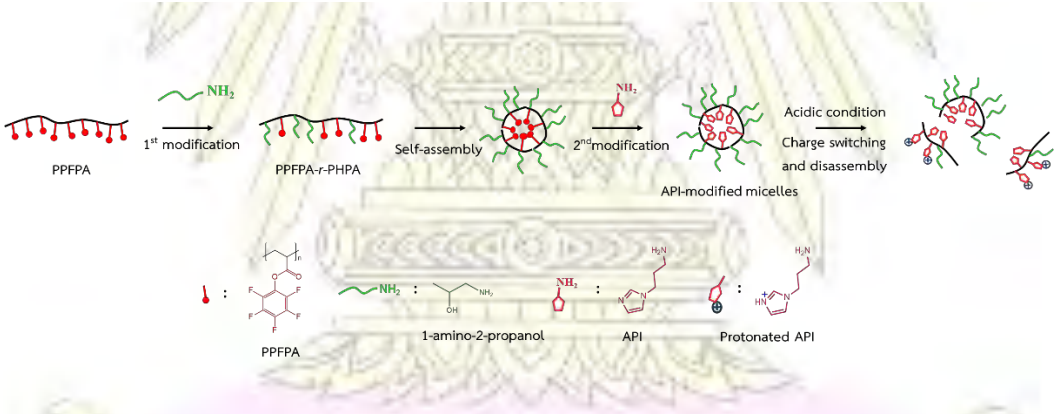


Figure 1.10 Schematic illustration of self-assembly and tumor cell uptake of the cRGD target that has been incorporated into pH-triggered surface charge-switchingpolymer micelles [19].

Herein, pH responsive polymeric micelles prepared from amphiphilic random copolymer was developed through a post-polymerization modification by simply starting with a single homopolymer precursor. Poly(pentafluorophenyl acrylate) (PPFPA) was synthesized via a reversible addition-fragmentation chain transfer (RAFT) polymerization as a polymer precursor and reacted with 1-amino-2-propanol to obtain amphiphilic random copolymer, poly(pentafluorophenyl acrylate)-*random*-poly(*N*-(2-hydroxypropyl) acrylamide) (PPFPA-*r*-PHPA). Subsequently, the residual active ester groups were modified with 1-(3-aminopropyl) imidazole (API) after micelle formation to form charge conversion moieties under an acidic condition [**Scheme 1**]. API is recognized as a pH-responsive ionizable group (pKa ≈ 6.7) and its primary amine is reactive towards the active ester on the polymer backbone to form an amide linkage under a mild condition. Additionally, the charge conversion property of imidazole ring has been studied in many previous works in terms of enhancing cellular uptake or acidic pH-triggered response [19-21]. Therefore, this work can be useful for the future development of pH-responsive micellar systems for target delivery applications.



Scheme 1 Illustration of the preparation of pH-triggered polymeric micelles by a post- polymerization modification.

1.2 Objectives

1. To synthesize and characterize amphiphilic random copolymer by the post-polymerization modification of PPFPA with 1-amino-2-propanol
2. To prepare and characterize polymeric micelles from the synthesized amphiphilic random copolymers
3. To modify polymeric micelles with 1-(3-aminopropyl) imidazole to yield pH-triggered polymeric micelles

1.3 Expected result

pH-triggered polymeric micelles prepared by the post-polymerization modification of pentafluorophenyl ester-containing polymer

Chapter 2

Experimental

2.1 Instruments

1. Balances (Precisa, Model XT220A, Switzerland)
2. Hot plate-stirrer (IKA, Model C-MAG HS 7, Germany)
3. Freeze dryer (LABCONCO, Model 77535-01, USA)
4. Nuclear magnetic resonance spectrometer (^{1}H NMR) (Model Mercury, Varian 400 MHz, USA)
5. Infrared spectrometer (FTIR) (Nicolet Impact 6700 FT-IR, USA)
6. FT-NMR spectrometer (Bruker AVANCE III HD 500 MHz, USA)
7. Gel Permeation Chromatography (GPC) (Model Waters 600, USA)
8. Transmission electron microscope (TEM) (JEOL JEM-1400, Japan)
9. Zetasizer Nano ZS (Malvern Instrument, U.K.)
10. Fluorescence spectrophotometer (Agilent, Model Cary Eclipse, USA)

2.2 Materials

All solvents used for reactions are analytical grade and used as received, unless otherwise specified. 1,4 Dioxane (anhydrous, 99.9%), pentafluorophenol (PFP), and 1-amino-2-propanol were commercially available from Merck, Germany. Dichloromethane (DCM) was dried over CaH_2 and reflux under N_2 atmosphere before use. 4,4'-Azobis (4-cyanovaleric acid) (ACVA), dicyclohexylcarbodiimide (DCC), anhydrous N,N'-dimethylformamide (DMF, 98.8%), 1-(3-aminopropyl) imidazole (API), phosphate buffered saline, pH 7.4 (PBS), and pyrene were purchased from Sigma-Aldrich, USA. Acrylic acid (AA) was obtained from Sigma-Aldrich, USA and distilled under vacuum before use. 4-cyanopentanoic acid dithiobenzoate (CPADB) was obtained from Santa Cruz Biotechnology, USA.

2.3 Characterization

The polymers before and after modification were characterized by nuclear magnetic resonance spectroscopy (NMR) using a Varian, model Mercury-400 nuclear magnetic resonance spectrometer (USA) operating at 400 MHz for ^{1}H NMR and Fourier transform infrared spectroscopy (FTIR) using a Nicolet Impact 6700 FT-IR spectrometer. ^{19}F NMR spectra were recorded on a Bruker AVANCE III HD (500 MHz) FT-NMR spectrometer. Fluorescence intensity was measured by a Fluorescence spectrophotometer (Agilent, Model Cary Eclipse, USA). Molecular weight of the polymers were measured by Waters 600 controller chromatograph equipped with two HR (Waters) columns (HR2 and HR4) (MW resolving range = 100-500,000 Da) at internal column temperature of 35°C and a refractive index detector (Waters 2414). THF was used as a solvent for the polymers and as an eluent for GPC analysis with the flow rate of 1.0 mL/min. Five polystyrene standards (996–188,000 Da) were used for generating a calibration curve. The hydrodynamic size and zeta potential of micelles were determined using a dynamic light scattering (DLS) instrument (Zetasizer Nano ZS, Malvern Instrument Ltd., U.K.) equipped with a He-Ne laser beam at 658 nm at a fixed scattering angle of 173°. The sample refractive index (RI) was set at 1.59 for polystyrene. All samples were filtered through a millipore 0.45 μm nylon membrane before analysis. The morphology of micelles was analyzed by transmission electron microscopy (TEM) by a JEOL JEM-1400 (Japan).

