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CHEMICAL MODIFICATION OF TEMPERATURE-SENSITIVE PLURONIC BLOCK COPOLYMERS AND THEIR AGGREGATION PROPERTIES

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Chemical Modification of Temperature-Sensitive Pluronic Block Copolymers and Their Aggregation Properties

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Abstract

In order to improve the hydrophobicity of Pluronic block copolymers, two kinds of hydrophobic blocks, poly(lactic acid) (PLA) and polycaprolactone (PCL) were grafted to both ends of Pluronic copolymers to decrease the critical micellization concentration (CMC) of Pluronics. The physical properties of the resulting biocompatible modified Pluronic block copolymers such as the aggregation behavior and morphologies were studied in detail in order to explore possible applications of modified Pluronics in the biomedical field.

PLA was successfully grafted to both ends of Pluronic F127 block copolymers (PEO-PPO-PEO) to obtain amphiphilic block copolymers PLA-F127-PLA with long hydrophobic PLA blocks. The structure and molecular weight of PLA-F127-PLA block copolymers were studied by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC) techniques. The narrow distribution of PLA-F127-PLA block copolymers (PDI: 1.34 ~ 1.54) from GPC results indicates the high quality of PLA-F127-PLA copolymers. From NMR spectra, the block composition of PLA-F127-PLA block copolymers was calculated from the peak intensity ratio of methyl protons of PLA ($\text{O-CH}(\text{CH}_3)\text{-CO-}$; $\delta = 1.58$ ppm) and methyl protons of F127 ($\text{-OCH}_2\text{-CH}(\text{CH}_3)\text{-}$; $\delta = 1.15$ ppm). Data from differential scanning calorimetric (DSC) and wide angle X-ray diffraction (WXR) measurements indicated that T_m and crystallinity of PLA blocks increase with increasing PLA block length in PLA-F127-PLA block copolymers.

The aggregation behavior of PLA-F127-PLA aqueous solutions was examined using laser light scattering (LLS), transmission electron microscopy (TEM) and surface tension techniques. The microstructure of PLAF127-29 (56.5wt% LA, $\bar{M}_n =$

29 kDa) and PLAF127-48 (73.7wt% LA, $\bar{M}_n = 48$ kDa) aggregates comprises of bilayer vesicles and that of PLAF127-23 (45.2wt% LA, $\bar{M}_n = 23$ kDa) aggregates is coated micelles with three layers.

The hydrolytic degradation of PLAF-127-PLA block copolymers in phosphate buffered saline (PBS) (pH 7.4) solution at 37 °C was studied using a combination of physical techniques, such as dynamic light scattering (DLS), TEM, NMR and GPC. It was found that the morphologies and particle size of PLAF127-23 nano-particles changed dramatically because of the initial rapid degradation of PLA blocks within 7 days. The morphology of the microstructure transformed from regular coated micelles to large compound vesicles. With increasing time, the degradation rate of LA-LA linkage was reduced, and the degradation mainly occurred at the interface of F127 and PLA blocks. The morphologies of PLAF127-23 nano-particles changed from the regular larger compound vesicles to irregular compound vesicles, and to regular large compound vesicles again as degradation progresses. Accordingly, the particle size of PLAF127-23 nano-particles decreased initially, and remained constant for a certain time period and it then increased again.

The release kinetics of both hydrophobic MAMA and hydrophilic PrHy model drugs from PLA-F127-PLA nano-particles show burst release within 10 hours and are believed to be mainly controlled by concentration gradient due to the slow hydrolytic degradation of PLA segments based on the mathematical fitting of the power law model.

PCL-F87-PCL and PLA-F87-PLA block copolymers with short hydrophobic PCL and PLA chains were also synthesized. The structure and molecular weight of PCL-F87-PCL and PLA-F87-PLA block copolymers were studied by NMR and GPC techniques. It was shown from DSC and WXRd analyses that PCL or PLA blocks

cannot form crystalline domain in the modified F87 block copolymers with low PCL or PLA content.

The aggregation behavior of PCL-F87-PCL and PLA-F87-PLA block copolymers in water was studied using the LLS technique. Several kinds of particles consisting of small micelles, medium and large aggregates were observed due to the complicated structure of these copolymers.

Importantly, the modified Pluronic copolymers with short hydrophobic chains exhibit temperature-sensitive behavior similar to that found in Pluronic systems. Compared with Pluronics, the CMT values of modified Pluronic copolymers obtained from surface tension measurements decrease largely due to the enhanced hydrophobicity, and this property enhances its usefulness for applications in controlled release system. A fully reversible sol-gel transition was detected from the hydrogels formed from PCL-F87-PCL and PLA-F87-PLA block copolymers.

The release behavior of hydrophobic MAMA loaded in PLA6F87 (10 wt% LA, $\bar{M}_n = 8560$ Da) nano-particles in PBS solution shows burst release and is controlled by the concentration driving force. The burst release was also observed for hydrophilic PrHy loaded in C4F87 (10.6wt% CL, $\bar{M}_n = 8610$ Da) nano-particles. The release mechanism is mainly diffusion controlled.

The release behaviors of model drug loaded in PCL-F87-PCL and PLA-F87-PLA hydrogels in PBS solution were examined by UV-vis spectroscopy, respectively. It was found that there is no burst release for hydrophobic MAMA from C4F87 hydrogel and the initial release rate is almost constant. This indicates that PCL-F87-PCL hydrogels possess attractive release profiles, which are beneficial for application in controlled release systems. The degradation control mechanism is dominant for the initial MAMA release from C4F87 hydrogel according to the power law model. There

is insignificant difference between the release rates of MAMA from C4F87 sol and gel, indicating that the diffusivity of hydrophobic drug from gels is not a strong function of the viscosity since the viscosity of C4F87 gel is much larger than that of C4F87 sol.

The release of hydrophilic model drug PrHy from PLA6F87 hydrogel is also at a constant rate and the initial release mechanism is mainly degradation controlled. Temperature has an effect on the release rate of PrHy, where PrHy is released faster from PLA6F87 gel at 37 °C than from PLA6F87 sol at 25 °C. Furthermore, the hydrophilic PrHy loaded in PLA6F87 hydrogel was found to increase the CGT of the polymer.

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4. Lu, F. Z.; Xiong, X. Y.; Li, Z. C., et al. A convenient method for the synthesis of amine-terminated poly(ethylene oxide) and poly(epsilon-caprolactone). **Bioconjugate Chem.** 13, 1159-1162 (2002)
5. Xiong, X. Y., Li, Z. C., Du, F. S., et al. Synthesis of new block copolymer containing glycopolymer segment PEO-b-P(lys-ML) and their aggregation behavior in water. **Acta. Polym. Sin.** 6, 787-792 (2001) (In Chinese).
6. Xiong, X. Y.; He, W.; Li, Z. C.; et al. Preparation and properties of vesicles from condensable amphiphilic amino acids. **Chinese J. Chem.** 19, 1108-1115 (2001).
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2. Xiong, X. Y.; Tam, K. C.; Gan, L. H. Synthesis and temperature-sensitivity of novel Pluronic F87/Poly(lactic acid) (PLA) block copolymers with short hydrophobic PLA blocks. (submitted to **Polymer**).

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List of Symbols and Abbreviations

A_2	second virial coefficient
ε -CL	ε -Caprolactone
CAC	critical association concentration
CGT	critical gelation temperature
CMC	critical micellization concentration
CMT	critical micellization temperature
DLS	dynamic light scattering
$\overline{DP}$	degree of polymerization
DSC	differential scanning calorimetry
GPC	gel permeation chromatography
ΔH	enthalpy change (kJ/mol)
HLB	hydrophilic-lipophilic balance
k	Boltzmann constant
LCST	lower critical solution temperature
LLS	laser light scattering
MAMA	9-(Methylaminomethyl) anthracene
$\overline{M}_n$	number-average molecular weight
$\overline{M}_w$	weight-average molecular weight
$\overline{M}_w / \overline{M}_n$	molecular weight distribution
n	refractive index of the solvent
N_A	Avogadro's constant (6.02×10^{23})

$N_{\text{aggregation}}$	the aggregation number
NMR	nuclear magnetic resonance
OsO_4	osmium tetroxide
PAA	poly(acrylic acid)
PAAm	polyacrylamide
PBS	phosphate buffered saline
PCL	polycaprolactone
PCL-PEO-PCL	polycaprolactone-poly(ethylene oxide)-polycaprolactone
PCL-Plu-PCL	polycaprolactone-pluronic-polycaprolactone
PCS	photon correlation spectroscopy
PDEAEM	poly((diethylamino)- ethyl methacrylate)
PDI	polymer distribution index
PEO	poly(ethylene oxide)
PLA	poly(lactic acid)
PLA-Plu-PLA	poly(lactic acid)-pluronic-poly(lactic acid)
PLLA-PN-PLLA	poly(l-lactic acid)-Pluronic-poly(l-lactic acid)
Pluronic-g-PAAm	Pluronic-g-polyacrylamide
Pluronic-PVA	Pluronic-poly(vinyl alcohol)
PPO	poly(propylene oxide)
PrHy	procaine hydrochloride
PVA	poly(vinyl alcohol)
R_g	z-average radius of gyration
R_h	hydrodynamic radius

SLS	static light scattering
$\text{Sn}(\text{Oct})_2$	stannous octoate
TEM	transmission electron microscope
WXRD	wide angle X-ray diffraction

Chapter 1 Introduction

1.1 Overview

Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer (PEO-PPO-PEO) (commercially available as Pluronics and Poloxamers) is a macromolecular surfactant that can aggregate into micelles in aqueous solution. The block polymers have been used widely as coating, foaming, detergent, solubilizing, thickening, and emulsifying agents. Furthermore, the block copolymers have excellent biocompatibility, so they are also potential candidates in biomedical applications such as drug delivery system, controlled release system and gene therapy [Alexandridis, 1995; Moghimi, 1995; Kabanov, 2002 a-b, 2003].

The physical properties of Pluronics block copolymers have been studied by many research groups. Alexandridis et al. [Alexandridis, et al. 1995] and Chu et al. [Chu, et al. 1995] had published extensive reviews on the properties of Pluronics copolymers. It is interesting that the micellization behavior of Pluronic copolymers changes with temperature. This is because both PEO and PPO homopolymers have a lower critical solution temperature (LCST) in water. The solubility of PEO and PPO decreases with increasing temperatures. The structure and shape of the Pluronics micelles depend on the composition and molecular weight of the polymers, concentration and temperature of the polymer solution.

For Pluronic copolymers, another interesting and important property is their thermo-reversible gelation behavior. This phenomenon is very useful in the pharmaceutical application, such as controlled release system. The gel of Pluronic F127 is only produced at the concentration greater than 18wt%. This indicates that high concentration is a necessary condition for gel formation.

To improve the physical properties of Pluronic copolymers (e.g. the critical micellization concentration, the gel strength), chemical modification of Pluronics has been reported. Bromberg et al. [Bromberg, et al. 1998] synthesized Pluronic-poly(acrylic acid) (PEO-PPO-PEO-g-PAA) graft polymers (M_w typically exceeds 10^6 g/mol) with lightly cross-linked PAA chains, which improves the properties of Pluronic gels. PEO-PPO-PEO-g-PAA graft polymers can form gels at moderately low concentrations (1 to 3 wt%) in aqueous solutions. Furthermore, the strength of gels formed from Pluronic-PAA graft copolymers is much higher than that formed from Pluronic copolymers. The Pluronic copolymers have also been hydrophobically modified with polycaprolactone (PCL) to obtain well-defined block copolymers. The block polymer PCL-Pluronic-PCL has very low CMC because the hydrophobicity of PCL is quite strong [Ha et al., 1999]. Lee and co-workers modified Pluronic F68 block copolymer with PLA blocks and studied the application of modified F68 in the adhesion-prevention membrane [Yamaoka et al., 2001].

1.2 Objectives of the project

There are two main objectives for the present research. Firstly, we seek to modify both ends of the Pluronics system with hydrophobic blocks to enhance the hydrophobicity of Pluronic block copolymers. As Pluronic block copolymers have attractive temperature-sensitive and biocompatible properties, they are potential candidates for biomedical applications such as drug delivery system. However, their CMC values are far too high due to the weak hydrophobicity of PPO block, hence Pluronic micelles are not very stable, which limits their application in drug delivery system. Therefore, hydrophobic blocks are selectively grafted to both ends of Pluronic copolymers to decrease the CMC values. Two kinds of hydrophobic polymers, i.e.

poly(lactic acid) (PLA) and polycaprolactone (PCL) were used to attach at both ends of the Pluronic copolymers. The reason for selecting PLA and PCL as the hydrophobic blocks is to impart good biocompatible and biodegradable properties to the modified Pluronic copolymers. Poly(lactic acid) and poly(ϵ -caprolactone) are well-known biodegradable and biocompatible polyesters.

The second objective is to study the physical properties of these new amphiphilic modified Pluronic block copolymers such as the aggregation behavior, and to explore possible applications in the biomedical field. The new amphiphilic block copolymers are expected not only to decrease the critical micellization concentration (CMC) and critical micellization temperature (CMT) of Pluronic copolymers but also to impart new and more attractive properties.

It is described above that other researchers have also modified Pluronic block copolymers with PCL and PLA blocks. However, these studies were limited and only focused on the applications of the modified system, thus the basis of this research is outlined below:

- 1) Although Lee and co-workers also synthesized modified Pluronic with PLA (PLLA-PN-PLLA), they only studied the physical properties of PLLA-PN-PLLA film to explore the application of PLLA-PN-PLLA as adhesion-prevention membrane [Yamaoka et al., 2001]. However, the main application for PLA-Plu-PLA block copolymers synthesized by our group is for drug delivery application. Therefore, the aggregation and morphologies of PLA-Plu-PLA aqueous solutions were studied in detail in this thesis.
- 2) Up to now, Pluronic block copolymers were only modified with long PCL and PLA. The resulting modified Pluronics do not possess temperature-sensitivity. Thus, Pluronic F87 was modified for the first time with short PCL and PLA

hydrophobic chains at both ends to produce PCL-F87-PCL and PLA-F87-PLA block copolymers respectively in this thesis. The purpose for grafting short hydrophobic chains to both ends of Pluronic copolymers is to ensure that the temperature-sensitive property of Pluronic copolymers is retained and the solubility of the PCL-Plu-PCL and PLA-Plu-PLA block copolymers is not compromised.

1.3 Survey of the thesis

The thesis consists of eight chapters. The main content of these chapters are as follows:

The review on the physical properties of Pluronic block copolymers and chemically modified Pluronics is summarized in Chapter 2. Pluronic block copolymers were modified chemically with long PLA blocks, short PCL and PLA blocks respectively. The detailed synthesis and characterization methods of the PLA-Plu-PLA and PCL-Plu-PCL block copolymers are given in Chapter 3. The synthesis and physical characterization of modified Pluronic block copolymers with long and short hydrophobic PLA and PCL chains are described in Chapter 4. The aggregation behavior and temperature-sensitivity of modified Pluronic block copolymers with PLA and PCL chains are presented in Chapter 5. Chapter 6 describes the hydrolytic degradation of modified Pluronic block copolymers with PLA and PCL chains. Following this, the drug release behavior of modified Pluronic block copolymers with PLA and PCL chains is discussed in Chapter 7. Finally, the conclusions of the present research and the recommendations for future work are presented in Chapter 8.

Chapter 2 Literature review

2.1 Properties and applications of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymers

Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer (PEO-PPO-PEO) is a macromolecular surfactant that is marketed as Pluronics by BASF and Poloxamers by ICI. Both PPO and PEO homopolymers have a lower critical solution temperature (LCST) in water. The LCST of PEO (about 100°C) is much higher than that of PPO (about -5°C), depending on their molecular weights. Both PEO and PPO are hydrophilic at low temperature but PPO is hydrophobic at higher temperature in contrast to hydrophilic PEO chains. Accordingly, the block copolymers PEO-PPO-PEO can aggregate into micelles in aqueous solution. The structure and shape of the micelles depend on the composition and molecular weight of the polymers, concentration and temperature of the polymer solution. Another interesting phenomenon for the block copolymers is that gels can be produced at high concentrations. All these properties make the polymers an attractive choice in coating, foaming, detergent, solubilizing, thickening and emulsifying applications. Furthermore, the block copolymers are one of the very few synthetic polymeric materials approved by the U. S. Food and Drug Administration for use as food additives and pharmaceutical ingredients. So they are also potential candidates in biomedical applications such as drug delivery system, controlled release system and even gene therapy due to their excellent biocompatibility [Alexandridis and Hatton, 1995;

Moghim, 1995; Kabanov et al, 2002 a-b, 2003].

Fig. 2.1 Reaction scheme for Pluronic copolymers PEO-PPO-PEO [Alexandridis and Hatton, 1995].

2.1.1 Types of Polymeric systems and their physical properties

There are more than 20 types of Pluronic PEO-PPO-PEO copolymers available commercially, with molecular weights ranging from 1000-20000 g·mol⁻¹. The copolymers are synthesized through a two steps process as shown in Figure 2.1. Initially, the monomer propylene oxide is initiated by propylene glycol to produce the PPO homopolymer and then the other monomer ethylene oxide is added to react with the hydroxyl groups on the two ends of PPO to form the final copolymer PEO-PPO-PEO. The copolymers with different molecular weights and PEO contents have different physical properties. Some of the properties of Pluronics copolymers are summarized in Table 2.1. In the nomenclature of Pluronics copolymers, the letters L, P or F represent liquid, paste or flakes, respectively. The first one or two numbers denote the molecular weight of the PPO block and the last number is indicative of the weight

faction of PEO block. The cloud point corresponds to the temperature of phase separation of the copolymer from water. For Pluronic copolymers, clouding is observed at moderately high temperatures. It can be seen that the cloud point increases with the PEO content and may even exceeds 100°C. The value of HLB (hydrophilic-lipophilic balance) denotes the hydrophilicity of Pluronics copolymers, which also increases as the PEO content increases [Alexandridis and Hatton, 1995].