2.4 Experimental procedure

2.4.1 Synthesis of pentafluorophenyl acrylate (PFPA)

Pentafluorophenyl acrylate (PFPA) was synthesized according to a modified method of Jochum and Theato [22]. In brief, Pentafluorophenol (26.7 g, 0.14 mol) was dissolved in dry dichloromethane (DCM) (10 mL) in a round bottom flask under cooling on an ice bath. Acrylic acid (AA) (9.9 mL, 0.14 mol) was added dropwise into the solution. Then, a solution of dicyclohexylcarbodiimide (DCC) (30.1 g, 0.14 mol) dissolved in dry dichloromethane (10 mL) was added dropwise into the solution mixture. After the solution was stirred for 2 h, a precipitate of dicyclohexylurea was removed by vacuum filtration and washed thoroughly with dichloromethane. The filtrate was evaporated to remove dichloromethane. The crude product was then purified by column chromatography (column materials: silica gel; solvent: hexane). Colorless liquid was obtained in 78% yield.

2.4.2 Synthesis of poly(pentafluorophenyl acrylate) (PPFPA)

Poly(pentafluorophenyl acrylate) (PPFPA) was synthesized via a RAFT polymerization according to the previously published method with a slight modification [24]. In brief, PFPA (2 M) dissolved in 2 mL of dioxane was added to a glass vial. Then, 1 mL of dioxane containing ACVA (16.8 mg, 0.06 mmol) as an initiator and CPADB (4.2 mg, 0.015 mmol) as a chain transfer agent were added. The sealed vial of the solution mixture was purged with N_2 gas for 30 min. Subsequently, the vial was immersed in a preheated oil bath at 70 °C for 7 h. The mixed solution was then cooled down to room temperature, precipitated in methanol, and then reprecipitated from THF in methanol twice. Pink powder product was obtained in 73% yield.

2.4.3 Post-polymerization modification of PPFPA with 1-amino-2-propanol

PPFPA (200 mg, 0.44 mmol, 1 eq. of PFP groups) was dissolved in 0.5 mL of anhydrous DMF. Then, 1-amino-2-propanol of varied equivalent to PFP groups of PPFPA (0.25, 0.50, and 0.75 eq.) in 0.5 mL of anhydrous DMF was added to the PPFPA solution. The solution mixture was stirred at room temperature for 24 h. The solution was precipitated in diethyl ether. Random amphiphilic copolymers of poly(pentafluorophenyl acrylate)-*random*-poly(*N*-(2-hydroxypropyl) acrylamide) (PPFPA-*r*-PHPA) having varied compositions were obtained after re-dissolving the precipitate in DMF and then reprecipitated in diethyl ether twice. The products were dried at 50°C in a vacuum oven overnight.

2.4.4 Micelle Formation

PPFPA-*r*-PHPA micelles were prepared by solvent exchange method. The copolymer, PPFPA-*r*-PHPA obtained in 2.4.3, having desired composition was dissolved in 0.1 mL DMF. Then, phosphate buffer saline (PBS, pH 7.4) was added dropwise under vigorous stirring. The micelle solution was stirred for 1 h and dialyzed against PBS with PBS being changed twice per day for 3 days (dialysis tube, MW cut off = 3500 Da).

2.4.5 Determination of critical micelle concentration (CMC)

The critical micelle concentration (CMC) was determined by fluorescence spectroscopy using pyrene as a hydrophobic fluorescence probe according to the previously published method with a slight modification [23]. Briefly, 6 µL of pyrene in acetone (1.2×10^{-4} M) was added into an empty glass vial and stirred to evaporate acetone. Then, micelles solution of different concentration (0.05 to 8 mg/mL) was added into dry pyrene in each vial. The vial was heated at 60 °C for 2 h to equilibrate the pyrene and the micelles. The solution was cooled down to room temperature and stirred for 24 h before fluorescence intensity was measured. The excitation was set at the fluorescence intensity of 336 nm and emission was recorded from 346 to

600 nm. The first inflection point on the plot intensity ratio of I_{374}/I_{394} against log concentration of polymer was determined as a CMC value.

2.4.6 Post functionalization of micelles with 1-(3-aminopropyl) imidazole

1-(3-Aminopropyl) imidazole (API) (100μL, 0.838 mmol) was added into the micelles solution of PPFPA-*r*-PHPA. The reaction was stirred for 24 h at room temperature in order to replace the remaining PFP moieties in the micelles with API. The modified micelles solution was purified by dialysis against PBS buffer with PBS being changed twice per for 3 days (dialysis tube, MW cut off = 3500 Da) to eliminate unreacted API. The suspension in dialysis bag was freeze-dried to obtain white powder of API-modified micelles as a product.

2.4.7 Determination of pH responsive property of API-modified micelles

The pH of a API-modified micelles suspension was adjusted by adding 0.1 M HCl solution or 0.1 M NaOH solution. The size and zeta potential measurements of the micelles were performed by dynamic light scattering (DLS) to monitor the charge conversion under pH variation.

Chapter 3

Results and Discussion

3.1 Synthesis of polypentafluorophenyl acrylate (PPFPA)

3.1.1 Synthesis of pentafluorophenyl acrylate (PFPA)

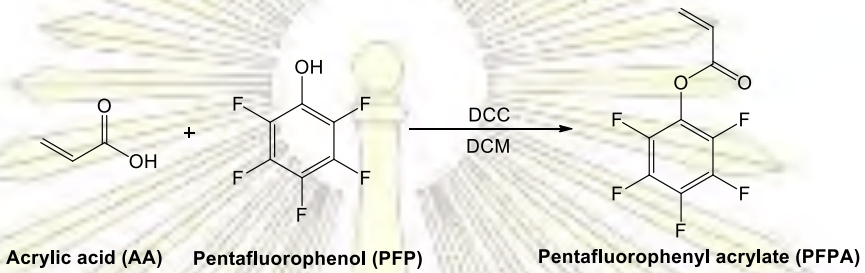


Figure 3.1 Synthesis of PFPA.

The PFPA monomer was synthesized by coupling reaction between acrylic acid and pentafluorophenol using DCC as a coupling reagent [**Figure 3.1**]. The crude product was purified by filtration to remove a by-product, dicyclohexylurea and column chromatography to get rid of unreacted acrylic acid, yielding colorless liquid product in 78% yield. The product was characterized by ^{1}H NMR spectroscopy. ^{1}H NMR spectrum [**Figure 3.2**] shows characteristic signals of PFPA monomer. The proton resonances, a, b, and c, are doublet of doublet with different coupling patterns. The signal of proton a was observed at δ of 6.2 ppm. This can be explained by coupling constant of geminal coupling, proton a and b (J_{ab}), and cis vicinal coupling of proton a and c (J_{ac}) which $J_{ab} < J_{ac}$. For the proton b, the signal was observed at δ of 6.7 ppm. This is due to coupling constant of geminal coupling, proton a and b (J_{ab}), and trans vicinal coupling (J_{bc}) which $J_{ab} << J_{bc.}$ The signal of proton c was observed at δ of 6.4 ppm explained by cis coupling constant (J_{ca}) and trans coupling constant (J_{cb}) which $J_{cb} > J_{ca}$.

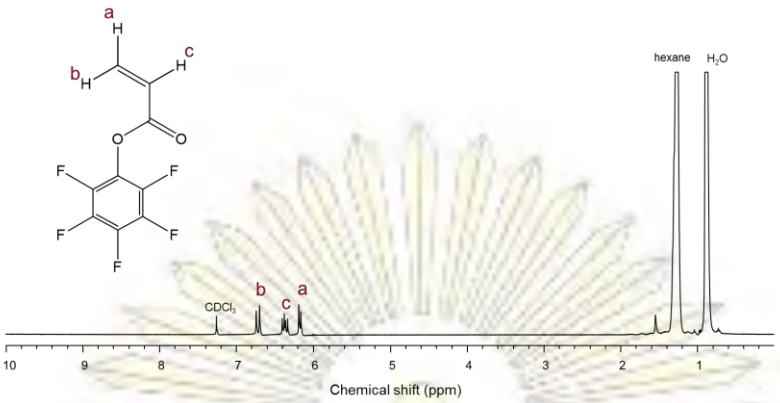


Figure 3.2 ^{1}H NMR spectrum in $CDCl_3$ of PFPA.

3.1.2 Synthesis of PPFPA by Reversible addition- fragmentation chain transfer (RAFT) polymerization

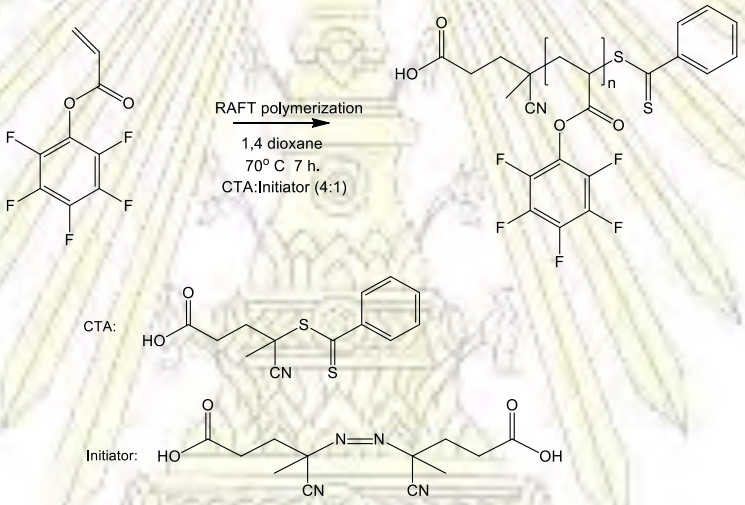


Figure 3.3 Preparation of PPFPA by a RAFT polymerization.

PPFPA homopolymer was synthesized by a RAFT polymerization using 4-cyano-4-phenyl carbothioylthiopentanoic acid (CPADB) as a chain transfer agent (CTA) and 4, 4' azobis(4-cyanovaleric acid) (ACVA) as an initiator with CPADB:ACVA in a ratio of 4:1. The synthetic method is shown in **Figure 3.3**. After stirring for 7 h at 70°C, the pink solution turned into an orange viscous solution. The solution sample was taken periodically at specific time intervals to determine the percentage conversion of monomer by using ^{1}H NMR spectroscopy and the residual solution was purified by

precipitation. The pink powder product was obtained with 73% yield. According to the ^{1}H NMR spectra [**Figure 3.4a**], the proton signals of polymer backbone (d and e) was observed in a range of δ of 2.0 to 3.2 ppm. After purification, the proton signals from PFPA monomer (6-6.8 ppm) disappeared as show in the ^{1}H NMR spectrum in **Figure 3.4b**, indicating that the PFPA was entirely removed from the product. The percentage conversion of monomer can be calculated from ratio of the resonance integration in ^{1}H NMR spectrum of crude polymer [**Figure 3.4a**] using equation (1). According to the kinetics of the RAFT polymerization of PFPA [**Figure 3.5**], the rate of monomer conversion reached about 93% after 20 h of reaction time. However, the linear relationship was observed from 1 to 7 h [**Figure 3.5B**], indicating that the RAFT polymerization can be well controlled only in this time range. Therefore, the optimal reaction time of 7 h yielded the reasonably high percentage conversion (80%) was selected for the preparation of PPFPA.

$$\%\text{Conversion} = \frac{\int \mathrm{H_e}/2}{\int \mathrm{H_e}/2 + \int \mathrm{H_a}} \times 100 \qquad (1)$$