Table 2.1 Some of the physical properties of Pluronic copolymers [Alexandridis and Hatton, 1995].

Pluronic	Mol Weight	PEO wt%	Cloud point (°C)	HLB
L31	1100	10	37	1-7
L35	1900	50	73	18-23
F38	4700	80	>100	>24
L64	2900	40	58	12-18
P65	3400	50	82	12-18
F68	8400	80	>100	>24
P84	4200	40	74	12-18
P85	4600	50	85	12-18
F87	7700	70	>100	>24
F88	11400	80	>100	>24
P103	4950	30	86	7-12
P104	5900	40	81	12-18
P105	6500	50	91	12-18
F108	14600	80	>100	>24
F127	12600	70	>100	>24

2.1.2 Micellization behaviour of block copolymers in aqueous solutions

2.1.2.1 Critical micelle concentration and critical micelle temperature

Amphiphilic block copolymers can aggregate into micelles in aqueous solution when the critical concentration, commonly referred to as the critical micellization concentration (CMC), is exceeded. The micellization of some of block copolymers can also be initiated by changing the temperature based on the critical micellization temperature (CMT). The structure of macromolecular micelles is similar to that produced from low-molecular-weight surfactants. However, the macromolecular micelles are much more stable than low-molecular-weight ones. So amphiphilic block copolymers attract considerable attention because of their wider range of applications [Alexandridis and Hatton, 1995; Chu and Zhou, 1995].

The aggregation properties of Pluronic polyols have been studied by many research groups. However, the aggregation behavior of commercially available Pluronic copolymers is very complex because of their compositional heterogeneity and polydispersity. In practice, a certain CMC or CMT range with some notable uncertainty is usually detected. The main reason may be attributed to the gradual increase of the hydrophobicity of PPO block as the temperature increases. Thus far, there are many methods that were used to study the CMC and CMT values, such as fluorescence spectroscopy [Bohorquez et al, 1999], light scattering [Pandya et al, 1993], surface tension [Linse et al, 1992], dye solubilization [Alexandridis et al, 1994 b] and FTIR [Su et al, 2002 a-b]. Slight variations in CMC or CMT values were obtained for the same

Pluronic copolymer due to different measuring techniques used, since the physical principles of each method are different.

Micelle formation was observed with increasing concentration of the copolymer Pluronic L64 at 25°C. In the dilute region, unimer, transition and micelle regions appeared in sequence with increasing temperature. The detailed dynamics of the micro- and macro-phase separation of Pluronic L64 were studied using the iodine laser temperature-jump and stopped flow techniques [Kositza et al, 1999]. There are three processes occurring as the temperature is increased. The first process is the micelle-unimer interactions. Unimers are associated into micelles and the aggregation number increases. The second process is the restructuring of micelles, which commences at the temperature close to the CMT, where the polydispersity of micelles decreases during this process. The last process is the micelle-micelle interactions. Larger aggregates form through the clustering of micelles, which produces macrophase separation. It was also found by Mortensen et al that just above the CMT, there is a region of 10 to 15 degrees corresponding to the unimer-to-micellar transition. Above this region, most Pluronic polymer chains exist as micelles composed of PPO core and a strongly hydrated PEO shell [Mortensen et al, 1993]. However, at the beginning of the unimer-to-micellar transition, it was proven by small-angle neutron scattering (SANS) that the PPO core contained greater than 60% of water [Goldmints et al, 1997]. With increasing temperature, the water in the core is gradually replaced by the hydrophobic polymeric chains. The CMC values of Pluronics copolymers decrease with increasing temperatures. The effect of the composition and molecular weight of Pluronic

copolymers on the CMC and CMT has been studied. At a given temperature, the CMC values increase with decreasing PPO block lengths and increasing PEO block length. For a constant PPO/PEO ratio, the CMC and CMT values decrease with increasing molecular weights of the Pluronics copolymers and the CMT values decreased with increasing content of PPO blocks at a given concentration [Alexandridis and Hatton, 1995; Chu and Zhou, 1995].

Figure 2.2 shows the CMC-CMT micellization transition of a number of Pluronic copolymers in aqueous solutions. The free polymer (unimer) exists in solutions at the copolymer concentrations and temperatures lower than the CMC-CMT boundary. The micelles that are in equilibrium with unimers exist above the boundary [Alexandridis and Hatton, 1995].

The PPO segment of Pluronics copolymers plays an important role in controlling the temperature-sensitive property because the dehydration of PPO blocks with increasing temperature is responsible for the micelle formation. The effect of PPO blocks on the micelle formation is more pronounced than that of PEO block. The CMC and CMT values increase marginally with increasing PEO block length.

The mechanism of micellization of Pluronics copolymers was also studied and reported in the literature. In general, there are two association models for block copolymers. One is closed association model, which is characterized by the equilibrium between free polymers (unimers) and macromolecular micelles. A critical micellization concentration exists for this model. The other is open association model, which is

Fig. 2.2 The CMC-CMT micellization transition of Pluronic copolymers

[Alexandridis and Hatton, 1995].

characterized by a stepwise association and a distribution of unimers, dimmers, trimers, etc. A series of corresponding equilibrium constants between the respective species exist for this kind of model. The mechanism of micellization of Pluronic copolymers belongs to the first model, which is the close association model. The aggregation process of Pluronic copolymers is strongly driven by entropy. For a 1 wt% copolymer solution at the critical micellization temperature, the reported ΔH° , ΔG° and ΔS° values are in the range $169\text{--}339 \text{ kJ}\cdot\text{mol}^{-1}$, $-24.5\text{--}28.8 \text{ kJ}\cdot\text{mol}^{-1}$ and $0.638\text{--}1.244 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ respectively [Alexandridis and Hatton, 1995]. The positive values of the standard enthalpy (ΔH°) indicate that the micellization of the polymers is an unfavorable endothermic process. The free energy (ΔG°) is negative due to the spontaneous

formation of the micelles. Therefore, the negative standard entropy (ΔS°) is the major driving force for the aggregation of the Pluronics copolymers [Alexandridis and Hatton, 1995; Chu and Zhou, 1995].

2.1.2.2 Water-air interface properties of polymers in aqueous solution

For pure non-ionic surfactants, the surface tension of solution decreases with an increase in concentration until CMC is reached. There after, the surface tension remains constant or decreases slightly because of the saturation of the surfactant molecules at the air-water interface. At the air-water interface, the process can be expressed by the Gibbs adsorption equation as described by equation (2.1):

$$d\sigma = -\Gamma RT d \ln C \quad (2.1)$$

where σ is the surface tension, Γ is the surface (excess) concentration of surfactant molecules absorbed at the surface, and C is the bulk concentration of the surfactant. Therefore, CMC can be obtained from the plot of σ versus the logarithm of C .

The surface tension of Pluronic solutions in water at 20°C had been studied extensively by Prasad and his coworkers, where complex surface tension curves were observed [Chu and Zhou, 1995]. They found that there were two breaks in the curves of surface tension versus $\log C$ for the concentration range of 10^{-7} % to 10 %. Also, for all the Pluronic block copolymers examined, no plateau region was observed in the concentration region. They interpreted the first transition to be related to the formation of monomolecular micelles with the hydrophobic PPO segments partitioned to the interior, shielded by hydrophilic PEO segments [Chu and Zhou, 1995].

Fig. 2.3 Surface tension σ for aqueous solutions of P 104 as a function of concentration at various temperatures [Wanka et al, 1994].

Hoffmann and co-workers studied the surface tension of Pluronic block copolymers (P104, P123, F127) in aqueous solution at different temperatures [Wanka et al, 1994]. For Pluronic P104, it was found that the shape of the curves of surface tension versus $\log C$ varied with temperature (see Fig. 2.3). It is shown that there are two apparent break points at 15 °C. However, there is only one break point at 40 °C and the surface tension remains constant beyond the CMC. This phenomenon is similar to that of normal non-ionic surfactants. It was believed that two break points of surface tension curves for Pluronic copolymers are related to the broad molecular weight distribution of these polymers. The most surface-active polymer chains begin to aggregate at the first break point. With further increase in the concentration, the amounts of the less

surface-active polymer chains increases until the second break point is reached, where all the polymer chains aggregate to form micelles [Hecht et al, 1994].

In order to gain more information on the conformation of Pluronic block copolymers on the air-water interface, Alexandridis et al. examined the surface tension measurements of two sets of Pluronic block copolymers in aqueous solutions at two temperatures, 25 °C and 35 °C [Alexandridis et al, 1994 a]. One set of Pluronic copolymers chosen is P103, P104, and F108 with the same size PPO block and increasing size PEO block. The other set of Pluronic copolymers is P105, P85, and P65 with the same PPO/PEO composition and with decreasing molecular weights. The plots of surface tension versus $\log C$ for the two sets of polymers are shown in Figure 2.4. At the first break point (bulk concentration around 0.001%), they believed that it is due to the phase transition occurring at the air-water interface. The block copolymer layer becomes more compact, where PEO blocks may protrude into the solution or fold around PPO block. The concentration at the second break point is the actual CMC, which is consistent with that obtained from dye solubilization experiments.

In general, for Pluronic block copolymers, there are two break points observed from the plots of the surface tension versus $\log C$. The second break point is believed to be the actual CMC value. The separation between this two break points decreases with increasing temperature and eventually disappears at 40 °C, which may be due to the increased micellization tendency at higher temperature.

Fig. 2.4 Surface tension data for various PEO-PPO-PEO copolymer solutions, plotted as a function of concentration for two temperatures (25 and 35 °C): P103, P104, F108, P105, P85, and P65. The solid lines at low copolymer concentrations indicate the range from which a per molecule values were obtained, while the solid lines at high concentrations underline the steady surface tension values attained after the formation of micelles in the bulk. The arrows mark the CMC obtained for the same copolymer using dye solubilization.

2.1.2.3 Structure and shape of micelles

The effect of temperature and concentration on the aggregation number, size and shape of micelles formed from Pluronic copolymers have been studied by many research groups. In general, the hydrodynamic radius R_h is between 2 and 15 nm in the temperature range 20-80 °C and the aggregation number varies from 4 to 400 in the temperature range 20-80 °C. The core radius r_c can be estimated by the following equation:

$$\frac{4\pi r_c^3}{3} = N_{PO} n_w \frac{M_0}{\rho N_A} \quad (2.2)$$

where M_0 is the molar mass of oxypropylene monomer, ρ is the density of liquid oxypropylene and n_w is the aggregation number. Hence, the thickness of outer corona can be roughly estimated by R_h and r_c [Chu and Zhou, 1995].

The hydrodynamic radius R_h of Pluronic L64 in aqueous solution remained constant over a wide concentration range at 35°C, which is a signature of a closed association process.

From the results of static and dynamic light scattering, it was established that the aggregation number increases with increasing temperature. The aggregation numbers of Pluronic L64 at the temperatures 21.0, 25.9, 40.0 and 60.0°C were calculated to be 2, 4, 19 and 85 respectively. However, the micellar radius R_h remained constant. The temperature independence of the hydrodynamic radius R_h is due to a dual effect of temperature. Although the aggregation number increases with temperature, the dehydration of the PEO block becomes more and more important with increasing temperature. Therefore, the dual effect of temperature makes it possible for the

hydrodynamic radius R_h to remain almost independent of temperature. The aggregation number of Pluronic micelles increases with increasing the PPO block length and decreasing the PEO block length [Alexandridis and Hatton, 1995; Chu and Zhou, 1995].

The structure of micelles of Pluronic copolymers is in the form of core-shell structure, whose core is the PPO block and the shell is the PEO block. However, some results from small angle neutron scattering on Pluronic L-64 in D_2O showed that the core-shell boundary in the PEO-PPO-PEO micelles is not sharp. The miscibility of the PO and EO groups may exist and water penetration into the PPO core may also occur [Chu and Zhou, 1995].

The shape of PEO-PPO-PEO micelles was also studied by many research groups. The Pluronic copolymers exist as unimers at low concentration and temperature. With increasing temperature, spherical micelles are formed at low concentration. The transition from spherical to rod-like micelles occurs with increasing the temperature. For example, the morphology of Pluronic P-85 micelles changed from sphere to rod at low concentration when the temperature was increased to about 70°C (see Fig 2.5). The reason for the change in morphology is that the PPO chains in the micellar core are fully stretched at high temperature. Further increase in the aggregation number must result in the transition from spherical to rod-like micelles [Chu and Zhou, 1995]. The important transition from micelles to “gels” occurs when the concentration of Pluronic copolymers is increased beyond a critical threshold. The interesting phenomenon will be discussed in detail in section 2.1.3.

Fig. 2.5 Phase diagram of aqueous P-85 solutions [Chu, 1995].

2.1.2.4 Effect of additives on micellization

The effects of electrolytes and surfactants on the micellization of the Pluronic copolymers solution have been studied by viscosity, cloud point, light scattering methods. Electrolytes with different sizes and polarizabilities were used as additives. Addition of KBr, KCl and KF decreases the CMT and CP of L64 and P85 solutions. However, addition of KCNS results in the increase of CMT and CP. A linear change (increase or decrease) in the cloud point with increasing salt concentration was observed. The effect of electrolytes was discussed in terms of “salting in” and “salting out”, but the exact mechanism is still unresolved [Alexandridis and Hatton, 1995]. Recently, Su et al studied the effect of salts on the micellization of Pluronic F127 block

copolymers by using FTIR [Su et al, 2002 c]. It was found that the addition of salts decreased the polarity of hydrated methyl groups of PPO block when the polymers existed in unimers.

The results for the addition of sodium dodecyl sulfate surfactant (SDS) to the Pluronic copolymer solutions reveal that SDS can suppress the formation of Pluronic micelles [Hecht et al, 1995]. This is because SDS binds to the free chains of the copolymer, and at the saturation condition, four to five SDS molecules bind to one F127 chain. The binding between the SDS and F127 begins at a concentration lower than the CMC of SDS. Cationic surfactant such as CTAB (N-hexadecyl-N, N, N-trimethylammonium bromide) also suppresses the micellization of F127. The effect of urea on the micellization of Pluronic copolymers was also examined, and the addition of urea results in the increase in the CMT and CMC. The mechanism could be related to the influence of urea on the water structure.

2.1.3 Thermoreversible gelation of the block copolymers in aqueous solutions

For Pluronic copolymers, another interesting and important property is their thermoreversible gelation. The phenomenon is very useful in the pharmaceutical fields such as drug delivery system.

Figure 2.6 shows the typical gelling behavior of Pluronic F127. From the figure, it is evident that gel is only formed at a concentration greater than 18 wt%, which indicates that high concentration is a necessary condition for gel formation. At a given concentration, the gels exist within a certain temperature range. The temperature for gel

Fig. 2.6 Phase behavior of the E₉₉P₆₅E₉₉ / water system. The solid circles refer to the cloud point [Chu, 1995].

formation decreases with increasing concentration of F127 solution, where the sol-gel transitions are thermoreversible [Alexandridis and Hatton, 1995].

The viscoelastic properties of Pluronic copolymers during the gel formation have been measured by Brown et al. [Brown, 1993]. The gelation temperature is defined as the temperature at which the storage modulus G' is equal to the loss modulus G'' . The storage modulus, G' remains high and the loss modulus G'' is lower in the gel region. Not all Pluronic copolymers possess such gelling behavior. Only copolymers with molar mass of PPO segment greater than 1750 g/mol can form gels at appropriate temperature and concentration. The gels are easier to form for the Pluronic copolymers with longer PPO block and larger content of the PEO block.

It is widely accepted that the structure of the Pluronic gels are cubic lattice. When the volume fraction of Pluronic micelles crosses the critical value for hard-sphere crystallization, the micelles pack into the cubic gels [Alexandridis et al, 1996]. The ^1H NMR results show that the dynamic state of the micellar core (the PPO block) does not change, while the dynamic state of the micellar shell (the PEO block) shows large fluctuations as gelation progresses. The motion of the PEO chains might be hindered due to the interpenetration of the PEO micellar shells [Cau et al, 1996].

The effects of electrolytes and homo-polymers on the gel formation have been investigated. Addition of NaCl, which is often referred to as a “salting-out” co-solute decreases the gelation temperature. NaSCN, a typical “salting-in” co-solute, results in the increase in the gelling temperature. Addition of PEO homo-polymer increases the gelation temperature and the addition of PPO homopolymer decreases the temperature of gel formation [Alexandridis and Hatton, 1995].

2.1.4 Applications

For Pluronic copolymers, the most important application is in medical field such as drug delivery system due to their excellent biocompatibility. The hydrophobic drugs can be dissolved in the core of micelles formed from Pluronic copolymers. A low-molecular-weight compound (ATP, adenosine triphosphate) that was dissolved in Pluronic micelles was effectively transported across the cells [Alexandridis and Hatton, 1995].

Pluronic micelles can also be used to protect microorganisms. Mammalian cells are

very useful in the biotechnology industry for the production of complex proteins, but they are easy to be destroyed under high shear. It was observed that Pluronic F68 can protect them from being destroyed under mechanical shearing. Wu et al. [Wu et al., 1997] found that Pluronic copolymers have the potential to be used as an effective separation medium in capillary electrophoresis (CE) for the separation of biomacromolecules such as DNA and proteins.

Recently, it was found that Pluronic block copolymers have great potential applications in gene and cancer therapies. Kabanov et al. published two detailed reviews on the applications of Pluronic block copolymers in these two potential application areas [Kabanov et al, 2002 a-b]. For example, Pluronics increase the expression of plasmid DNA in skeletal muscle in mice. A formulation consisting of Pluronic L61 and F127 has been found to increase gene expression by 5-20-fold compared to pure DNA system [Kabanov et al, 2002 a]. It is known that chemotherapy is the main method for cancer treatment. However, the efficacy of chemotherapy is severely limited by the existence of multiple drug resistance (MDR) cells. Fortunately, Pluronic block copolymers were found to inhibit the multiple mechanisms of drug resistance in MDR cells. Pluronic P85 and L61 sensitize resistant cancer cell lines and increase the cytotoxic activity of cancer drugs by 2 to 3 orders of magnitude [Kabanov et al, 2002 b].

Gels formed from Pluronic copolymers have significant potential applications in controlled drug release and drug targeting systems because of its thermoreversible sol-gel transition. More importantly, it was found that there is no burst release for the

drug release from Pluronic gels, that is, the loaded drug is released at a constant rate [Moore et al, 2000; Anderson et al, 2001]. As we know, burst release of drug release is a major problem for most drugs loaded in polymeric micelles because the concentration of injected drugs decreases rapidly. Thus, frequent injection of drug is needed, which exacerbates the risk of infection. Fortunately, the constant release of drugs loaded in the Pluronic gels maintains the concentration of drugs in a satisfactory level for prolonged periods, thus avoiding the frequent injection of drugs.

The dissolution of gel during the study of drug release in aqueous solution is a problem encountered by many research groups. Various approaches were adopted; some studied the process by entrapping the gel in a membrane [Gilbert et al, 1986; Pandit et al, 1998], while others did not use any membrane to separate the gel from the aqueous solution. Moore and Anderson studied the release behavior of drug loaded in Pluronic F127 gel by taking into consideration the effect of gel dissolution [Moore et al, 2000; Anderson et al, 2001].

It was discovered that the release rate of model drugs from F127 gel decreased with increasing Pluronic F127 concentration, which is probably because the size and number of micelles in gels increase and subsequently lead to the decrease in size and number of aqueous channels. The hydrophobic molecules with higher hydrophobicity show a lower release rate due to a greater partitioning into the micellar region within the gel structure [Gilbert et al, 1986]. Salts show significant effect on the release rate of drug loaded in Poloxamer P407 gel. The release rate decreases with an increase in the salt concentration, which is believed to be due to the increase of the microviscosity within

the aqueous region of the gel [Pandit et al, 1998]. Moore et al. studied the release of propranolol (PNL) loaded in F127 gel without using a membrane [Moore et al, 2000], where the drug release obeyed a zero-order rate diffusion, which is controlled by the dissolution of gel (see Fig. 2.7).

Fig. 2.7 Profiles for the dissolution of a 20% F127 gel (in g) and simultaneous release of PNL (in mg) from the gel, versus time at 37°C and a stirring speed of 20 rpm. The gel contained 0.4% PNL and had a surface area of 24.6 cm². The error bars show the relative variation of data and the line represents a linear regression fit [Moore, 2000].

2.2 Chemical modification of PEO-PPO-PEO block copolymers

There are some disadvantages in the applications for Pluronic copolymers, namely:

1. The CMC of Pluronic copolymers is too high due to the weak hydrophobicity of PPO block.
2. The strength of gels formed from Pluronic copolymers is weak because they are only physically cross-linked.

Chemical modifications of Pluronic copolymers not only improve the properties of the polymers but also impart new and more attractive properties to them. Bromberg et al. [Bromberg, 1998 a-b] have synthesized Pluronic-PAA graft polymers with light cross-linking by PAA chains. The strength of gels formed from Pluronic-PAA graft copolymers is much higher than produced from Pluronic copolymers. The Pluronic copolymers have also been hydrophobically modified with polycaprolactone (PCL) and Poly(l-lactic acid) (PLA) to obtain well-defined block copolymers. The block polymer PCL-Pluronic-PCL has very low CMC because the hydrophobicity of PCL is quite strong [Ha et al, 1999]. The chemical modifications of Pluronics published up to now are summarized in Table 2.2. However, only very limited study on the well-defined chemical modification of Pluronic copolymers has been reported.

2.2.1 Copolymers composed of PEO-PPO-PEO and anionic polyelectrolyte, poly(acrylic acid)

Bromberg et al. [Bromberg, 1998 a-b] chemically modified the PEO-PPO-PEO block copolymers with poly(acrylic acid) (PAA), thereby imparting a dual responsive property (i.e. pH and temperature) to the polymer. The new material can be potentially used in eye drops because the dilute aqueous solution of the Pluronic-PAA polymers

can quickly form a thin gel layer at the temperatures of the cornea in the cul-de-sac of the eye (33-34°C).

Table 2.2 Chemical modifications of Pluronic block copolymers.

Polymers used	Examples	Applications
Anionic polyelectrolyte	PAA	Drug delivery, Bio-adhesive application
Cationic polyelectrolyte	PAAm, PDEAEM	DNA separation Drug delivery
Nonionic polyelectrolyte	PVA	Drug delivery
Polyester	PLA, PCL	Drug delivery adhesion-prevention membrane

The PEO-PPO-PEO-g-PAA graft polymer was synthesized by dispersion/emulsion polymerization of acrylic acid along with simultaneous grafting of poly(acrylic acid) onto Pluronic backbone. The synthetic mechanism is that of free-radical polymerization of acrylic acid with chain transfer to the Pluronic copolymer resulting in the cross-linked C-C bonding between PAA chains and Pluronic copolymers (see Figure 2.8). One PEO-PPO-PEO-g-PAA chain contains mixtures of PAA and Pluronic segments. The PEO-PPO-PEO-g-PAA graft polymer possesses a high molecular weight (M_w typically exceeds 10^6 g/mol, measured by GPC on a Shimadzu LC-10A Series HPLC, using 0.05 M NaNO_3 as the elution phase and a pump speed of 0.8 ml/min.) and extreme temperature sensitivity [Bromberg, 1998 a-b].

Fig. 2.8 The synthetic mechanism of PEO-PPO-PEO-g-PAA graft copolymers. $R\bullet$ is the free radical, X_mH is Pluronic, and AA is the acrylic acid monomer, XL is a divinyl cross-linker [Bromberg, 1998 b].

Compared with Pluronic copolymers, PEO-PPO-PEO-g-PAA graft polymers can form reversible gels with significant elastic moduli in water at lower concentration above a certain temperature. For example, F127-PAA polymer exhibits enhanced viscosity in moderately low (1 to 3 wt%) aqueous solutions. However, Pluronic F127 can form gels at body temperature in aqueous solution only above 16wt%. It was explained that the PAA chains were attached to several Pluronic chains and bridged several micelles. Thus, the negative change in the heat capacity is the result of the exposure of the PPO segments to water, which is consistent with the mechanism of micellization. Evidence from oscillatory shear and DSC studies, indicates that the gel point correlates with the CMT in the parent Pluronic and confirms that the appearance

Fig. 2.9 Phase diagram for aqueous solutions of F127-PAA at pH 7.0. T_{gel} and CMT stand for critical gelation and micellization temperature, respectively [Bromberg, 1998 e].

of micelles in the solution is responsible for the gelation (Fig. 2.9). As evidenced by NMR and SANS, the spherical core of these aggregates consists of collapsed and substantially dehydrated chains of PPO, while the adjacent PEO blocks provide a more hydrated layer around the core (an “inner corona”), and ionized PAA chains constitute an expanded corona. The core radius of the Pluronic-PAA micelles (ca. 3 nm) is similar to that of the parent Pluronic micelles [Bromberg, 1998 a-e; Bromberg et al, 1998f, 1999 a-d]. Recent results obtained from fluorescence techniques (steady-state intensity and depolarization and fluorescence lifetimes) also show that the aggregates formed from Pluronic-PAA copolymers above CMT are micelle-like with substantially

dehydrated PPO core and hydrated PEO-PAA shell. The shell provides the physical cross-linking required for gelation. However, small hydrophobic domains were detected at a temperature below the CMT. The smaller domains are even more hydrophobic than the core of the micelle-like aggregates. It is believed that the hydrophobic domains are produced during the PAA blocks being grafted onto the Pluronic copolymers [Ho et al, 2002].

The effects of pH and salt addition on the gel property of the Pluronic-PAA polymers have also been studied. The strength of gel increases with pH until a critical pH value, where the strength of gel decreases. The elasticity of the gel formed from Pluronic-PAA polymers is proportional to the number of polymer chains connected to both ends of the micelle-like aggregates. PAA segments expand with increasing pH due to the repulsive interactions between the ionized carboxylic groups, indicating the ability of PAA chains to participate in expanding the size of the aggregates, which results in the enhanced viscoelasticity of the gels. However, at complete ionization after pH 11, PAA chains become more compact due to electrostatic screening. The folding of the PAA causes the gel elasticity to decrease. Addition of low concentration of salt (NaCl) increases the viscoelasticity of the gels. This is due to the enhancement of hydrophobic interactions between the PPO segments. At higher salt concentration, the elasticity of the gels decreases because of the folding of the PAA chains caused by the electrostatic screening [Bromberg, 1998 a-e; Bromberg et al, 1998f, 1999 a-d].

The solubilization of hydrophobic compounds (pyrene and steroid hormones) into the micelles formed from the Pluronic-PAA polymers was also studied. The saturated

concentration of the probe in the Pluronic-PAA solution increases largely upon the formation of micelles above the critical temperature (CMT) [Bromberg et al, 1999 b-d]. More interestingly, the hydrogel formed from PEO-PPO-PEO-g-PAA graft copolymers shows attractive advantage for oral drug delivery due to its pH sensitivity and ability to adhere to the mucosal surfaces. It was found that the biocompatible polymers with COOH group were able to prolong residence time of topical formulations because of adherence to mucosal surfaces [Chen et al, 1995]. The drug loaded in the PEO-PPO-PEO-g-PAA hydrogel was not released because the low pH environment in the stomach (pH $\sim$ 1-2) induced the hydrogel to adopt a compact configuration. However, when the hydrogel loaded drug enters to the intestine (pH $\sim$ 6.2-7.4) and adheres to the mucosal surfaces, drug is released out due to the swelling of the hydrogel [Bromberg et al, 1998 g].

Recently, Bromberg et al. modified PEO-PPO-PEO-g-PAA graft copolymers by introducing a cross-linker (ethylene glycol dimethacrylate) to produce an interesting microgel system [Bromberg et al, 2002 a]. Microgel is defined as a intramolecularly cross-linked macromolecules with a globular structure. This kind of microgel is capable of volume phase transition in aqueous solution. The size of microgel is about hundreds of microns when it is in a swollen state. Therefore, the smaller microgel adheres more strongly to the mucosa and possesses a longer residence time in the intestine compared to the previous hydrogel. It was observed that there were Pluronic chains dangling on the chemically cross-linked PAA network within the microgel below the CMT. When the temperature increases beyond the CMT, the dangling Pluronic chains aggregate

together to form hydrophobic domains in the microgel (see Figure 2.10). These physically cross-linked hydrophobic domains improve the loading capacity of hydrophobic drugs such as taxol in the microgel [Bromberg et al, 2002a, 2003].

Fig. 2.10 A scheme representing thermoreversible aggregation of Pluronic dangling chains (shown by solid lines) into aggregates within gel microparticles [Bromberg et al, 2002 a].

2.2.2 Copolymers composed of PEO-PPO-PEO block and cationic polyelectrolyte

Bromberg et al. modified Pluronic copolymers (F88, F108 and F127) with hydrophobic cationic polyacrylamide segments using the same method as the PEO-PPO-PEO-g-PAA graft copolymers they studied [Bromberg, 1998h]. The molecular weight of the resulting Pluronic-g-PAAm graft copolymers is in the broad range of 135,000 to 2,050,000 g/mol. The F88-g-PAAm copolymer with low concentration (5 w/v%) shows gelation behavior at 44 °C due to the aggregation of

Pluronic segments, which is similar to that of PEO-PPO-PEO-*g*-PAA graft copolymers. The gels formed from Pluronic-*g*-PAAm graft copolymers show potential feasibility of DNA separation by capillary electrophoresis [Bromberg, 1998h].

Very recently, cationic poly((diethylamino)- ethyl methacrylate) (PDEAEM) blocks were attached to both ends of Pluronic F127 block copolymer through oxyanionic polymerization by Anderson et al. [Anderson et al, 2003]. The chemical structure of the resulting block copolymers is shown in Fig. 2.11. The molecular weight of PDEAEM-F127-PDEAEM block copolymers is in the range 12,840 – 19,730 g/mol with narrow polydispersity (1.18 – 1.20). The CMT of PDEAEM-F127-PDEAEM block copolymers obtained from DSC are slightly smaller than that of F127. The drug release of PDEAEM-F127-PDEAEM gels shows pH sensitivity (see Fig. 2.12). The lower pH values, the higher the rate of drug release. This is because PDEAEM blocks become protonated at low pH and then the electrostatic repulsion of cationic blocks results in the swelling and subsequent dissolution of PDEAEM-F127-PDEAEM gels. Therefore, the entrapped drugs in the gels are easier to be released [Anderson et al, 2003].

Fig. 2.11 The chemical structure of PDEAEM-F127-PDEAEM block copolymers [Anderson et al, 2003].

Fig. 2.12 Release of Nile blue chloride from PDEAEM-*b*-PEO-*b*-PPO-*b*-PEO-*b*-PDEAEM pentablock copolymer gels at a pH of (◆) 6.2, (■) 7.4, and (▲) 8.2. Error bars represent standard errors [Anderson, 2003].

2.2.3 Copolymers composed of PEO-PPO-PEO and nonionic poly(vinyl alcohol)

In order to avoid the strong polyelectrolyte effects for PEO-PPO-PEO-*g*-PAA graft copolymers, nonionic poly(vinyl alcohol) segment was attached to one end of Pluronic L122 copolymer to obtain Pluronic-PVA block copolymers by Bromberg et al [Bromberg et al, 1999e]. The molecular weight of Pluronic-PVA block copolymers is in the range 8,870 – 19,400 g/mol with the molecular weight distribution range 1.3 – 1.6. As expected, Pluronic-PVA block copolymers exhibit higher CMT values than Pluronic block copolymers. Also, the temperature-sensitivity of Pluronic-PVA block copolymers

is more dramatic than that of Pluronic block copolymers due to the entanglement of hydrophilic PEO and PVA blocks. The gelation of Pluronic-PVA block copolymers occurs at higher concentration, which is also caused by the formation of large aggregates with interpenetrating shells. The large amount of hydroxy groups on the surface of Pluronic-PVA aggregates were expected to provide some novel strategies for the design of supramolecular structures [Bromberg et al, 1999e].

2.2.4 Block copolymers composed of PEO-PPO-PEO and biodegradable poly(ϵ -caprolactone)

Poly(ϵ -caprolactone)s (PCL) are attached to both ends of the block copolymers PEO-PPO-PEO to obtain the block copolymers PCL-PEO-PPO-PEO-PCL (PCL-Pluronic-PCL) [Ha et al, 1999]. Poly(ϵ -caprolactone) is a well-known biodegradable polyesters, which has attracted attention in controlled drug delivery due to its good biocompatible property. Furthermore, the biodegradability of PCL can be improved by copolymerization with other polymers such as PEO, and PLA.

The copolymers PCL-Pluronic-PCL were synthesized by the ring opening polymerization of the monomer ϵ -caprolactone using the Pluronics copolymers as the initiator and stannous octoate ($\text{Sn}(\text{Oct})_2$) as the catalyst. There are four kinds of Pluronics copolymers modified by PCL. They are F127, F87, F88 and F68. The structure of the synthesized PCL-Pluronic-PCL copolymers was characterized by ^1H NMR, FT-IR, GPC and DSC.

The hydrophobically modified copolymers PCL-Pluronic-PCL can self-assemble

into micelles in water at lower CMC than the Pluronics copolymers. The CMC values determined from fluorescence spectroscopic technique are 1.630×10^{-4} M, 6.289×10^{-5} M and 2.503×10^{-6} M for CF87 (M_n 12,786), CF127 (M_n 20,800) and CF87-1 (M_n 25,596) respectively [Kim et al, 2000]. The structure of the micelle consists of a core-shell structure, with the inner core containing PCL and the outer shell comprising PEO-PPO-PEO segments. The size of micelles for PCL-Pluronic-PCL copolymers is in the range of 116-196 nm and it increases with the molecular weight of the PCL-Pluronic-PCL copolymers. The temperature-sensitive property of the block copolymers PCL-Pluronic-PCL has also been studied [Kim et al, 2000]. It is surprising that the size of micelle decreased with increasing the temperature. This may be attributed to the hydrophobicity of PPO block, which increases with increasing temperature. Hence, the interaction between PCL block in the core and PPO block in the shell is strengthened [Kim et al, 2000].

The diffusional behaviors of indomethacin (IMC) from the PCL-Pluronic-PCL micelles were studied by Kim et al [Kim et al, 2000]. IMC exhibits a slow release from the micelles and the releasing rate is dependent on temperature. The release amount of IMC from the PCL-Pluronic-PCL micelles decreases with increasing temperature. There are two possible for this phenomenon, namely (1) the micelle size decreases with increasing temperature, which retards the diffusion of the drug diffusion through the micelles, (2) the hydrophobicity of PPO block increases with temperature resulting in the stronger interaction between the IMC loaded in the core and the hydrophobic polymer chains [Kim et al, 2000].

2.2.5 Block copolymers composed of PEO-PPO-PEO and biodegradable poly(l-lactic acid)

The reported studies on the modification of Pluronic block copolymers by Poly(l-lactic acid) (PLA) is limited. Yamaoka and Lee et al. synthesized poly(l-lactic acid)-Pluronic-poly(l-lactic acid) (PLLA-PN-PLLA) block copolymers by attaching PLA blocks to both ends of Pluronic F68 block copolymer [Yamaoka et al, 2001].