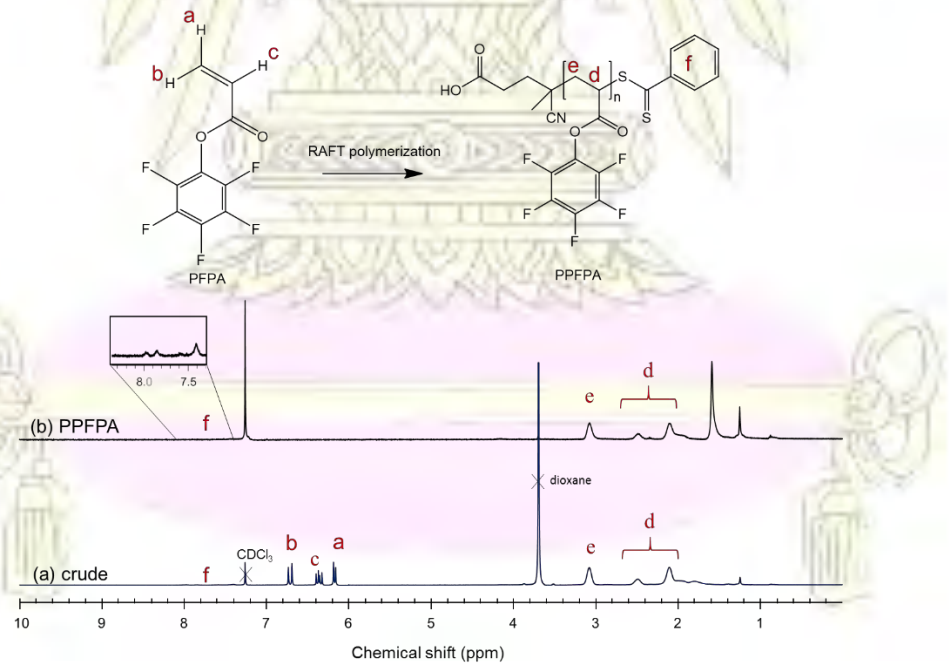


Figure 3.4 ^{1}H NMR spectra in $CDCl_3$ of (a) crude PPFPA and (b) PPFPA after purification.

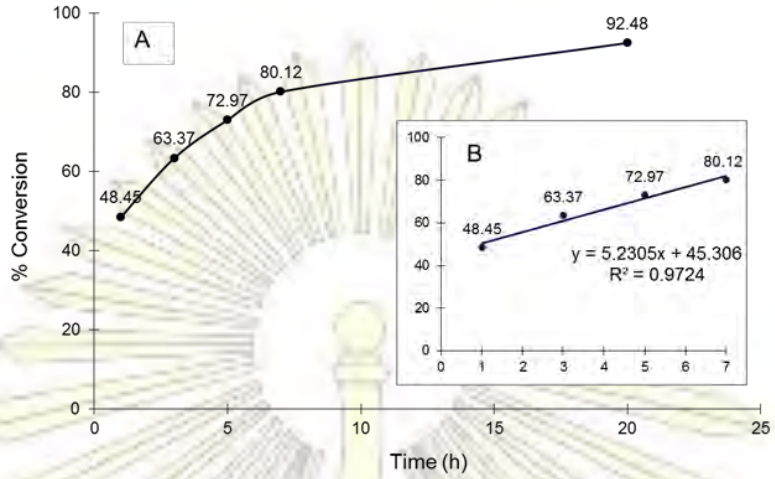


Figure 3.5 Kinetics plot for RAFT polymerization of PFPA.

Number average molecular weight (M_n) of PPFPA was determined by GPC. The results showed that M_n of PPFPA obtained from GPC (24,605 Da) is close to the targeted molecular weight of PPFPA (24,090 Da) calculated from equation (2) for the targeted degree of polymerization of 100. Molecular weight distribution is quite narrow with polydispersity index (PDI) of 1.27. This molecular weight information suggested that RAFT polymerization is well-controlled.

$$M_{n,targeted} = [M_w PFPA \times \text{Targeted degree of polymerization } (100)] + M_w CTA\ (279.38) \quad (2)$$

3.2 Synthesis of amphiphilic copolymers by post-polymerization modification of PPFPA with 1-amino-2-propanol

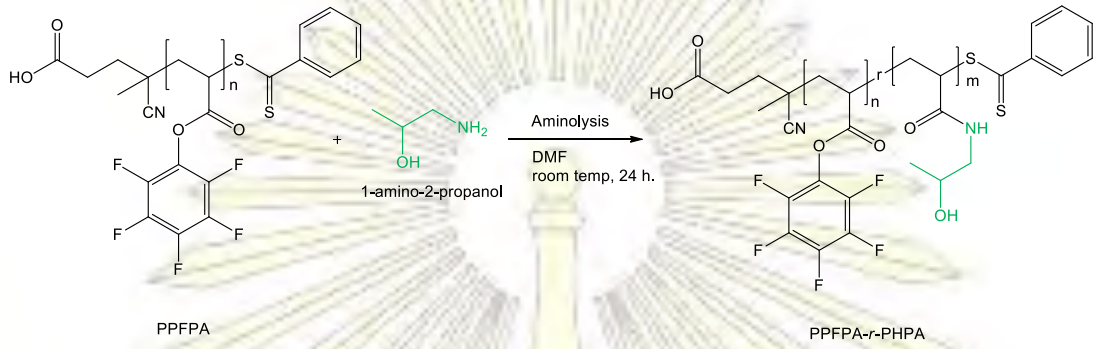


Figure 3.6 Post-polymerization modification of PPFPA with 1-amino-2-propanol.

The PPFPA homopolymer was used as polymer precursor to react with 1-amino-2-propanol in order to prepare amphiphilic random copolymer (PPFPA-*r*-PHPA). The post-polymerization modification of PPFPA was performed at room temperature in anhydrous DMF for 24 h by reacting pentafluorophenyl groups of PPFPA with varied equivalents of 1-amino-2-propanol. The extent of reaction was monitored by ^{1}H NMR and FT-IR spectroscopy.

The ^{1}H NMR spectra of PPFPA both before and after being modified with 1-amino-2-propanol are shown in **Figure 3.7**. The ^{1}H NMR spectrum of 1-amino-2-propanol [**Figure 3.7a**] showed the characteristic signals at δ of 1.2, 2.6-2.8, and 3.8 ppm, which can be assigned to methyl protons (e), methylene protons (c), and methine proton (d), respectively. Upon post-functionalization with 1-amino-2-propanol, the chemical shifts of the methylene protons (a) from the polymer backbone have shifted upfield. The signals of methyl protons (e) and methine proton (d) from 1-amino-2-propanol also emerged in all ^{1}H NMR spectra of the copolymers shown in **Figure 3.7 c-e**.