The purpose for synthesizing PLLA-PN-PLLA block copolymers is for their application as adhesion-prevention membrane. It was stated that PLLA homopolymer is one of the most accessible biomedical polymer due to its excellent biocompatibility and biodegradability. PLLA has been used as an implant for a long time, and up to now, there is no apparent disadvantage or problem related to toxicity, severe acute inflammation, or immunogenicity. However, PLLA chains are very rigid and the hydrophobicity of PLLA is too high, therefore, the PLLA polymeric membrane was not effective in the prevention of pelvic adhesion. As we know, Pluronic block copolymers are more hydrophilic and the polymer chains are more flexible, thus PLLA was modified using Pluronic F68. It was shown that the receding contact angle of PLLA-PN-PLLA film against water was lower than that of PLLA film. The copolymer films exhibit a low level of initial cell attachment and a slow rate of cell growth, depending on the Pluronic content in the copolymers (see Figure 2.13).

Fig. 2.13 Time profiles of the cell growth on (●) TCPS, (○) PLLA, (▲) LN(t)-10, (△) LN(t)-15, and (■) LN-20 films [Yamaoka et al, 2001].

2.3 Properties of amphiphilic triblock and star-shaped copolymers composed of PEO, PCL and PLA blocks

The properties of amphiphilic tri-block copolymers composed of PCL/PEO or PLA/PEO segments have been studied by several research groups. Their structures are similar to those of PCL-Pluronic-PCL block copolymers. The hydrophilic PEO polymer has a unique biocompatibility and reaction characteristics. The particles with the PEO block in the shell possess a high stability in human bodies because PEO can reduce protein adsorption and resist cell adhesion. The hydrophobic blocks PCL and PLA are polyesters with good biocompatibility and biodegradability. Polyesters such as poly(D, L-lactic acid) (PDLLA) have been safely used in humans for a long time

because they can degraded into small molecules in human body and eventually removed by renal clearance [Lavasanifar et al, 2002]. Therefore, PCL/PEO and PLA/PEO amphiphilic triblock copolymers have great potential for use in drug delivery system.

2.3.1 Properties of the PCL-PEO-PCL triblock copolymers

PCL-PEO-PCL triblock copolymers have been synthesized by ring-opening polymerization of ϵ -caprolactone using dihydroxy PEO polymer as the initiator without catalysts [Ryu et al., 2001]. Three kinds of PCL-PEO-PCL triblock copolymers were synthesized, where the molecular weight of these copolymers range from 12,810 to 23,860 g/mol. The aggregation behavior was studied by fluorescence spectroscopy, transmission electron microscopy (TEM) and photon correlation spectroscopy (PCS). The results indicated that PCL-PEO-PCL triblock copolymers can self-assemble into spherical micelles with a core-shell structure. The values of critical aggregation concentration (CAC) were determined by the fluorescence probe technique. The CAC values decrease with decreasing proportions of PEO block with aggregate size ranging from 20 to 70 nm. Two kinds of particles with different sizes have been observed from the TEM micrographs [Ryu et al., 2001].

Drug loading into the nano-particles produced from the PCL-PEO-PCL triblock copolymers and drug released from the particles have also been investigated. The drug loading contents increase with increasing PCL block length. This may be attributed to the longer PCL block, which has a stronger hydrophobic interaction with the

hydrophobic drug. However, the higher PCL block length induces slower drug release. Furthermore, it was observed that the drug release decreased with increasing drug loading contents. Drug of high concentration in the core of particles can form crystals, which may hinder the release of the drug from the nano-particles [Ryu et al, 2001].

The degradation behavior of the aggregates formed from PCL-PEO-PCL triblock copolymers in PBS (0.1M, pH 7.4) was also reported. The PCL blocks in the nano-particles degraded slowly, confirming that the drug release from the nano-particles of PCL-PEO-PCL triblock copolymers is controlled by a diffusion mechanism rather than the degradation mechanism [Ryu et al, 2001].

Zhao and Wu [Zhao et al, 2001] have studied the self-assembly of PCL-PEO-PCL triblock copolymers by laser light scattering (LLS). The R_g/R_h values of PCL-PEO-PCL nano-particles obtained by static light scattering (SLS) and dynamic light scattering (DLS) are around 0.95, which are larger than the value (0.774) predicted a non-draining and uniform sphere. This indicates that the particles are slightly draining due to its swollen PEO shell. The aggregation number of the particles is in the range of 100 – 420 nm [Zhao et al, 2001]. They have also studied the biodegradation of the polymers in the presence of an enzyme, lipase PS. The results showed that the nano-particles produced from the polymers were degraded rapidly and the degradation rate can be adjusted by the concentration of the enzyme PS [Zhao et al, 1999].

2.3.2 Properties of the PLA-PEO-PLA triblock copolymers

The synthesis method of PLA-PEO-PLA triblock copolymer is similar to one of

PCL-Pluronic-PCL copolymer. PLA-PEO-PLA triblock copolymers were obtained by the ring opening polymerization of the monomer l-lactide using dihydroxy PEO polymer as the initiator and stannous octoate ($\text{Sn}(\text{Oct})_2$) as the catalyst [Hubbell et al, 1993]. Rashkov and Vert [Rashkov et al, 1996] reported another synthesis method of PLA-PEO-PLA triblock copolymer. The catalyst used is zinc or CaH_2 instead of $\text{Sn}(\text{Oct})_2$ because zinc and CaH_2 are nontoxic. But the reaction time is longer (4-7 days).

Vert's group have studied the degradation and hydrogels properties of PLA-PEO-PLA triblock copolymer. Hydrolysis of PLA-PEO-PLA copolymer with short PLA chains was performed in DMSO/ H_2O in the presence of trifluoroacetic acid. The degradation process was monitored and confirmed by ^1H and ^{13}C NMR. There are

Fig. 2.14 Two kinds of hydrolysis sites of PLA-PEO-PLA copolymers [Rashkov et al, 1996].

two types of sites where hydrolysis could occur, A and B respectively (see Fig 2.14). The results indicate that hydrolysis reactions at sites A and B proceed with comparable rates [Rashkov et al, 1996]. PLA-PEO-PLA copolymer with long PLA chains was made into plates and soaked in distilled water. Results obtained from monitoring the

hydrolytic process show that the ester bonds of PLA blocks are cleaved at random in the early stages. At the later stage of degradation, a highly swollen hydrogel layer is formed from the surface of a compact, partially degraded specimen. The hydrogel is composed of PLA-PEO-PLA copolymers with short PLA blocks resulting from the degradation of parent long blocks [Rashkov et al, 1996]. Protein release from the hydrogels of PLA-PEO-PLA copolymers was studied. The hydrogels were obtained by adding small amounts of water into miscible PLA-PEO-PLA organic solution. The hydrogel prepared by the phase separation is more hydrophilic than the hydrogel obtained by just putting the polymer plate into water. Generally, hydrogel is composed of two different domains. The hydrophilic part of gel is PEO chains, which can delivery hydrophilic drugs. PLA blocks assemble to form nano-sized physical junctions in the gel, which can accommodate hydrophobic drugs. Two proteins, bovine serum albumin (BSA) and fibrinogen were used. The release of BSA exhibits a parabolic-type profile and obeys the diffusion mechanism due to the gel-BSA compatibility. In contrast, fibrinogen shows a linear release from gels indicating that it is incompatible with the hydrogels [Molina et al, 2001].

Recently, Li et al. synthesized bioresorbable hydrogels obtained from both poly(L-lactide)/PEG and poly(D-lactide)/PEG triblock copolymers [Li et al, 2003]. The mixture of L₂₀EO₁₈₂L₂₀ and D₁₈EO₁₈₂D₁₈ in aqueous solution (15%) form a hydrogel at 37°C. It is confirmed by Raman spectroscopy that the formation of stereocomplexation occurring between poly(L-lactide) and poly(D-lactide) blocks leads to the gelation of copolymers.

2.3.3 Properties of the star-shaped PLA-PEO block copolymer

The properties of biodegradable star-shaped PLLA-PEO block copolymers have been investigated by Park and Kim et al. [Park et al, 2001]. Star-shaped PLLA homopolymer was first synthesized by using glycerol as the initiator and stannous octoate as the catalyst. Then PEO polymers were attached onto the end of PLLA to obtain the star-shaped PLLA-PEO block copolymer. The structure of star-shaped PLLA-PEO block copolymers is showed in Fig. 2.15.

The temperature-sensitive properties of star-shaped PLLA-PEO block copolymers in aqueous solution were examined by dye solubilization method and dynamic light scattering technique. The CMT values were obtained by a UV/vis spectrophotometer and using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe molecule. It was observed that the CMT decreased with increasing the PLA block length at a certain concentration for the polymer series with the same length of PEO blocks (see Figure 2.16). The diameter of the particles formed from star PLLA-PEO polymers decreases with increasing temperature. The reason is believed to be the partial dehydration of PEO blocks with increasing temperature.

It is interesting that temperature-sensitive sol-gel transition of the polymer was observed (see Fig. 2.17). Gel-to-sol transition behavior with increasing temperature was found over the critical gelation concentration (CGC). The CGC of the star-shaped polymers increases with increasing temperature [Park et al, 2001].

Fig. 2.15 The structure of star-shaped PLLA-PEO block copolymer [Park et al, 2001].

Fig. 2.16 The CMT of aqueous solution of star PLLAPEO2K series: star PLLA5-PEO2K (■), star PLLA7-PEO2K(▲), and star PLLA9-PEO2K (◆) [Park et al, 2001].

Fig. 2.17 Temperature-sensitive sol-gel transition behavior of aqueous solution of star PLLA-PEOs: ■, star PLLA5-PEO5K; ▲, star PLLA7-PEO5K; ●, star PLLA9-PEO5K [Park, 2001].

2.3.4 Properties of PEG-PLLA-PEG and PEG-PLGA-PEG triblock copolymers

Novel PEG-PLLA-PEG block copolymers with thermogelation, biodegradability and no toxicity have been synthesized by Jeong et al. [Jeong et al, 1997]. PEG-PLLA diblock copolymer was synthesized first by the ring-opening polymerization of monomer L-lactide onto poly(ethylene glycol) (PEG, M_w 5000), followed by the coupling of PEG-PLLA diblock copolymers with hexamethylene diisocyanate to obtain the resulting PEG-PLLA-PEG triblock copolymers. The single sol-gel transition was observed for the PEG-PLLA-PEG triblock copolymers (see Fig. 2.18). For polymers

with different composition, the gel-sol transition occurs at a higher temperature when the polymer concentration is increased [Jeong et al, 1997, 2002].

Fig. 2.18 Gel-sol transition curves of PEG-PLLA-PEG triblock copolymers: (●) 5000-2040-5000; (▲) 5000-3000-5000; (■) 5000-5000-5000 [Jeong et al, 1997].

Jeong et al. also partially changed the middle hydrophobic PLLA block of PEG-PLLA-PEG triblock copolymers to produce the next generation of PEG-PLGA-PEG triblock copolymers [Jeong et al, 1999a]. The chemical structure of PEG-PLGA-PEG triblock copolymers is shown in Figure 2.19. The sol-to-gel transition of PEG-PLGA-PEG triblock copolymers in aqueous solutions is believed to be due to the micellar expansion accompanying an increase in aggregation number driven by hydrophobic interaction. They studied the release behavior of hydrophobic

spironolactone loaded in the PEG-PLGA-PEG hydrogels for a period of two months (see Fig. 2.20). Biphasic mechanism of drug release was observed. The initial stage of release is controlled by the diffusion only while the later stage is controlled by the combination of diffusion and degradation [Jeong et al, 1999b]. Furthermore, it was found that the hydrophilic drug Ketoprofen was released completely after only two weeks, which is much faster than that of hydrophobic drug spironolactone. This is mainly due to the different partitioning behavior of the two drugs in the hydrogel, where the more hydrophobic spironolactone partitioned more into the hydrophobic core of the hydrogel.

Fig. 2.19 The chemical structure of PEG-PLGA-PEG triblock copolymers. For the sake of simplicity, the coupling part of the PLGA middle block via hexamethylene diisocyanate was omitted [Jeong et al, 1999 a].

Fig. 2.20 Spironolactone release from a PEG–PLGA–PEG (550–2810–550) triblock copolymer hydrogel. The legend indicates the initial concentration of polymer in the PBS buffer. The drug load was fixed at 0.25 wt%. The lines are theoretical curves derived from a mathematical model [Jeong et al, 1999 b].

2.4 Summary

The physical properties, temperature-sensitivity and aggregation behavior of Pluronic block copolymers in water have been reviewed in this chapter based on two reviews published in 1995 and others papers published since 1995. The macromolecular surfactants have been extensively studied as potential drug delivery vehicles due to their excellent biocompatibility, and the application of Pluronic micelles and hydrogel in drug delivery systems was reviewed.

However, the CMC values of Pluronic block copolymers are typically very large, due to the low hydrophobicity of PPO blocks. This limits the application of Pluronic micelles because they are not stable and are easily destroyed by dilution when injected into the human body. It is for this reason that there is a need for the chemical modifications of Pluronic block copolymers. The chemical modification of Pluronic copolymers and their application in biomedical field have been reviewed. However, not much research has been conducted on the chemical modification of Pluronic block copolymers, thus this provides a strong motivation for the current research.

In this thesis, hydrophobic PLA and PCL chains were grafted on both ends of Pluronic F127 and F87 yielding PLA-Plu-PLA and PCL-Plu-PCL block copolymers. The main reason for selecting F127 and F87 is that these two Pluronic copolymers have high PEO content, which is desirable since PEO is widely used for drug delivery applications. Some of the reasons for selecting PLA and PCL are; (a) they are well-known biodegradable and biocompatible polyesters, (b) they can enhance the hydrophobicity of modified Pluronic copolymers, thereby lowering the CMC value. Therefore, PLA-Plu-PLA and PCL-Plu-PCL block copolymers should be potential candidates for biomedical applications such as drug delivery and controlled release systems.

Chapter 3 Experimental Details

3.1 Materials

The Pluronic block copolymer series (F127, F87) were kindly supplied by BASF Corporation (Mount Olive, New Jersey, US) and they were dried overnight under vacuum before use. L-Lactide (LA) was purchased from Aldrich and recrystallized from ethyl acetate (EtAc). The purified L-Lactide was stored at 4-5 °C under argon environment. ϵ -Caprolactone (ϵ -CL) was purchased from Aldrich and dried over calcium hydrogen (CaH₂) under stirring at room temperature for 48 hours and distilled under reduced pressure. The purified ϵ -CL was stored at room temperature under argon environment. Stannous octoate (Sn(Oct)₂) was purchased from Aldrich and used as received. Model drug procaine hydrochloride (PrHy, M_w = 273 g/mol, water solubility 1 g/ml) and 9-(methylaminomethyl)anthracene (MAMA, M_w = 221g/mol, water solubility 1.4×10^{-5} g/ml) were purchased from Aldrich and used as received. Osmium tetroxide (OsO₄) in 2-methyl-2-propanol (2.5 wt%) needed for staining purpose was purchased from Aldrich and used as received.

3.2 Polymerization – Chemical modification of Pluronic copolymers

Pluronic copolymers were modified by two kinds of biocompatible hydrophobic polyesters, poly(lactic acid) (PLA) and polycaprolactone (PCL). The Pluronics chosen are F127 (M_n 12,600 Da, 70 wt% PEO) and F87 (M_n 7,700 Da, 70 wt% PEO). Both of these block copolymers contain high PEO content, which is favorable for the application in drug delivery system.

3.2.1 Pluronic block copolymers modified by long and short poly(lactic acid) (PLA) hydrophobic chains

Poly(lactic acid) (PLA) hydrophobic chains were attached at both ends of Pluronic copolymers using Pluronic copolymers as the initiator and stannous octoate ($\text{Sn}(\text{Oct})_2$) as the catalyst to obtain amphiphilic block copolymers PLA-Pluronic-PLA. Two sets of PLA-Pluronic-PLA block copolymers with long and short PLA blocks were synthesized respectively. Pluronic F127 was modified by long PLA chains to produce three kinds of PLA-F127-PLA block copolymers with different compositions. Pluronic F87 was modified by short PLA chains to obtain three kinds of PLA-F87-PLA block copolymers.

The detailed synthesis procedure of PLAF127-29 is as follows. A round bottom flask with a stopcock was heated to remove the moisture under reduced pressure. After cooling to room temperature, argon was introduced into the flask. Following this, L-Lactide (86.6mmol) and F127 (0.50mmol) were added and heated with continuous stirring to produce a well-mixed molten phase. The mixture was then cooled, and $\text{Sn}(\text{Oct})_2$ (0.1wt% of L-Lactide) was added to the flask under argon environment. Then the vacuum-purge cycle was repeated for at least six times, and the mixture was degassed and heated to 180 °C. After stirring for 15h, the reaction product was cooled to room temperature and the product was dissolved with methylene chloride, precipitated twice in 10-fold volume of methanol and once in 10-fold volume of diethyl ether. The polymer PLAF127-29 was filtered and dried overnight under vacuum. A light yellowish powder with a yield of 80% was obtained. In the nomenclature for the polymer “PLAF127-29”, the numeric “29” corresponds to the molecular weight of the polymer obtained from NMR result in kDa.

The other PLA-Pluronic-PLA copolymers with different block composition were synthesized using the same synthesis method as PLAF127-29.