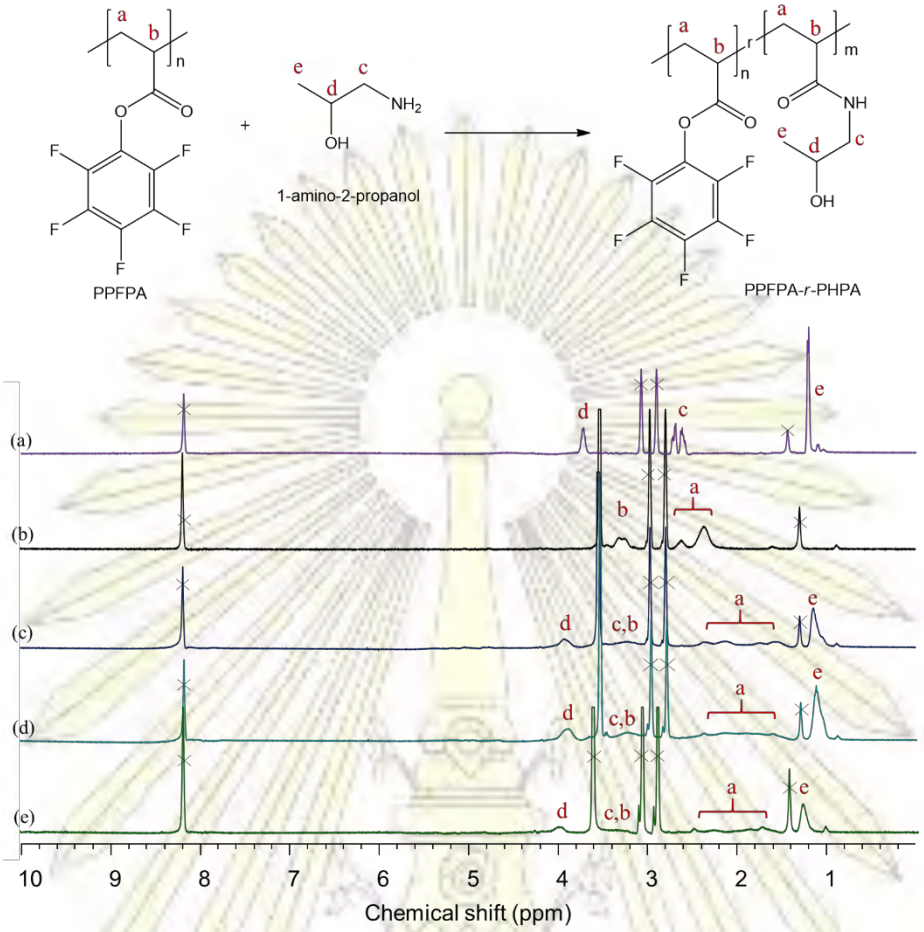


Figure 3.7 ^{1}H NMR spectra in DMF-d_7 of (a) 1-amino-2-propanol and PPFPA both (b) before and after post modified with varied equivalent of 1-amino-2-propanol: (c) 0.25, (d) 0.5, and (e) 0.75.

The copolymer composition can be estimated from ^{1}H NMR peak integration using equation (3). It was found that the post-functionalization with 0.25, 0.5, and 0.75 equivalents of 1-amino-2-propanol to the PFP groups yielded PPFPA-*r*-PHPA with PHPA composition of 22, 53, and 76 %, respectively. These results strongly indicated that the copolymer composition can be effectively tuned by the amount of 1-amino-2-propanol employed for the post-polymerization modification.

$$\%\text{PHPA} = \frac{\int \mathrm{H_d}}{\int \mathrm{H_a}/2} \times 100 \qquad (3)$$

The variation of copolymer composition as a function of 1-amino-2-propanol employed in the step of post polymerization modification can also be verified by FT-IR analysis. The FT-IR spectra [**Figure 3.8**] of PPFPA showed a characteristic C=O stretching signal of an ester bond at 1780 cm^{-1}. Once partially modified with 1-amino-2-propanol to form the copolymer, a new C=O stretching of amide bond emerged at 1660 cm^{-1}, confirming the successful amidation happened between PFP pendant groups of the PPFPA and amino groups of 1-amino-2-propanol. This particular signal proportionally increased as the mole equivalent of 1-amino-2-propanol increased. The copolymer composition can also be evaluated from the relative ratio of these two C=O stretching signals. From the calculation, the composition of PHPA in the copolymer was found to be 27, 55, and 77% upon the post functionalization of PPFPA with 0.25, 0.50, and 0.75 equivalents of 1-amino-2-propanol, respectively. These composition values are in excellent agreement with those determined from the ^{1}H NMR data. Broad OH stretching signal appearing in all spectra of the copolymers also signified the presence of hydroxyl groups inherited from 1-amino-2-propanol in the copolymers.

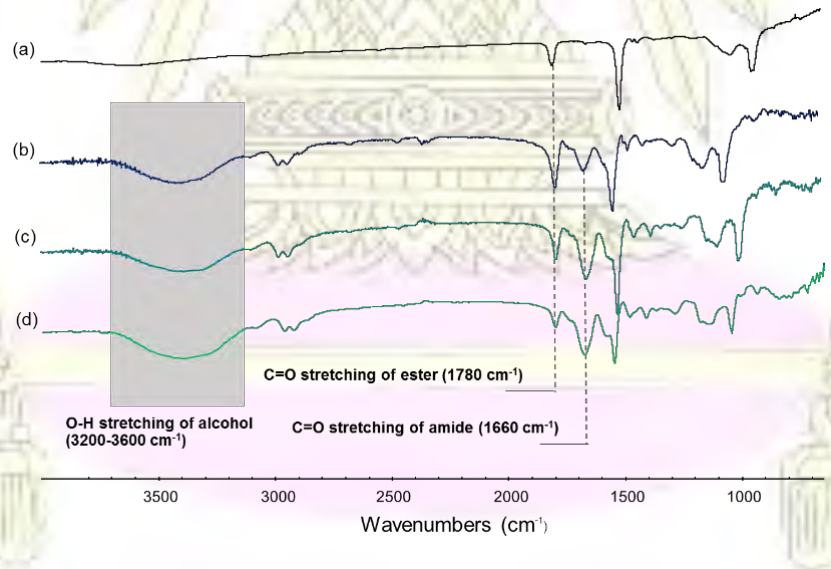


Figure 3.8 FT-IR spectra of PPFPA both (a) before and after post modified with varied equivalent of 1-amino-2-propanol: (b) 0.25, (c) 0.5, and (d) 0.75.

3.3 Micelle formation

Three PPFPA-*r*-PHPA with different composition of PHPA were tested for their amphiphilic character and ability to self-assemble into micelles in aqueous media (PBS, pH 7.4). Morphology of the micelles from all copolymers was investigated by TEM. As shown in TEM [**Figure 3.9A**], spherical-like nanoparticles were formed in all cases. As evaluated by DLS [**Table 3.1**], the size as well as polydispersity index (PDI) of the micelles proportionally increased as the PHPA composition was elevated. This may be describable to the swelling effect in aqueous media of the micelles having higher content of hydrophilic PHPA entities, which in principle should locate exteriorly as shell of the micelles. Moreover, the fact that DLS profiles of all micelles [**Figure 3.9B**] appear as a single peak implies that they have unimodal distribution.

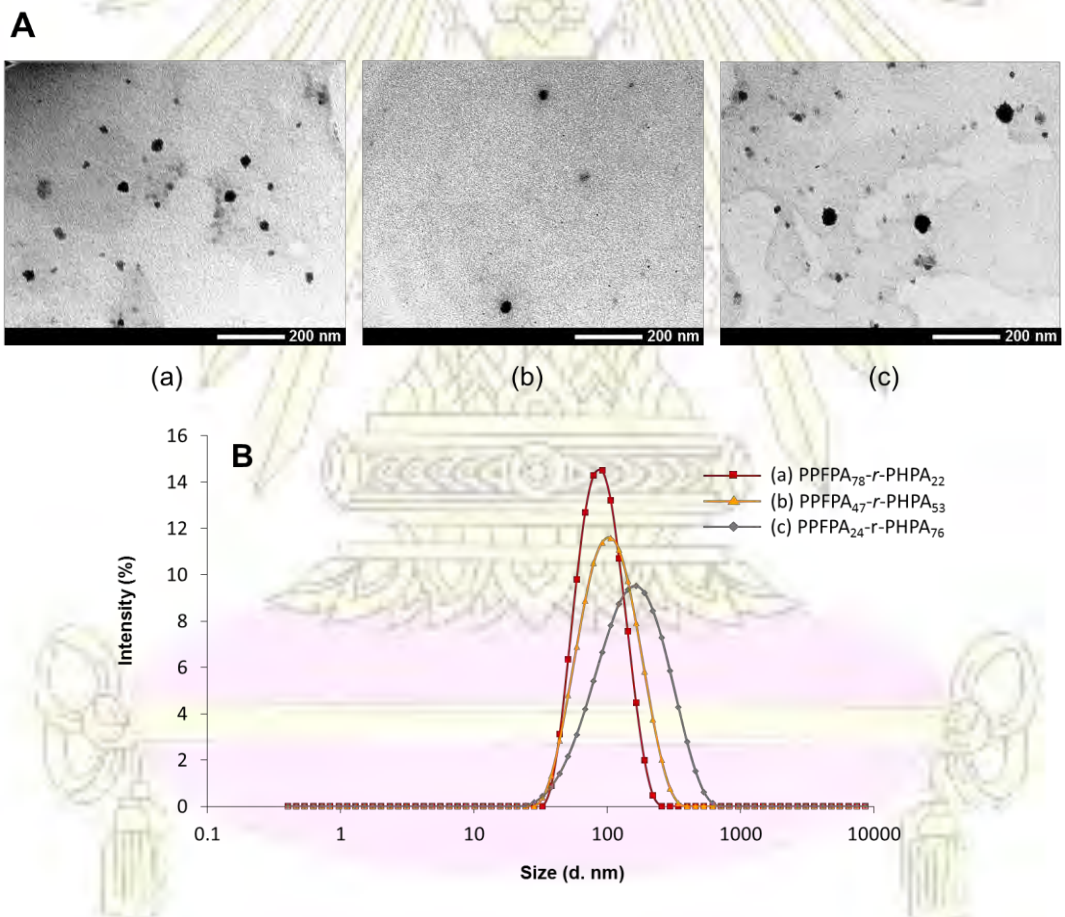


Figure 3.9 (A) TEM images, scale bar is 200 nm and (B) DLS profiles of (a) $PPFPA_{78}$-*r*-$PHPA_{22}$ (b) $PPFPA_{47}$-*r*-$PHPA_{53}$, and (c) $PPFPA_{24}$-*r*-$PHPA_{76}$ micelles.

Table 3.1 Particle size of micelles investigated by DLS

Copolymer	Average Size (nm)	PDI
PPFPA$_{78}$-*r*-PHPA$_{22}$	83±1.51	0.119
PPFPA$_{47}$-*r*-PHPA$_{53}$	90±0.24	0.228
PPFPA$_{24}$-*r*-PHPA$_{76}$	123±0.66	0.265

The critical micelle concentration (CMC) of the selected amphiphilic copolymer was examined by fluorescence spectroscopy. Pyrene was used as a fluorescence probe. Its absorption and emission spectra change depends on the media environment. The CMC was determined from the intersection of the plot of fluorescence intensity ratio (I_{374}/I_{394}) against the log of copolymer concentration. As shown in **Figure 3.10** (a) and (b), the CMC of PPFPA$_{78}$-*r*-PHPA$_{22}$ and PPFPA$_{47}$-*r*-PHPA$_{53}$ were about 0.7 and 2 mg/mL, respectively. The increasing of hydrophilic PHPA moieties in the copolymer apparently reduces hydrophobicity of the amphiphilic copolymer thus decreases hydrophobic driving force for self-assembly of the micelles formation [24]. And this is the reason why CMC of PPFPA$_{47}$-*r*-PHPA$_{53}$ is higher than that of PPFPA$_{78}$-*r*-PHPA$_{22}$.