3.2.2 Pluronic F87 block copolymer modified by short polycaprolactone (PCL) hydrophobic chains

Short Polycaprolactone (PCL) hydrophobic chains were attached at both ends of Pluronic F87 copolymers to obtain amphiphilic block copolymers, i.e. PCL-F87-PCL. According to the literature procedure [Ha, 1999], the block copolymers, PCL-F87-PCL were synthesized by the ring opening polymerization of the monomer ϵ -caprolactone using Pluronic F87 block copolymers as the initiator and stannous octoate ($\text{Sn}(\text{Oct})_2$) as the catalyst.

The detailed synthesis procedure of C6F87 is as follows. A round bottom flask with a stopcock was heated under vacuum condition to remove the moisture. After cooling to room temperature, argon was introduced into the flask. Then ϵ -CL (24 mmol) and F87 (1.5 mmol) were added and heated under stirring, in order to achieve a well-mixed molten phase. After the mixture was cooled, $\text{Sn}(\text{Oct})_2$ (at 0.1wt% of ϵ -CL) was added to the flask under argon environment and the vacuum-purge cycle was repeated three times. The mixture was degassed and heated to 140 °C. After stirring for 16h, the reaction product was cooled to room temperature and subsequently dissolved with methylene chloride, precipitated twice in 10-fold volume of methanol and once in 10-fold volume of diethyl ether. The polymer C6F87 was filtered and dried overnight under vacuum. A white powder with a 73% yield was obtained. The numeric “6” in the polymer “C6F87”, corresponds to the number of repeat unit “CL” on each end of C6F87 block copolymer.

The other PCL-F87-PCL copolymers with different block composition were synthesized using the same synthesis method as C6F87.

3.3 Characterization

3.3.1 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectra were recorded at room temperature using a Bruker ACF-400 (400 MHz) Fourier Transform Spectrometer. Chemical shifts (δ) were given in ppm using tetramethylsilane (TMS) as an internal reference. The polymer sample was dissolved in deuteriochloroform (CDCl_3) for measurement and the concentration used is about 10 mg/ml.

3.3.2 Gel Permeation Chromatography (GPC)

Gel Permeation Chromatography (GPC) of the copolymers was performed on an Agilent 1100 apparatus (Germany) equipped with a differential refractometer as the detector. Tetrahydrofuran (THF) was used as the mobile phase with a flow rate of 1.0 ml/min. The standard polymer used is polystyrene (PS). The samples were dissolved in THF first and then filtered through a filter (0.45 μm) to remove impurities. The sample concentration is around 10 mg/ml.

3.3.3 Differential Scanning Calorimetric (DSC)

Differential Scanning Calorimetric (DSC) thermograms were obtained using the modulated DSC 2920 (TA Instruments, Inc., New Castle, DE). The scanning rate was set at 10 $^{\circ}\text{C}/\text{min}$. The sample (ca. 10mg) was placed in an aluminum pan that was sealed using a sample pan crimper. The seal should be tight but not hermetic.

3.3.4 Wide Angle X-ray Diffraction measurement (WXR)

The wide angle X-ray diffraction measurements (WXR) were conducted using the Philips PW1830 powder diffractometer equipped with a Cu K α radiation source. The X-ray diffractometer was operated at 20 kV and 10 mA. The polymer sample (ca. 1g) was put into a sample holder and then melted at high temperature. A flat platelet was formed in the sample holder after it was cooled down to room temperature and used for measurement. The samples were measured at room temperature (25 °C).

3.3.5 Tensiometer

The CMC was measured using a Dataphysics DCAT-21 tensiometer. A stock PLAF127-23 aqueous solution of high concentration (0.01 wt%) was initially prepared. Then, a series of sample solutions of predetermined lower concentrations ($5.2 \times 10^{-6} - 10^{-4}$ wt%) were obtained through the dilution of 0.01 wt% polymer stock solution. The surface tension of the sample solutions was measured using a Du Noüy ring fixture by successive titration of the PLAF127-23 concentrated polymer solution to 60 ml of distilled deionized water. The CMC of PLAF127-23 polymer aqueous solution was determined from the plot of surface tension versus the logarithm of solution concentration.

3.3.6 Transmission electron microscope (TEM)

Transmission electron microscope (TEM) was performed on a JEOL JEM-2010 electron microscope at an acceleration voltage of 200 kV. The copper grid (400 meshes) with a carbon film was used. The copper grid was immersed in a drop of the aqueous polymer solution for 2 mins, and then removed and dried. A drop of osmium tetroxide (OsO $_4$) in 2-methyl-2-propanol (2.5 wt%) was placed on the copper grid for

2 mins. The copper grid was then dried overnight at room temperature prior to measurement.

3.3.7 Rheometer

The rheological properties were studied by using a TA (Carrimed CSL 500) controlled stress rheometer. The geometry used is a rough steel plate with 2 cm diameter. Silicon oil was applied around the sample and plate to avoid the evaporation of solvent. Temperature scans were carried out over the range 10 to 55 °C at a ramp rate of about 2 degrees per minute.

3.4 Sample preparation

3.4.1 The preparation of aggregates formed from PLA-Pluronic-PLA and PCL-F87-PCL block copolymers in aqueous solutions

A sample of PLA-Pluronic-PLA block copolymer (10 mg) was dissolved in 2 ml of tetrahydrofuran (THF). The PLA-Pluronic-PLA solution was added drop-wise to 10 ml of distilled water under gentle stirring. THF was then removed under reduced pressure and the PLA-Pluronic-PLA nano-particles in water (0.1 wt%) were formed at the same time.

PCL-F87-PCL aggregates were prepared in the same way as PLA-Pluronic-PLA aggregates.

3.4.2 Hydrolytic degradation of PLA-Pluronic-PLA and PCL-F87-PCL block copolymers in phosphate buffered saline aqueous solutions

The nano-particles of PLA-F127-PLA block copolymers in phosphate buffered saline (PBS, pH 7.4, 0.01M) aqueous solutions were prepared as follows: PLA-F127-

PLA block copolymers were initially dissolved in tetrahydrofuran (THF). The PLA-F127-PLA solutions were added drop-wise to PBS solutions under gentle stirring, after which THF was removed under reduced pressure. Then the PLA-F127-PLA copolymers in PBS solutions were kept in the shaking water bath at 37 °C. At predetermined time intervals, polymer solutions (30ml) were taken out and filtered to remove the insoluble degraded PLA blocks. The filtrate solutions were dried and dissolved in CDCl₃ for NMR measurement and THF for GPC measurement, respectively. The insoluble inorganic salts from PBS solutions were filtered before NMR and GPC measurements.

The samples for the hydrolytic degradation of PLA-F87-PLA and PCL-F87-PCL block copolymers were prepared in the same way as PLA-F127-PLA copolymers. The change in the particle size for PLA-F87-PLA and PCL-F87-PCL aggregates with degradation was monitored by dynamic light scattering.

3.4.3 The preparation of hydrogels formed from PLA-F87-PLA and PCL-F87-PCL block copolymers

The copolymers were first dissolved in acetone. A predetermined amount of distilled water was added slowly into the polymer solutions in acetone under gentle stirring. After this, acetone was removed under reduced pressure. Then the hydrogels formed from copolymers were equilibrated for two days.

3.4.4 In vitro drug release study from PLA-Pluronic-PLA and PCL-F87-PCL aggregates in PBS aqueous solutions

A certain amount of copolymer and a hydrophobic model drug MAMA was dissolved in THF. The solution with polymer and MAMA was added drop-wise to

distilled water under gentle stirring and THF was removed under reduced pressure. The excess MAMA precipitate was removed by filtration. The drug loaded polymer aggregates in water were dialyzed against distilled water using a dialysis membrane (molecular weight cut-off 12,000 – 14,000) to remove the free drug outside the particles. The initial loading amount of drug in polymer aggregates was determined in the following manner. A certain amount of drug loaded polymer aggregates in water was dialyzed against distilled water using a dialysis membrane to remove the free drug outside the particles. Then the polymer aggregates within the dialysis membrane were dried and subsequently dissolved in acetone. The reason to dissolve the drug loaded aggregates in acetone is to destroy polymeric aggregates to unimers. The amount of drug loaded in the polymeric aggregates was determined from the calibration curve obtained in acetone measured using the UV-Vis spectrophotometer. Then the polymer aggregates loaded with MAMA within the dialysis membrane (15 g) were put in PBS (PBS, pH 7.4, 0.01M) aqueous solution (150 g). The whole solution was then placed in the shaking water bath at 37 °C for the drug release study. At predetermined time intervals, released MAMA solution (5 ml) outside the dialysis membrane was taken out and measured at wavelength 254 nm by ultra-violet spectrophotometer (HP UV, series 4) to determine the concentration of released MAMA. The calibration curve is shown in Figure 3.1 (A). The fresh PBS solution (5 ml) was added into the release solution to replace the sample that was removed so as to maintain a constant release volume. The error in the concentration profile attributed to this method of dilution is around 3%.

The preparation method of polymer aggregates loaded with hydrophilic model drug PrHy is slightly different from that of MAMA loaded polymer aggregates. A certain amount of copolymer was dissolved in THF. The polymer solution was added

drop-wise to PrHy aqueous solution under gentle stirring and THF was then removed under reduced pressure. The following method for the PrHy release study is similar to one for MAMA release study. The sample solution was measured at wavelength 290 nm by UV spectrophotometer to determine the concentration of released PrHy. The calibration curve is shown in Figure 3.1 (B).

Fig. 3.1 The calibration curve of model drugs in PBS solution: (A) MAMA; (B) PrHy.

3.4.5 In vitro drug release study from PLA-Pluronic-PLA and PCL-F87-PCL hydrogels in PBS aqueous solutions

A certain amount of copolymer and model drug (hydrophobic MAMA or hydrophilic PrHy) was first dissolved in acetone. A predetermined amount of distilled water was added slowly into the polymer solutions in acetone under gentle stirring and acetone was removed under reduced pressure. Then the drug loaded hydrogels formed from copolymers were equilibrated for two days.

A predetermined amount of drug loaded hydrogel was introduced into a dialysis membrane (molecular weight cut-off, 8000) and the sample was placed in PBS solution. Then the whole solution was placed in the shaking water bath at 37 °C for the drug release study. At predetermined time intervals, drug released PBS solution (2 ml) outside the dialysis membrane was taken out and measured by UV to determine the concentration of released drug. The solution was then poured back to the release system.

3.5 Laser light scattering measurements

3.5.1 Theory of Laser light scattering

When a laser beam passes through a dilute polymer solution, the electron clouds of the molecules are polarized and become a secondary light source. The secondary light source emits scattered light in different directions.

3.5.1.1 Static light scattering

Static light scattering (SLS) was used to measure and analyze the time-average scattered intensities. The method is often used to determine microscopic properties of particles such as the z-average radius of gyration (R_g), the weight-average molecular weight ($\bar{M}_w$) and the second virial coefficient (A_2).

According to the Einstein's fluctuation theory, an equation of the form shown in equation (3.1) can be used:

$$\frac{KC}{R_\theta} = \frac{1}{M_w} \left[1 + \frac{16\pi^2 n^2 \langle R_g^2 \rangle \sin^2(\frac{\theta}{2})}{3\lambda^2} \right] + 2A_2C \quad (3.1)$$

where, Rayleigh ratio, $R_\theta = (I_s r^2 / I_i \sin \theta)$; $K = [4\pi^2 n^2 (\partial n / \partial C)^2 / (N_A \lambda^4)]$; C , the concentration of the polymer solution; n , the refractive index of the solvent; θ , the angle of measurement; λ , the wavelength of laser light; N_A , the Avogadro's constant and $(\partial n / \partial C)$, the refractive index increment of the polymer solution. A plot of (KC/R_θ) versus $[\sin^2(\theta/2) + kC]$ (where k is a plotting constant) can be used to analyze the data to determine the molecular parameters. By extrapolating the data to zero angles and concentrations, the R_g and A_2 can be obtained from the slopes. A simultaneous extrapolation to zero angle and concentration yields an intercept, which is the inverse of the $\bar{M}_w$.

3.5.1.2 Dynamic light scattering

The frequency of scattered light fluctuates slightly around that of the incident light with time due to the constant motion of the polymer molecules. Dynamic light scattering (DLS) measures the intensity fluctuations with time and correlates these fluctuations to the properties of the scattering objects. These fluctuations can be caused by translational diffusion of particle as they move in and out of the scattering volume. They could also be due to the rotation of non-spherical particles in the scattering volume. In general, the terms of correlation functions of dynamic variables are always used to describe the response of the scattering molecules to the incident light.

The averaged intensities $\langle I \rangle$ over long observation time T can be expressed by the equation below [Brown, 1993]

$$\langle I(\theta) \rangle = \frac{1}{T} \lim_{T \rightarrow \infty} \left(\int_0^T I(\theta, t) dt \right) \quad (3.2)$$

However, in order to study the dynamic properties of the scattering system, correlation functions have to be determined from the time dependent measurements. These functions can then be transformed using Fourier transformation techniques to yield appropriate scattering spectra that contain molecular information on the dynamics of the molecules. The appropriate normalized correlation function is described below [Brown, 1993; Akcasu, 1993]:

$$g_2(t) = \frac{\langle I(t)I(t+\tau) \rangle}{\langle I(t)^2 \rangle} \quad (3.3)$$

where $I(t)$ is an average value of the product of the scattered intensity at an arbitrary observation time, t , and $I(t+\tau)$ is the intensity registered at decay time τ . The above expression can be simplified using the Siegert relations:

$$G_2(t) = B(1 + \beta |g_1(t)|^2) \quad (3.4)$$

$$g_2(t) = 1 + \beta |g_1(t)|^2 \quad (3.5)$$

For a system consisting of non-interacting homogenous mono-disperse spherical particles,

$$g_1(t) = \exp(-\Gamma t) = \exp(-\frac{t}{\tau}) \quad (3.6)$$

where τ is the decay time of the process and $\Gamma = \tau^{-1}$ its decay rate. Combining equation 3.4 with 3.6, one obtains:

$$G_2(t) = B(1 + \beta \exp(-2\Gamma t)) \quad (3.7)$$

The decay rate is related to the translational diffusion coefficient, D by:

$$\Gamma = Dq^2 \quad (3.8)$$

and Stokes-Einstein expression can be expressed as:

$$D_0 = \frac{kT}{6\pi\eta R_h} \quad (3.9)$$

where k , the Boltzmann constant; T , the absolute temperature; η , the solvent viscosity; R_h , the polymer hydrodynamic radius [Noggle, 1996].

For polydisperse systems, more than one-decay processes are present. The relaxation behaviors observed from light scattering are more complex, since light scattering is sensitive to both diffusive and non-diffusive processes. Several decay rates and decay times, Γ_i , τ_i , respectively are present in the system under investigation, thus the field correlation function $g_1(t)$ is the weighted sum of the individual contributions,

$$g_1(t) = \sum w_i \exp(-\Gamma_i t) \quad (3.10)$$

where w_i is the amplitude corresponding to the decay rate, Γ_i . For multiple decay processes, the summation in equation 3.10 can be replaced by an integral, leading to the following expression [Chu, 1991; Brown, 1993]:

$$g_1(t) = \int w(\Gamma) \exp(-\Gamma t) d\Gamma \quad (3.11)$$

where $w(\Gamma)$ is a continuous distribution function of decay rate Γ . If the relationship between $w(\Gamma)$ and Γ , is desired, the mathematical transformation needed to obtain such a relationship is rather complex and will not be discussed here. Significant research has been devoted to this field and much success has been achieved and it is now possible to obtain such a relationship without much difficulty [Stepanek, 1993].

3.5.2 Equipment and sample preparation

The Brookhaven laser light scattering system was used in this study. The equipment consists of a BI-200SM goniometer (Version 2.0), BI-9000AT digital correlator and other supporting data acquisition and analysis software and accessories. The setup of the system is shown in Figure 3.2. The goniometer system includes focusing optics, a rotating arm, detector optics, detector, neutral density filter, beam

focus, sample cell assembly, cells, and a high voltage power supply for the detector. The accessories include a 200mW 488nm Argon-ion laser, digital temperature controller water bath and the matching liquid filter.

Fig. 3.2 Schematic representation of a static and dynamic light scattering system.

The sample solutions were placed into a glass test tube and centrifuged in order to remove any dust that may exist in the test solutions. The concentration used in the laser light scattering experiments is equal or lower than 0.1 wt%, which is in the dilute solution regime. The behavior of individual particles can be characterized under this concentration [Dai, 2000]. The measured temperature was controlled by a water bath, and all the test solutions were allowed to equilibrate for at least 24 hours.

The BI-9000AT digital correlator (Ver 1.6) includes the 25ns fixed channels, BI-CON stepping motor controller, PCS analysis software, ISDA size distribution software, IST alignment/stability software, and the BI-ZP Zimm/Berry/Debye plot software. The Zimm fitting method is the most popular method for the analysis of light scattering data. It works well for medium-sized particles (radii of up to ~20-50 nm). Berry fitting method is useful for larger particles. The digital correlator, which has a maximum channel of 522 and maximum count rate of 40 millions counts per second, is considered the heart of the instrument as the raw data is processed and analyzed to yield useful information. This system can be operated automatically or manually in either DOS prompt or in windows mode.

The data obtained from the DLS measurement are the correlation functions represented by either the $G_2(t)$ or $g_2(t)$ function. The REPES Inverse Laplace Transformation routine supplied with the GENDIST software was used to analyze the DLS results.

Chapter 4 Synthesis and Characterization of Modified Pluronics Block Copolymers with Hydrophobic PLA and PCL Chains

4.1 Introduction

Amphiphilic Pluronic block copolymers (PEO-PPO-PEO) have been widely used in drug delivery systems due to their excellent biocompatibility [Moghimi, 1995; Kabanov, 2002 a-c, 2003]. However, the CMC values of Pluronic copolymers are high, which decreases the stability of micelles/aggregates formed by the Pluronic copolymer chains in-vivo [Alexandridis, 1995]. Carriers with insufficient stabilities are easy to be fragmented and removed rapidly from blood by renal exclusion. The molecular weight of polymeric micelles ($>10^6$ g/mol) prevents renal elimination unless the micelle structure dissociates to unimers [Kwon, 1993; Xu, 1991].