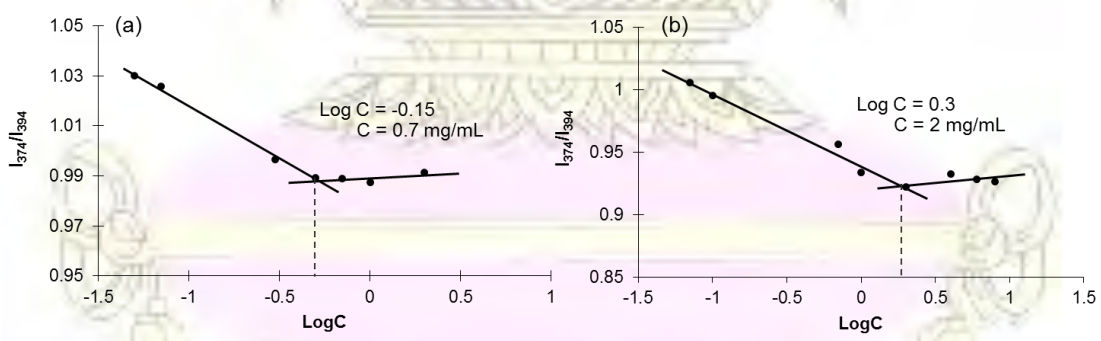


Figure 3.10 Plot of fluorescence intensity ratio (I_{374}/I_{394}) against the log of copolymer concentration for CMC determination of (a) PPFPA$_{78}$-*r*-PHPA$_{22}$ and (b) PPFPA$_{47}$-*r*-PHPA$_{53}$ using fluorescence spectroscopy.

3.4 Post-functionalization of micelles with 1-(3-aminopropyl) imidazole

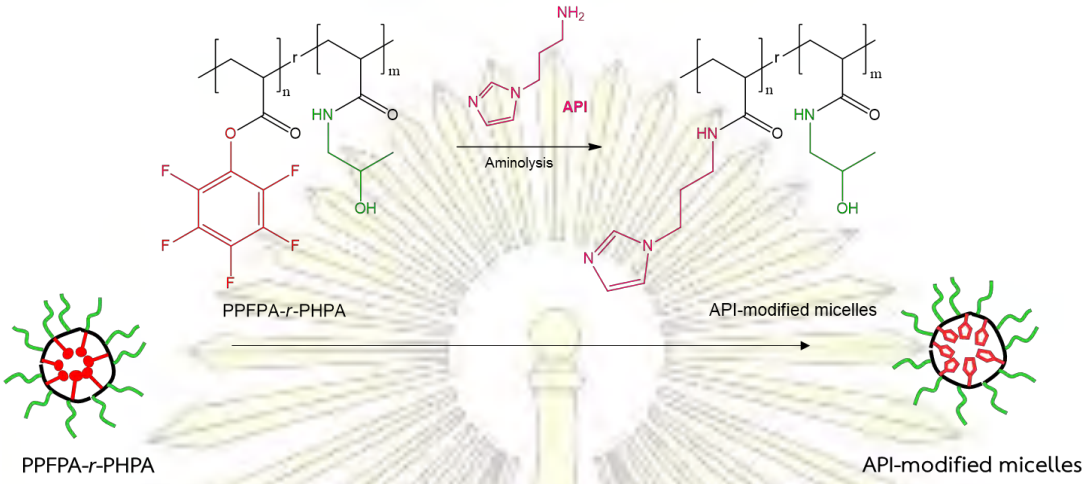


Figure 3.11 Post-functionalization of micelles with API.

Second post-polymerization modification was performed in suspension. In this procedure, the micelles prepared from $PPFPA_{78}$-*r*-$PHPA_{22}$ were selected to react with 1-(3-aminopropyl) imidazole (API) because of their smallest size, with highest residual active PFP groups and low CMC value. The reaction was performed by aminolysis [**Figure 3.11**]. As shown in FT-IR spectrum of the micelles obtained after modification with API, the peak of C=O stretching of ester at 1780 cm^{-1} disappeared [**Figure 3.12c**] with increasing intensity of C=O stretching of amide, indicating that all of the active PFP groups in the micelles were substituted by API. Moreover, the ^{19}F NMR analysis was used to confirm the success of this post functionalization. The result showed that the characteristic signals of PFP groups disappeared from the spectrum after aminolysis with API [**Figure 3.13**].

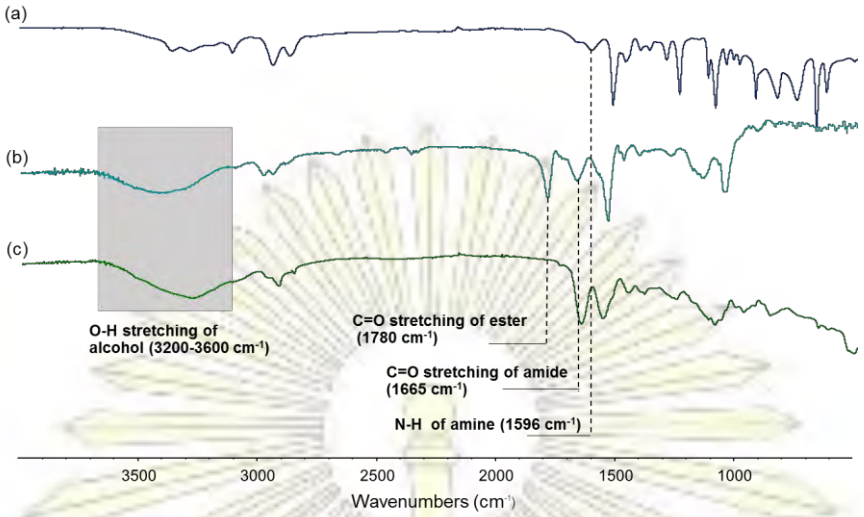


Figure 3.12 FT-IR spectra of (a) API, PPFPA$_{78}$-*r*-PHPA$_{22}$ both (b) before and (c) after post functionalized with API.