In order to increase the CMC of Pluronic copolymers, hydrophobic modification of Pluronic block copolymers is necessary. The chemical modification of Pluronic copolymers had been performed by several research groups. For example, Ha et al. have modified Pluronic copolymers with PCL chains to obtain PCL-Pluronic-PCL block copolymers [Ha, 1999]. Very recently, cationic poly((diethylamino)-ethyl methacrylate) (PDEAEM) blocks were attached to both ends of Pluronic F127 block copolymer through oxyanionic polymerization to produce PDEAEM-F127-PDEAEM block copolymers by Anderson et al [Anderson, 2003].

In this study, hydrophobic polymers, poly(lactic acid) (PLA) were attached at both ends of Pluronic F127 block copolymers to obtain the block copolymers PLA-F127-PLA with long PLA blocks. Poly(lactic acid) is a well-known biodegradable

and biocompatible polyester. Thus block copolymers comprising of the commercial Pluronic types and polyesters have great potential for application in the biomedical field such as drug delivery and controlled release systems.

In addition, Pluronic F87 was modified for the first time with short PCL and PLA hydrophobic chains at both ends to produce PCL-F87-PCL and PLA-F87-PLA block copolymers respectively using the same synthesis method as PLA-F127-PLA block copolymers. The purpose for grafting short hydrophobic chains to both ends of Pluronic copolymers is to ensure that the temperature-sensitive property of Pluronic copolymers is retained and the solubility of the PCL-Plu-PCL and PLA-Plu-PLA block copolymers is not compromised.

The block composition, molecular weight and molecular weight distribution of modified Pluronic block copolymers were characterized by NMR and GPC techniques. The melting temperature (T_m), glass temperature (T_g) and crystallinity of modified Pluronic block copolymers were studied by DSC and WXR techniques.

4.2 Results and Discussion

4.2.1 Synthesis

4.2.1.1 Synthesis of PLA-F127-PLA block copolymers with long PLA chains

The block copolymers PLA-F127-PLA were synthesized by ring opening polymerization of the monomer L-lactide using Pluronic copolymer F127 as the initiator and stannous octoate ($\text{Sn}(\text{Oct})_2$) as the catalyst. The mechanism of the reaction is that of co-ordination polymerization (Fig. 4.1) [Kricheldorf, 1995, 2000]. Many other catalysts such as SnO , SnO_2 , Ab_2O_3 and PbO have been used in the

Fig. 4.1 Synthesis mechanism of PLA-F127-PLA block copolymers.

synthesis of PEO/PCL and PEO/PLA block copolymers [Xie, 1999; Rashkov, 1996]. However, Sn(Oct)_2 appears to have the advantage of producing polymer of higher molecular weight with better yield [Li, 1996]. It is important to note that the amount of Sn(Oct)_2 added has a significant effect on the polymer produced. At low Sn(Oct)_2 concentration, the polymer obtained has a narrower polydispersity index (PDI). However, insufficient amount of Sn(Oct)_2 would lead to failure in the polymerization. It was found that an optimum amount of Sn(Oct)_2 would be around 0.1wt% of the monomer.

Three kinds of PLA-F127-PLA block copolymers with different PLA block length were synthesized. They are PLAF127-23, PLAF127-29 and PLAF127-48, respectively. In the nomenclature of the three polymers “PLAF127-23, 29, 48”, the

numeric “23, 29, 48” corresponds to the molecular weight of the three polymers obtained from NMR results in kDa.

4.2.1.2 Synthesis of modified F87 block copolymers with short PCL and PLA chains

The block copolymers, PCL-F87-PCL were synthesized by the ring opening polymerization of the monomer ϵ -caprolactone using Pluronic copolymer F87 as the initiator and stannous octoate ($\text{Sn}(\text{Oct})_2$) as the catalyst. The mechanism of the reaction is similar to that for PLA-F127-PLA block copolymer. The appropriate amount of the catalyst $\text{Sn}(\text{Oct})_2$ is 0.1wt% of the monomer.

PCL-F87-PCL block copolymers with three different block compositions were synthesized. They are designated as PCL4F87, PCL6F87 and PCL11F87. For the nomenclature of the polymer “PCL4F87, PCL6F87 and PCL11F87”, the numbers “4, 6 and 11” corresponds to the number of repeating unit “CL” on each end of PCL-F87-PCL block copolymers respectively.

PLA-F87-PLA block copolymers with different block composition were synthesized using the same method as PCL-F87-PCL. Three copolymers, PLA6F87, PLA9F87 and PLA14F87, were produced. Similarly, the numeric “6, 9, and 14” in “PLA6F87, PLA9F87 and PLA14F87” corresponds to the number of repeat unit “LA” on each end of PLA-F87-PLA block copolymers respectively.

4.2.2 Characterization

The composition, structure and molecular weight of modified Pluronic block copolymers were characterized by NMR and GPC techniques.

4.2.2.1 NMR results

4.2.2.1.1 PLA-F127-PLA block copolymers

The NMR results of PLA-F127-PLA block copolymer are as follows:

^1H NMR (400 MHz, CDCl_3 , TMS), δ (ppm): 1.13-1.15 (m, $-\text{OCH}_2-\text{CH}(\text{CH}_3)-$), 1.54-1.63 (m, $-\text{O}-\text{CH}(\text{CH}_3)-\text{CO}-$ and $\text{HO}-\text{CH}(\text{CH}_3)-\text{CO}-$), 3.35-3.75 (m, $-\text{OCH}_2-\text{CH}_2-$ and $-\text{OCH}_2-\text{CH}(\text{CH}_3)-$), 4.25-4.35 (m, $\text{HO}-\text{CH}(\text{CH}_3)-\text{CO}-$ and $-\text{CO}-\text{OCH}_2-\text{CH}_2-\text{O}-$), 5.15-5.19 (m, $-\text{O}-\text{CH}(\text{CH}_3)-\text{CO}-$) (Fig. 4.2).

^{13}C NMR (400 MHz, CDCl_3), δ (ppm): 17.03 ($-\text{O}-\text{CH}(\text{CH}_3)-\text{CO}-$), 17.85 ($-\text{OCH}_2-\text{CH}(\text{CH}_3)-$), 69.41 ($-\text{O}-\text{CH}(\text{CH}_3)-\text{CO}-$), 70.97 ($-\text{OCH}_2-\text{CH}_2-$ and $-\text{OCH}_2-\text{CH}(\text{CH}_3)-$), 169.98 ($-\text{O}-\text{CH}(\text{CH}_3)-\text{CO}-$) (Fig. 4.3).

Figure 4.2 shows a ^1H NMR spectrum of a typical PLA-F127-PLA in CDCl_3 . The small peak at δ of 4.30 ppm belongs to methylene protons of PLA- $\text{CO}-\text{OCH}_2-\text{CH}_2-\text{O}$ -F127 segment, indicating the successful synthesis of PLA-F127-PLA block copolymer. The absence of a peak at δ of 4.9-5.0 ppm, which could have been contributed by the methine proton of the PLA- $\text{O}-\text{CH}(\text{CH}_3)-\text{COOH}$ group, suggests that there was negligible or no PLA homopolymer in the PLA-F127-PLA block copolymers [Raskov, 1996; Li, 1996]. The degree of polymerization (n) of PLA in $\text{PLA}_n\text{-F127-PLA}_n$ copolymers was calculated from the peak intensity ratio of methyl protons of PLA ($\text{O}-\text{CH}(\text{CH}_3)-\text{CO}-$: $\delta = 1.58$ ppm) and methyl protons of F127 ($-\text{OCH}_2-\text{CH}(\text{CH}_3)-$: $\delta = 1.15$ ppm). The number-average molecular weight ($\bar{M}_n$) of the $\text{PLA}_n\text{-F127-PLA}_n$ copolymers was obtained by using the following expression:

$$\bar{M}_n = \bar{M}_n(\text{F127}) + 144n \quad (4.1)$$

Fig. 4.2 ^1H NMR spectrum of PLAF127-29 (CDCl_3).

Fig. 4.3 ^{13}C NMR spectrum of PLAF127-29 (CDCl_3).

The calculated block contents of PLA in PLAF127-23, PLAF127-29 and PLAF127-48 block copolymers are in good agreement with the PLA contents in the synthesis composition (Table 4.1).

Table 4.1 Characterization of PLA-F127-PLA block copolymers.

Sample	W _{LA} % in feed	Unit ratio for PLA: PEO: PPO	$\bar{M}_n$ (theory)	$\bar{M}_n$ (NMR) ^a	$\bar{M}_w / \bar{M}_n$ (GPC)	$\bar{M}_w$ ^b	Yield (%)
PLAF127-23	50	142: 200: 65	25200	22800	1.34	30600	85
PLAF127-29	66.7	230: 200: 65	37800	29000	1.54	44700	80
PLAF127-48	80	500: 200: 65	50400	48000	1.41	67700	82.5

a: Determined by the integration ratio of the peak at 1.58 ppm (-O-CH(CH₃)-CO-group in PLA block) and the peak at 1.15 ppm (-OCH₂-CH(CH₃)- group in Pluronic F127 block) in the ¹H NMR spectrum.

b: $\bar{M}_w = \bar{M}_n$ (NMR) $\times$ [$\bar{M}_w / \bar{M}_n$ (GPC)]

The ¹³C NMR spectrum of typical PLA-F127-PLA in CDCl₃ is shown in Figure 4.3. The peaks at 17.03, 69.41 and 169.98 ppm are associated to the PLA blocks. The peaks at 17.85 and 70.97 ppm belong to the F127 segment.

4.2.2.1.2 Modified F87 block copolymers with short PCL and PLA chains

The NMR result of PCL-F87-PCL block copolymer is as follows:

¹H NMR (400MHz, CDCl₃, TMS), δ (ppm): 1.15 (m, -OCH₂-CH(CH₃)-), 1.25-1.35 (m, -O-(CH₂)₂-CH₂-(CH₂)₂-CO-), 1.59-69 (m, -O-CH₂-CH₂-CH₂-CH₂-CH₂-CO-), 2.30-2.38 (m, -O-(CH₂)₄-CH₂-CO-), 3.35-3.85 (m, -OCH₂-CH₂- and -OCH₂-CH(CH₃)-), 4.01-4.08 (m, -O-CH₂-(CH₂)₄-CO-). 4.21-4.28 (t, -CO-OCH₂-CH₂-) (Fig. 4.4).

Fig. 4.4 ^1H NMR spectrum of PCL4F87 (CDCl_3).

A ^1H NMR spectrum of PCL4F87 in CDCl_3 is shown in Figure 4.4. The small peak at 4.23 ppm belongs to the methylene protons of the $\text{PCL-CO-OCH}_2\text{-CH}_2\text{-O-F87}$ group, which indicates the formation of the PCL4F87 block copolymer. From the intensity ratio of $-\text{CO-OCH}_2\text{-CH}_2-$ (δ 4.25 ppm) and $-\text{OCH}_2\text{-CH}(\text{CH}_3)-$ (δ 1.15 ppm), we confirm that PCL blocks were attached to both ends of Pluronic F87. The polymerization degree ($\overline{DP}$, k) of PCL in $\text{PCL}_k\text{-F87-PCL}_k$ copolymers was calculated from the peak intensity ratio of methylene protons of PCL ($-\text{O}-(\text{CH}_2)_4\text{-CH}_2\text{-CO}-$: δ =2.32 ppm) and methyl protons of F87 ($-\text{OCH}_2\text{-CH}(\text{CH}_3)-$: δ =1.15 ppm). The number-average molecular weight ($\overline{M}_n$) of the $\text{PCL}_k\text{-F87-PCL}_k$ copolymer was obtained using the same method as PLA-F127-PLA block copolymers.

The calculated weight contents of PCL in PCL4F87, PCL6F87 and PCL11F87 block copolymers are showed in Table 4.2.

Table 4.2 Characteristics of PCL-F87-PCL block copolymers

Sample	Number of CL on one end	$\bar{M}_n$ (NMR) ^a	$\bar{M}_w / \bar{M}_n$ (GPC)	$\bar{M}_w$ ^b	Yield (%)
PCL4F87	4	8610	1.04	8950	75
PCL6F87	6	9070	1.06	9610	73
PCL11F87	11	10210	1.08	11030	70

a: Determined by the integration ratio of the peak at 2.32 ppm (-O-(CH₂)₄-CH₂-CO-group in PCL block) and the peak at 1.15 ppm (-OCH₂-CH(CH₃)- group in Pluronic F87 block) in the ¹H NMR spectrum.

b: $\bar{M}_w = \bar{M}_n$ (NMR) $\times$ [$\bar{M}_w / \bar{M}_n$ (GPC)]

The NMR result of PLA-F87-PLA block copolymer is as follows:

¹H NMR (400 MHz, CDCl₃, TMS), δ (ppm): 1.15-1.17 (d, -OCH₂-CH(CH₃)-), 1.52-1.61 (m, -O-CH(CH₃)-CO-), 3.35-3.85 (m, -OCH₂-CH₂- and -OCH₂-CH(CH₃)-), 4.22-4.37 (m, HO-CH(CH₃)-CO- and -CO-OCH₂-CH₂-), 5.12-5.21 (m, -O-CH(CH₃)-CO-) (Fig. 4.5).

Figure 4.5 shows a ¹H NMR spectrum of PLA-F87-PLA in CDCl₃. One part of the small peak at 4.30 ppm belongs to the methylene protons of the PLA-CO-OCH₂-CH₂-O-F87 group. This peak indicates the formation of the PLA-F87-PLA block copolymer. From the intensity ratio of the peak at 4.28 ppm (HO-CH(CH₃)-CO- and -CO-OCH₂-CH₂-) and the peak at 1.15 ppm (-OCH₂-CH(CH₃)-), we can conclude that PLA blocks were attached to both ends of Pluronic F87. There was also no

Fig. 4.5 ^1H NMR spectrum of PLA-F87-PLA (CDCl_3).

homopolymer of PLA in the PLA-F87-PLA block copolymer because no peak was detected at 4.9-5.0 ppm, which belongs to the methine proton of the PLA-O- $\text{CH}(\text{CH}_3)\text{-COOH}$ group [Rashkov, 1996].

The polymerization degree ($\overline{DP}$, n) of PLA in $\text{PLA}_n\text{-F87-PLA}_n$ copolymers was calculated from the peak intensity ratio of methyl protons of PLA (O- $\text{CH}(\text{CH}_3)\text{-CO-}$, 1.58 ppm) and methyl protons of F87 ($-\text{OCH}_2\text{-CH}(\text{CH}_3)\text{-}$, 1.15 ppm). The number-average molecular weight ($\overline{M}_n$) of the $\text{PLA}_n\text{-F87-PLA}_n$ copolymer was determined using the same method as PLA-F127-PLA.

The calculated weight contents of PLA in PLA6F87, PLA9F87 and PLA14F87 block copolymers are showed in Table 4.3.

Table 4.3 Characteristics of PLA-F87-PLA block copolymers

Sample	Number of LA on one end	$\bar{M}_n$ (NMR) ^a	$\bar{M}_w / \bar{M}_n$ (GPC)	$\bar{M}_w$ ^b	Yield (%)
PLA6F87	6	8560	1.05	9000	73.3
PLA9F87	9	9000	1.05	9450	80
PLA14F87	14	9720	1.07	10400	82

a: Determined by the integration ratio of the peak at 1.58 ppm (-O-CH(CH₃)-CO- group in PLA block) and the peak at 1.15 ppm (-OCH₂-CH(CH₃)- group in Pluronic F87 block) in the ¹H NMR spectrum.

b: $\bar{M}_w = \bar{M}_n$ (NMR) $\times$ [$\bar{M}_w / \bar{M}_n$ (GPC)]

4.2.2.2 Gel Permeation Chromatography

4.2.2.2.1 PLA-F127-PLA block copolymers

Figure 4.6 shows the GPC trace of PLAF127-48 block copolymer. The standard used for GPC measurement is polystyrene. The PDI (polymer distribution index) of PLAF127-23, PLAF127-29 and PLAF127-48 block copolymers were determined by GPC measurements (Table 4.1). $\bar{M}_w / \bar{M}_n$ of Pluronic 127 is 1.32. The narrow and symmetric single peak in Fig. 4.6 indicates that PLA-F127-PLA block copolymers are pure, without PLA homopolymer and Pluronic F127 copolymer present.

4.2.2.2.2 Modified F87 block copolymers with short PCL and PLA chains

GPC traces of PCL-F87-PCL block copolymers and Pluronic F87 are shown in Fig. 4.7. The molecular weight distributions ($\bar{M}_w / \bar{M}_n$) of PCL4F87, PCL6F87 and PCL11F87 block copolymers were obtained using the GPC (see Table 4.2). From these

Fig. 4.6 The GPC trace of PLAF127-48 block copolymer (RI signal).

Fig. 4.7 GPC traces of (a) F87, (b) PCL4F87, (c) PCL6F87 and (d) PCL11F87.

Fig. 4.8 GPC traces of (a) F87, (b) PLA6F87, (c) PLA9F87 and (d) PLA14F87.

narrow and symmetric single peaks shown in Fig. 4.7, we confirmed that there was no PCL homopolymer and Pluronic F87 copolymer present in the PCL-F87-PCL block copolymers.