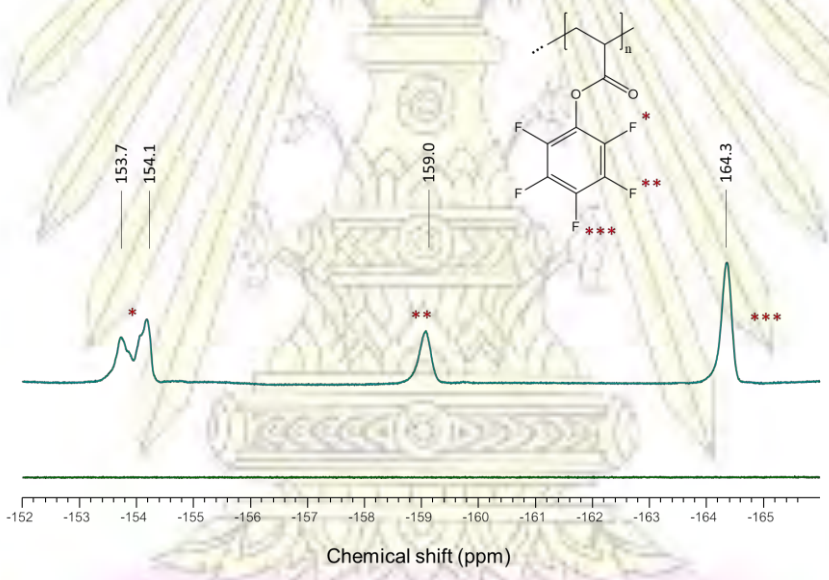


Figure 3.13 ^{19}F NMR spectra in DMF-d$_7$ of PPFPA$_{78}$-*r*-PHPA$_{22}$ (a) before and (b) after post functionalization with API.

3.5 Determination of pH responsive property of API-modified micelles

The $PPFPA_{78}$-*r*-$PHPA_{22}$ micelles of which the PFP groups were entirely replaced with API can be designated as API-modified micelles. As determined by DLS [**Table 3.2**], the micelles having API groups as core are apparently larger than those having PFP groups. Higher PDI and slightly lower zeta potential values are observed after functionalization. The fact that the micelles maintained their negatively charged zeta potential value after the post-functionalization suggested that the micelles nanostructure is still preserved and have hydroxyl groups dominating outside as shell. As the pH of the media was adjusted to be more acidic at pH 5, the micelles integrity were apparently destroyed [**Figure 3.14**] as can be evidenced from DLS profile [**Figure 3.15**] which became multimodal, suggesting disassembly and agglomeration of the micelles under an acidic condition happened. As expected, the surface of micelle turned from negatively charged to positively charged (7.14 mV) implying the imidazolyl groups became hydrophilic after being protonated under an acidic condition and were no longer hydrophobic enough to hold the micelles together. This micelles disintegration was also promoted by charge repulsion among protonated imidazolyl groups.

Table 3.2 Particle sizes and zeta potential values of $PPFPA_{78}$-*r*-$PHPA_{22}$ micelles before and after the post-modification with API.

Copolymer micelles	pH	Average size (nm)	PDI	Zeta potential (mV)
$PPFPA_{78}$-*r*-$PHPA_{22}$	7.4	83±1.51	0.119	-27.2
API-modified micelles	7.4	127±2.6	0.385	-21.6
API-modified micelles	5.0	223±9.6	0.488	7.14

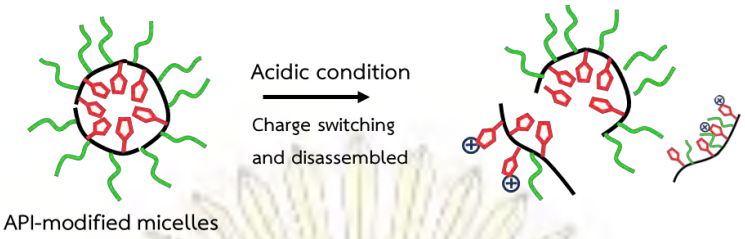


Figure 3.14 Illustration of disintegration of API-modified micelles under acidic condition.

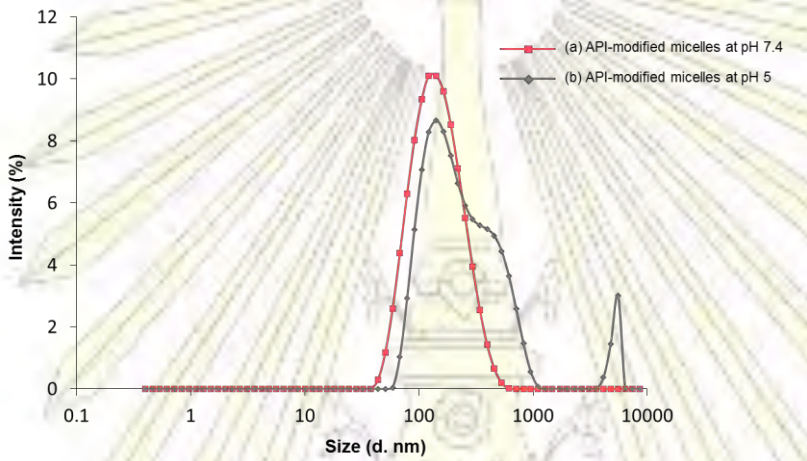


Figure 3.15 DLS profiles of API-modified micelles at (a) pH 7.4 and (b) pH 5.

Chapter 4

Conclusion and Suggestion

PPFPA as a polymer precursor was successfully synthesized by a RAFT polymerization. The molecular weight of PPFPA analyzed by GPC (24,605 Da) was close to the targeted value (24,090 Da) with a narrow PDI value (1.27). Amphiphilic random copolymers composed of PPFPA and PHPA were successfully prepared by post-polymerization modification of PPFPA with 1-amino-2-propanol. The composition of PHPA in copolymer was found to be 22, 53, and 76% estimated by ^{1}H NMR spectroscopy and 27, 55, and 77% estimated by FT-IR spectrometry upon the post-functionalization of PPFPA with 0.25, 0.50, and 0.75 equivalents of 1-amino-2-propanol, respectively. As verified by DLS and TEM, the spherical-like nanoparticles assembled from these amphiphilic copolymers had hydrodynamic diameter in the range below 200 nm.

pH Responsive micelles prepared by the post-functionalization of $PPFPA_{78}$-*r*-$PHPA_{22}$ micelles with API had lager size and showed lower zeta potential value at pH 7.4. As investigated by DLS, the surface of API-modified micelles became positively charged (7.14 mV) under an acidic condition (pH 5) and showed multimodal distribution. These can be used as an indication of pH responsive behavior of the API-modified micelles

This work demonstrated that the sequential post-polymerization modification of single homopolymer precursor is an efficient and facile approach to prepare pH responsive polymeric micelles.

For the future work, our investigation is to study the cytotoxicity and encapsulate hydrophobic drug molecules to be further developed into a potential pH-responsive drug carrier.

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