Figure 4.8 shows the GPC traces of PLA-F87-PLA block copolymer and Pluronic F87. The molecular weight distribution ($\bar{M}_w / \bar{M}_n$) of PLA6F87, PLA9F87 and PLA14F87 block copolymers were determined by GPC (see Table 4.3). It is evident that PLA-F87-PLA block copolymers are very pure, where no PLA homopolymer and Pluronic F87 copolymer are present as shown by the narrow and symmetric single peaks (Fig. 4.8).

4.2.3 Thermal properties

4.2.3.1 PLA-F127-PLA block copolymers

Thermal characteristics of PLA-F127-PLA block copolymers were studied using a differential scanning calorimeter (DSC). The DSC results of PLA-F127-PLA block copolymers are shown in Figure 4.9. In order to remove the influence of thermal history, all the DSC curves shown were obtained at the second heating run. There are two endothermic peaks shown for PLAF127-29 block copolymer. The double melting peaks at 128 and 141 °C belong to the crystalline PLA blocks and the double peaks at 34 °C are probably associated with the glass transition of the amorphous PLA region. The results are in agreement with those of PLA-PEO-PLA triblock copolymers obtained by Li et al. [Li, 1996]. The double peak at 39 °C belongs to the melting crystalline F127 block. The wide angle X-ray diffraction pattern of PLAF127-29 confirmed the existence of both crystalline PLA and F127 structure (see Figure 4.10). The peaks at $2\theta = 14, 15.9$ and 16.5° belong to the crystalline PLA blocks and the two weak peaks at $2\theta = 18.3$ and 22.8° belong to crystalline F127 block.

There is a broad single melting peak at 137 °C for crystalline PLA blocks of PLAF127-23 block copolymer. For PLAF127-48 copolymer, the glass transition of amorphous PLA region shows at much smaller temperature (13 °C) than that of the other two block copolymers, which could be due to the increase of crystalline PLA domain and thus the decrease of amorphous PLA domain.

It is shown that the T_m of PLA blocks increases with increasing the PLA block length. This should be related to the degree of crystallinity of PLA blocks in the three block copolymers. The degree of crystallinity of PLA blocks was calculated by the following two equations:

Fig. 4.9 The DSC results of PLA-F127-PLA block copolymers

Fig. 4.10 The wide angle X-ray diffraction pattern of PLAF127-29 and F127 block copolymers.

$$\Delta H_f = \Delta H / w$$

$$\text{Degree of crystallinity} = \Delta H_f / \Delta H^0$$

where ΔH^0 is the enthalpy of fusion for pure PLA crystalline polymer, J/g; ΔH is the measured enthalpy of fusion for the sample by DSC, J/g; w is the weight fraction of PLA blocks in the block copolymer [Pannu, 1999]. ΔH^0 of PLA is 202.8 J/g [Polymer Handbook, 1999].

The T_m values and degree of crystallinity of PLA-F127-PLA block polymers are summarized in Table 4.4. As expected, the T_m of the main crystalline domains becomes higher as the degree of crystallinity of PLA blocks increases.

Table 4.4 DSC results of PLA-F127-PLA block copolymers

Sample	T_m (°C)		ΔH (J/g) of PLA	ΔH_f (J/g) of PLA	Crystallinity of PLA	T_g (°C) of PLA
	F127	PLA				
PLAF127-23	46	137	1.2	12.2	0.06	42
PLAF127-29	39	128, 141	3.6	48.6	0.24	34
PLAF127-48	37	143, 155	3.6	50.2	0.25	13

It was observed that the degradation rate of polyesters increased when they were grafted to flexible hydrophilic PEO backbones because PEO block with low T_g and T_m values is able to increase the permeability and chain mobility of polyesters in the PEO-*b*-polyester copolymers. The chain mobility and permeability will then affect the rate of degradation by controlling accessibility of water to hydrolytically unstable bonds [Li, 2002 a; Wang, 2001]. Compared to the T_g and T_m of PLA homopolymer (62 and 179 °C respectively as shown in Figure 4.9), those of PLA-F127-PLA block polymers are lower, which is consistent with the published results. Thus, the

degradation rate of PLA-F127-PLA block polymers in aqueous solution is higher, which is favorable for their application in drug delivery application.

4.2.3.2 Modified F87 block copolymers with short PCL and PLA chains

Thermal characteristics of modified F87 with short PCL blocks were studied by DSC. Figure 4.11 shows the DSC results of F87 and modified F87 with short PCL blocks. In order to remove the influence of thermal history, all the DSC curves shown in Figure 4.11 were obtained at the second heating run. There is only one endothermic peak for F87 at 50 °C, which is the melting temperature (T_m) of F87. For PCL4F87 and PCL6F87 block copolymers, there is still one endothermic peak, where T_m of PCL4F87 and PCL6F87 is 41 and 39 °C respectively. PCL is a known semi-crystalline polymer ($T_m = 55$ °C) [Polymer Handbook, 1999], however, there is no melting peak of PCL block for PCL4F87 and PCL6F87. This could be because PCL blocks in PCL4F87 and PCL6F87 block copolymers are not long enough to form crystalline structure. However, there are two endothermic peaks for PCL11F87 at 31 and 38 °C respectively, and the main peak at 38 °C is the melting peak of F87 block in PCL11F87. The small shoulder peak is the melting peak of PCL blocks, indicating that the PCL blocks in PCL11F87 are long enough to form crystalline structure. The crystallizability of PCL11F87 was confirmed by the wide angle X-ray diffraction pattern shown in Figure 4.12. Pluronic F87 shows two characteristic crystalline peaks at $2\theta = 19.1$ and 23.2° . The other two small peaks at $2\theta = 20.5$ and 21.4° belong to the crystalline PCL blocks. It is shown that T_m of F87 block in PCL4F87, PCL6F87 and PCL11F87 block copolymers decreases with increasing block content of PCL. This should be related to the degree of crystallinity of F87 block in the three block copolymers.

Fig. 4.11 The DSC results of PCL-F87-PCL block copolymers

Fig. 4.12 The wide angle X-ray diffraction pattern of PCL11F87 and F87 block copolymers.

The degree of crystallinity of polymer was calculated using the same method described in section 4.2.3.1. ΔH^0 of PEO, PPO is 182.7 and 144.8 J/g [Polymer Handbook, 1999]. There is only one melting peak for Pluronic F87, indicating that PEO and PPO have very good compatibility. Thus ΔH^0 of Pluronic F87 can be obtained by averaging ΔH^0 of PEO and PPO, with an average value of 163.8 J/g., and ΔH^0 of PCL is 135.1 J/g [Polymer Handbook, 1999]. The T_m values and degree of crystallinity of F87 block and PCL in PCL4F87, PCL6F87 and PCL11F87 block copolymers are summarized in Table 4.5. The double peaks in the DSC curve of PCL11F87 was analyzed by Origin software and fitted by Lorentzian formulation, where the curve was deconvoluted into two separate curves with identifiable peak. It is shown that the crystallinity of Pluronic F87 block copolymer is very high (0.99). After being modified by PCL chains, the crystallinity of F87 block in the resulting PCL-F87-PCL block copolymers decreased by about 50%. As expected, the degree of crystallinity of F87 blocks in PCL-F87-PCL block copolymers decreases with increasing the block length of PCL, which is consistent with the changing trend of T_m of F87 block.

Table 4.5 DSC results of PCL-F87-PCL block copolymers

Sample	T_m (°C)		ΔH (J/g)		ΔH_f (J/g)		Crystallinity	
	F87	PCL	F87	PCL	F87	PCL	F87	PCL
F87	50		161.8				0.99	
PCL4F87	41		83.0		92.8		0.57	
PCL6F87	39		71.8		84.6		0.52	
PCL11F87	38	31	56.1	10.8	74.4	44.1	0.45	0.33

Fig. 4.13 The DSC results of PLA-F87-PLA block copolymers

Fig. 4.14 The wide angle X-ray diffraction pattern of PLA14F87 and F87 block copolymers.

Similarly, the thermal properties of modified Pluronic F87 with PLA were also examined by DSC (see Figure 4.13). There is one endothermic peak for PLA6F87 and PLA9F87, two endothermic peaks for PLA14F87, indicating that only F87 forms crystals in PLA6F87 and PLA9F87 copolymers while both F87 and PLA blocks form crystals in PLA14F87 copolymer. The DSC result of PLA14F87 is consistent with its X-ray diffraction pattern shown in Figure 4.14. The two strong peaks at $2\theta = 19.1$ and 23.3° belong to the crystalline F87 and the small peak at $2\theta = 16.6^\circ$ belongs to the crystalline PLA blocks. This means the PLA content in PLA14F87 is sufficiently long to form the crystalline structure. Table 4.6 summarizes up the T_m and the crystallinity of PLA6F87, PLA9F87 and PLA14F87 block copolymers. The degree of crystallinity of F87 block in PLA14F87 block copolymer was not calculated because the double peaks in the DSC curve of PLA14F87 cannot be deconvoluted into two separate peaks using the Lorentzian formulation. It is shown that the T_m and crystallinity of F87 block decrease with increasing the PLA block length.

Table 4.6 DSC results of PLA-F87-PLA block copolymers

Sample	T_m ($^\circ\text{C}$)		ΔH (J/g) of F87 block	ΔH_f (J/g) of F87 block	Crystallinity of F87 block
	F87	PLA			
F87	50		161.8		0.99
PLA6F87	36		72.0	80.1	0.49
PLA9F87	34		65.9	77.0	0.47
PLA14F87	32	39			

Chapter 5 Aggregation behaviors and Temperature-Sensitive Property of Modified Pluronic Block Copolymers with PLA and PCL in Aqueous Solutions

5.1 Introduction

Amphiphilic block copolymers have been widely studied in the past several decades due to their excellent capacity to self-assemble to produce nano-particles containing a hydrophobic core and a hydrophilic shell. Polymeric nano-particles with good biocompatibility have the potential for many different applications, such as in enhanced drug delivery systems [Gan, 1999; Rosler, 2001].

Pluronic block copolymers were modified with biodegradable PCL and PLA hydrophobic chains at both ends, which were described in chapter 4. The resulting biocompatible block copolymers are expected to have significant potential for application in the biomedical field such as drug delivery, and controlled release systems. Therefore, in this chapter, the aggregation behavior of modified Pluronic block copolymers in water was studied in detail by laser light scattering (LLS) and transmission electron microscopy (TEM) techniques. [Note: Some sections of this chapter have been published in Macromolecules [Xiong, 2003]].

It is well-known that Pluronic block copolymers show very good temperature-sensitive property. Thus, the temperature-sensitivity of modified Pluronic block copolymers was also studied. The critical micellization temperature (CMT) and

critical gelation temperature (CGT) of modified Pluronic block copolymers with PCL and PLA were measured by tensiometer and rheometer, respectively.

5.2 Results and Discussion

5.2.1 Aggregation behavior of modified Pluronic block copolymers with PCL and PLA in aqueous solutions

Pluronic block copolymers (F127 and F87) were modified with hydrophobic PCL and PLA blocks to obtain PCL-Plu-PCL and PLA-Plu-PLA block copolymers. The PCL-Plu-PCL and PLA-Plu-PLA aggregates were prepared according to the method described in section 3.4.1 of chapter 3. The aggregation behavior of the resulting copolymers in water was studied by LLS, TEM and tensiometer.

5.2.1.1 Morphologies and particles size of modified Pluronic block copolymers with PCL and PLA in water

5.2.1.1.1 PLA-F127-PLA block copolymers with long PLA chains

Static and dynamic light scattering studies on the PLA-F127-PLA block copolymers in water were carried out. The concentration used in the laser light scattering experiments is lower than 0.1 wt%, which is in the dilute solution regime. The behavior of individual particles can be characterized under this concentration [Dai, 2000].

In the SLS mode, time-averaged intensity measurements were made at various scattering angles and analyzed using the software provided, adopting either the Zimm, Berry, Debye plots. Such evaluations using measured angular or concentration

dependencies of the intensity of the scattered light provide important information on particles such as the weight-average molecular weight ($\bar{M}_w$), z-average radius of gyration (R_g) and the second virial coefficient (A_2). The Zimm plot is based on the following equation:

$$\frac{KC}{R_\theta} = \frac{1}{\bar{M}_w} \left[1 + \frac{16\pi^2 n^2 \langle R_g^2 \rangle \sin^2(\frac{\theta}{2})}{3\lambda^2} \right] + 2A_2C \quad (5.1)$$

where R_g and A_2 were obtained from the slopes of $(KC/R_\theta)_{C \rightarrow 0}$ versus $\sin^2(\theta/2)$ and $(KC/R_\theta)_{\theta \rightarrow 0}$ versus C , respectively. The weight-average molecular weight ($\bar{M}_w$) of particles was determined by the extrapolation of $(KC/R_\theta)_{C \rightarrow 0, \theta \rightarrow 0}$.

The Zimm plot of PLAF127-48 is shown in Figure 5.1. Table 5.1 summarizes the values of R_g , $\bar{M}_w$ and the aggregation number for PLAF127-23, PLAF127-29 and PLAF127-48.

Fig. 5.1 Zimm plot of aggregates formed from PLAF127-48 block copolymer in aqueous solution at room temperature, where the concentration range was from 0.01 to 0.06 mg/ml.

Table 5.1 Laser light scattering results of PLA-F127-PLA block copolymers in aqueous solutions at room temperature.

Sample	R_h (nm)	R_g (nm)	R_g/R_h	$\bar{M}_w$ (aggregates)	$N_{\text{aggregation}}^a$
PLAF127-23	50	95	1.9	3.9×10^7	1.3×10^3
PLAF127-29	56	56	1.0	4.4×10^7	9.9×10^2
PLAF127-48	56	48	0.85	3.3×10^7	4.8×10^2

a: $N_{\text{aggregation}} = \bar{M}_w(\text{aggregates}) / \bar{M}_w(\text{polymer})$, where $\bar{M}_w(\text{polymer})$ is shown in Table 4.1 of Chapter 4 (Page 66).

The dynamics of PLA-F127-PLA block copolymers in aqueous solution can be successfully examined by DLS. Figure 5.2 shows the relaxation time distribution functions of PLAF127-23, PLAF127-29 and PLAF127-48 block copolymers in aqueous solutions at different scattering angles.

One single peak was observed and the peak relaxation times shifted to lower values with increasing scattering angles, suggesting that only one type of particle is present. The dependence of the relaxation rate Γ (the reciprocal of peak relaxation time) on the square of the scattering vector (q^2) was examined to verify if the scattering objects were related to translational diffusion. The plots of Γ versus q^2 for PLAF127-23, PLAF127-29 and PLAF127-48 block copolymers are shown in Figures 5.3 (A) to (C). All the three polymers exhibit good q^2 dependence of Γ , confirming that the relaxation time peaks correspond to the translational diffusion of the scattering objects. The slopes of the straight line in Figures 5.3 (A) to (C) represent

Fig. 5.2 Relaxation time distribution functions of PLAF127-23 (A, 0.01 wt%), PLAF127-29 (B, 0.01 wt%) and PLAF127-48 (C, 0.006 wt%) block copolymers in aqueous solutions at different scattering angles.

Fig. 5.3 Plots of Γ versus q^2 for PLAF127-23 (A, 0.01 wt%), PLAF127-29 (B, 0.01 wt%) and PLAF127-48 (C, 0.006 wt%) block copolymers in aqueous solution.

the translational diffusion coefficients D of micelles. Thus, the hydrodynamic radius, R_h of these particles were calculated from equation (5.2):

$$R_h = \frac{kT}{6\pi\eta D_0} \quad (5.2)$$

The hydrodynamic radii R_h of the micelles determined from equation 5.2 for PLAF127-23, PLAF127-29 and PLAF127-48 aggregates are 50, 55.7 and 56 nm, respectively (Table 5.1), which indicates that the aggregate size increases marginally with increasing PLA block length. The R_g/R_h values of the three copolymers are all larger than that predicted for a non-draining hard sphere (0.774), meaning that PLA-F127-PLA nano-particles are swollen (Table 5.1).

In order to confirm the structure of PLAF127-23, PLAF127-29 and PLAF127-48 aggregates in aqueous solutions, samples were examined by TEM. The hydrophobic PLA segments containing double bonds were stained with OsO_4 and the TEM micrographs of PLAF127-23, PLAF127-29 and PLAF127-48 aggregates are shown in Figures 5.4 (A) to (C).

TEM micrographs reveal that the structure of PLAF127-29 and PLAF127-48 aggregates is that of vesicle with a diameter of about 80 nm for PLAF127-29 (Figure 5.4 B) and 100 nm for PLAF127-48 (Figure 5.4 C). The thickness of the hydrophobic layer is approximately 5-10 nm. The size distribution of PLAF127-48 aggregates is broader. However, PLAF127-23 aggregates (diameter of ~100 nm) have the appearance of coated micelles with three layers (Figure 5.4 A). The thickness of the outside hydrophobic layer is about 5-10nm and the radius of internal hydrophobic core is about 30 nm. Based on these results, we proposed two possible microstructures, which are schematically illustrated in Figures 5.5 (A) and (B).

(A)

(B)

(C)

Fig. 5.4 TEM micrographs of aggregates formed from PLAF127-23 (A), PLAF127-29 (B) and PLAF127-48 (C) block copolymers in water.

Fig. 5.5 Possible microstructure of vesicles formed from PLA-F127-PLA block copolymers in aqueous solution: (A) coated micelles from PLAF127-23; (B) bilayer vesicles from PLAF127-29 and PLAF127-48.

The particle size and the morphology determined from TEM studies are consistent with the results derived from LLS data. It is reasonable to observe the vesicular structure for PLAF127-29 and PLAF127-48 block copolymers with hydrophobic blocks on both ends because their chemical structure is more similar to that of liposome with one hydrophilic head and two long hydrophobic tails compared to that of diblock copolymers. It is reported by Shen et al. that PS-PAA diblock copolymers with longer hydrophobic PS block are easier to form vesicles

[Shen, 2000]. This is consistent with our results because the aggregation number of PLAF127-48 (480) aggregates is smaller than that of PLAF127-29 (990) as shown in Table 5.1. The reason that the structure of PLAF127-23 aggregates is different from that of PLAF127-29 and PLAF127-48 is most likely related to the length of PLA segments. Because the PLA block length on one end for PLAF127-23 is about half of F127 block length and shorter than that of PLAF127-29 and PLAF127-48, the chemical structure of PLAF127-23 is more like star-like amphiphilic copolymers with long hydrophilic block and short hydrophobic block. Thus, PLAF127-23 may tend to form normal core-shell micelles. However, the existence of hydrophobic PPO block makes the aggregation behavior of PLAF127-23 more complicated, which probably results in the formation of coated micelles to stabilize the micelles with an outer bilayer. The relationship between morphologies and block composition needs to be further studied, which is beyond the scope of the present work. This is the subject for the recommendation for future studies.

The effect of concentration of PLAF127-48 block copolymer in aqueous solutions on the particle size is shown in Figure 5.6. The relaxation time peak of the copolymers at different concentrations is identical, indicating that the particles size of PLAF127-48 is independent of polymer concentration. This confirms that the aggregation mechanism of the polymer corresponds to the closed association model.

5.2.1.1.2 PCL-F87-PCL and PLA-F87-PLA block copolymers with short hydrophobic chains

Static and dynamic light scattering of PCL-F87-PCL and PLA-F87-PLA block copolymers in aqueous solutions were also studied extensively. The concentration used is equal or less than 0.1 wt%, which is in the dilute solution regime.

Fig.5.6 Relaxation time distribution functions of PLAF127-48 in aqueous solutions with different concentrations at a scattering angle of 90° .

In the SLS, time-averaged intensity measurements were made at different scattering angles and concentrations. The z-average radius of gyration (R_g), $\bar{M}_w$ and aggregation number of PCL4F87, PCL6F87 and PCL11F87 aggregates are summarized in Table 5.2. The R_g values of the three polymers are large and there is no obvious trend.

Table 5.2 Laser light scattering results of PCL-F87-PCL block copolymers in aqueous solutions at room temperature.

Sample	R_h (nm)			R_g (nm)	$\bar{M}_w$ (aggregates)	$N_{\text{aggregation}}^a$
	Small	Middle	Large			
PCL4F87	6.8	97		155.6	8.33×10^5	90
PCL6F87	7.3	156		116.9	2.43×10^6	250
PCL11F87	14	80.5	454	139.4	6.88×10^7	6240

a: $N_{\text{aggregation}} = \bar{M}_w(\text{aggregates}) / \bar{M}_w(\text{polymer})$, where $\bar{M}_w(\text{polymer})$ is given in Table 4.2 of Chapter 4 (Page 68).

Figure 5.7 shows the relaxation time distribution functions of PCL4F87, PCL6F87 and PCL11F87 block copolymers in aqueous solutions obtained at different scattering angles. Two relaxation time peaks were detected for PCL4F87 and PCL6F87 block copolymers with varying angles. The relaxation time distribution functions of PCL11F87 block copolymer are more complicated with three relaxation time peaks. The relaxation time of each peak shifts to lower value with increasing angles.

Whether the relaxation time peak corresponds to true particles is dependent on the observation of q^2 dependence of Γ (the reciprocal of peak relaxation time). Hence, the parameter, Γ was plotted as the function of q^2 for PCL4F87, PCL6F87 and PCL11F87 block copolymers (see Fig. 5.8) and good q^2 dependence of Γ is observed for all the relaxation peaks, indicating that these relaxation time peaks are attributed to true particle. The R_h values of PCL4F87 aggregates were determined to be 6.8, 97nm,

Fig. 5.7 Relaxation time distribution functions of PCL4F87 (A, 0.1 wt%), PCL6F87 (B, 0.1 wt%) and PCL11F87 (C, 0.1 wt%) block copolymers in aqueous solutions at different angles.

Fig. 5.8 Plots of Γ versus q^2 for PCL4F87 (A, 0.1 wt%), PCL6F87 (B, 0.1 wt%) and PCL11F87 (C, 0.1 wt%) block copolymers in aqueous solution.

while PCL6F87 is made up of aggregates with size of 7.3, and 156nm. For PCL4F87 and PCL6F87 block copolymers, the size of small particle is similar to that of Pluronic F87 micelles. The large particles observed in PCL4F87 and PCL6F87 systems are believed to possess rather complex structure. For PCL11F87 block copolymer, three kinds of particles are produced, whose R_h values are 14, 80.5, 454 nm, respectively. The existence of very big aggregates (R_h of 454 nm) is consistent with the large aggregation number of PCL11F87 aggregates as shown in Table 5.2. It is believed that such complicated morphologies are attributed to the complex structure of these polymers. For the three PCL-F87-PCL block copolymers, there are five blocks, two of which are hydrophilic PEO and three are hydrophobic PPO and PCL. Although the hydrophobicity of PCL is stronger than PPO, the block length of PCL is much shorter than that of PPO. Thus the hydrophobicity of PPO and PCL blocks competes and controls the aggregation behavior of PCL-F87-PCL block copolymers, resulting in the complicated aggregation. There is also small peak at several scattering angles as evident in Fig. 5.7. The Γ of the peaks are not q^2 dependent, thus these peaks do not correspond to true particles. They may be induced by the internal vibrational modes of the particles.

The R_g , $\bar{M}_w$ and aggregation number of aggregates composed of PLA6F87, PLA9F87 and PLA14F87 block copolymers are summarized in Table 5.3. R_g values decrease with increasing PLA block length. This trend is identical to that observed for the PLA-F127-PLA block copolymers with long hydrophobic PLA chains.

Figure 5.9 shows the relaxation time distribution functions of PLA6F87, PLA9F87 and PLA14F87 block copolymers in aqueous solutions at different scattering angles. There is one major peak detected for PLA6F87, three peaks for

Table 5.3 Laser light scattering results of PLA-F87-PLA block copolymers in aqueous solutions at room temperature.

Sample	R_h (nm)			R_g (nm)	$\bar{M}_w$ (aggregates)	$N_{\text{aggregation}}^a$
	Small	Middle	Large			
PLA6F87		160		226	7.18×10^6	800
PLA9F87	10	77	409	223.4	1.13×10^7	1200
PLA14F87	13	81		86	1.95×10^6	190

a: $N_{\text{aggregation}} = \bar{M}_w(\text{aggregates}) / \bar{M}_w(\text{polymer})$, where $\bar{M}_w(\text{polymer})$ is shown in Table 4.3 of Chapter 4 (Page 70).

PLA9F87 and two peaks for PLA14F87. Γ was plotted as a function of q^2 for PLA6F87, PLA9F87 and PLA14F87 block copolymers (see Fig. 5.10). Good q^2 dependence of Γ is observed for the three block copolymers, indicating that the relaxation time peaks correspond to true particles. R_h values of these particles were calculated and summarized in Table 5.3. The aggregation behavior of PLA-F87-PLA is also very complicated, similar to that of PCL-F87-PCL block copolymers. The sample shown in Figure 5.11 (A) was stained with OsO_4 . The real structure of large aggregates can be seen, but the contrast of picture is not very good. In order to improve the contrast of TEM picture, PLA9F87 sample was also stained by Platinum as shown in Figure 5.11 (B). The small micelles with a diameter of around 20 nm were evident. The net-like complicated aggregates are observed in Figures 5.11 (A) and (B). The diameter of net-like big aggregates varies from about 300 to 700 nm, which is consistent with that observed from DLS measurements. Therefore, several

Fig. 5.9 Relaxation time distribution functions of PLA6F87 (A, 0.1 wt%), PLA9F87 (B, 0.1 wt%) and PLA14F87 (C, 0.1 wt%) block copolymers in aqueous solutions at different scattering angles.

Fig. 5.10 Plots of Γ versus q^2 for PLA6F87 (A, 0.1 wt%), PLA9F87 (B, 0.1 wt%) and PLA14F87 (C, 0.1 wt%) block copolymers in aqueous solution.

(A)

(B)

Fig. 5.11 TEM micrographs of aggregates formed from PLA9F87aqueous solution (0.15 wt %): (A) sample was stained by OsO₄; (B) sample was stained by Platinum.

kinds of aggregates coexist for PCL-F87-PCL and PLA-F87-PLA block copolymers in aqueous solution.

The morphologies of the aggregates formed in other PCL-F87-PCL and PLA-F87-PLA block copolymer systems will need to be determined and studied by TEM and other techniques.

In order to elucidate whether the PPO block in the modified F87 block copolymers with short PCL and PLA chains is responsible for the complicated aggregation behaviors of the resulting PCL-F87-PCL and PLA-F87-PLA, the PPO block in the copolymers was replaced by PEO block to obtain the simpler PLA-PEO-PLA triblock copolymer with short PLA chains. The PLA-PEO-PLA triblock copolymer is designated as PLA9PEO8K ($\bar{M}_w / \bar{M}_n$ 1.06), where the numeric “9” corresponds to the amount of repeating units of PLA block on one end of the triblock copolymer and “8K” represents the molecular weight of PEO block (8000 g/mol), which is similar to the molecular weight of F87 ($\bar{M}_n$ 7700). The aggregation behavior of PLA9PEO8K in aqueous solution was detected by DLS at different scattering angles (50 to 100°). The relaxation time distribution function of PLA9PEO8K at 90° is shown in Figure 5.12. There is only one peak observed for the triblock copolymer and R_h of PLA9PEO8K particles is 25 nm, indicating that the aggregation behavior of PLA9PEO8K ($\bar{M}_n$, 9300) triblock copolymer is much simpler than that of PLA9F87 ($\bar{M}_n$, 9000) block copolymer with the same length of PLA blocks. The only difference between these two polymers is the existence of hydrophobic PPO block between two hydrophilic PEO blocks in PLA9F87. Thus, the PPO block should be responsible for the complex aggregation behavior of PCL-F87-PCL and PLA-F87-PLA block copolymers.

Fig. 5.12 Relaxation time distribution function of PLA9PEO8K (0.1 wt%) in aqueous solutions at 90°.

Compared to the modified Pluronic block copolymers with short hydrophobic chains (PCL-F87-PCL and PLA-F87-PLA), the aggregation behavior of modified Pluronic block copolymers with long hydrophobic chains (PLA-F127-PLA) is simpler. Since the PLA chain length in PLA-F127-PLA copolymers is longer than PPO segment, the hydrophobic interaction of PLA blocks dominates the aggregation process. Thus, the PLA blocks assemble to form the hydrophobic core through their strong hydrophobic interaction, which produces vesicles or even coated micelles with three layers.

The effect of concentration of PCL4F87 and PLA14F87 aqueous solutions on the particle size was studied by dynamic light scattering (see Fig. 5.13). For each of the two polymers, the relaxation time peak remains constant at all concentrations,

Fig. 5.13 Relaxation time distribution functions of PCL4F87 (A) and PLA14F87 (B) in aqueous solutions with different concentrations at constant angle 90° .

indicating that the particles size of the two polymers is not concentration dependent. This also proves that the aggregation mechanism of the two polymers obeys the closed association model.

5.2.1.2 Surface Tension Measurements and the Critical micellization concentration (CMC)

5.2.1.2.1 PLA-F127-PLA block copolymers with long PLA chains

The CMC values of PLA-F127-PLA block copolymers were determined by surface tension measurements. The surface tension of PLAF127-23, PLAF127-29 and PLAF127-48 aggregates was plotted against the logarithm of the polymer

Fig. 5.14 Surface tension as a function of the logarithm of concentrations of PLAF127-23 (◆), PLAF127-29 (■) and PLAF127-48 (▲) block copolymers in aqueous solutions at room temperature.

concentration (Fig. 5.14), and the CMC values of PLAF127-23, PLAF127-29 and PLAF127-48 block copolymers were determined to be 7.3×10^{-5} , 2.2×10^{-5} , 1.7×10^{-6} wt %, respectively. As expected, the CMC of PLA-F127-PLA block copolymers at room temperature is much lower than that of F127 (CMC ~ 1 wt%), due to the greater hydrophobicity of the modified F127 system. The CMC decreases with increasing PLA block length, due to the enhanced hydrophobicity of PLA-F127-PLA block copolymers. The extremely low CMC values of these modified Pluronic polymers augur well for their potential applications as vesicles for drug delivery applications because PLA-F127-PLA nano-particles will be very stable. These aggregates comprising of different kinds of vesicles are potential candidates for enhanced drug

delivery applications because they can be used to deliver both hydrophobic and hydrophilic drugs. In addition, the size of PLA-F127-PLA aggregates is ideal for such applications as we can achieve better circulation time and bioavailability of the aggregates in biological system. For drug delivery applications, the preferred range of particle size is ~ 10 to 100 nm, and the size of PLA-F127-PLA aggregates lies within this range [Kabanov, 2002 c].

The relationship between surface tension and $\log(C)$ is shown in Figure 5.14, where the surface tension at the water-air interface of the polymer solutions decreases with increasing polymer concentration due to the partitioning of polymer chains to the air-water interface. According to the modified Gibbs adsorption equation [Alexandridis, 1994 a; Ghosh, 2002], the maximum surface (excess) concentration, Γ_{max} , can be calculated from:

$$\Gamma_{max} = -(1 / 2.303 RT)(d\gamma / d \log C) \quad (5.3)$$

where γ is the surface tension of solution; C is the concentration, R is the molar gas constant, and T is the absolute temperature.

For a multi-component system, $dC_1 = dC_2 = \dots = dC_{total}$, equation 5.3 can be expressed as follows:

$$\sum_i \Gamma_{max} = -\frac{1}{2.303 RT} \frac{d\gamma}{d \log C_{total}} \quad (5.4)$$

Thus, for PLA-F127-PLA block copolymers, although there are two kinds of hydrophobic blocks (PLA and PPO), the A_{min} values calculated from Γ_{max} will be the averaged surface area of PLA-F127-PLA polymer chains.

$d\gamma / d \log C$ can be obtained from the slope of the linear plot of γ versus $\log(C)$ at concentration below the CMC as shown in Figure 5.14.

The minimum area per molecule, A_{min} , can then be calculated using equation (5.4):

$$A_{min} = 1 / N \Gamma_{max} \quad (5.5)$$

where N is Avogadro's number. The calculated Γ_{max} and A_{min} are summarized in Table 5.4.

Table 5.4 CMC and interfacial properties of PLA-F127-PLA block copolymers in aqueous solutions at room temperature.

Sample	CMC $\times 10^5$ (wt %)	$d\gamma / d \log(C)$	$\Gamma_{max} \times 10^6$ (mol $\cdot$ m $^{-2}$)	A_{min} ($\text{\AA}^2$)
PLAF127-23	7.3	-0.0148	2.59	64
PLAF127-29	2.2	-0.0122	2.14	78
PLAF127-48	0.17	-0.0069	1.21	137

The calculated A_{min} for PLA-F127-PLA block copolymers at the air-water interface is close to that for Pluronic block copolymers reported by Alexandridis et al. However, it is smaller than that for nonionic surfactants with aliphatic tail group and PEO head group of comparable size to the PEO blocks of Pluronic block copolymers [Alexandridis, 1994a]. Similarly, this means that there exists a certain amount of folded PEO chains at the air-water interface or desorption of PEO blocks in the water phase for PLA-F127-PLA block copolymers. Another possibility could be that a large amount of polymers are absorbed to the wall of cuvette on the air-water surface at these low concentrations, resulting in the decrease of actual bulk concentration. Thus, the slope of the surface tension curves would be affected.

The surface properties of PLA-F127-PLA block copolymers obtained need to be further examined.

It is observed that the area that each copolymers chain occupies increases with the hydrophobic PLA block length for PLA-F127-PLA block copolymers with the same hydrophilic PEO content. However, the trend for Pluronic block copolymers is the reverse [Alexandridis, 1994 a]. Their explanation is that PEO blocks become more compact at the interface and even part of them may fold around PPO blocks or desorbs into the aqueous sub-phase. Compared to the triblock chemical structure of Pluronic (PEO-PPO-PEO), the structure of PLA-F127-PLA (PLA-PEO-PPO-PEO-PLA) is more complex. Thus, it should be more difficult for the PEO blocks in PLA-F127-PLA to fold around hydrophobic PLA or PPO blocks. It was assumed that Pluronic molecules were absorbed at the interface as an inverted “U”. The orientation of PLA-F127-PLA polymer chains at the interface may be in the double-connected “U” or more complicated configuration, thereby occupying a larger surface area.

5.2.1.2.2 PCL-F87-PCL and PLA-F87-PLA block copolymers with short hydrophobic chains

The CMC values of PCL-F87-PCL block copolymers were determined by surface tension measurements at room temperature. Figure 5.15 shows the surface tension of aggregates as a function of the logarithm of polymer concentration for solutions consisting of PCL4F87, PCL6F87 and PCL11F87 block copolymers. There are two break points observed on the plots of the surface tension versus $\log C$, which is similar to Pluronic block copolymers [Wanka, 1994; Alexandridis, 1994 a]. The

Fig. 5.15 Surface tension as a function of the logarithm of concentrations of PCL4F87 (A), PCL6F87 (B) and PCL11F87 (C) block copolymers in aqueous solutions at room temperature.

Fig. 5.16 Surface tension as a function of the logarithm of concentrations of PLA6F87 (A), PLA9F87 (B) and PLA14F87 (C) block copolymers in aqueous solutions at room temperature.

second break point is believed to be the real CMC value, which was described in detail in section 2.1.2.2 of Chapter 2 [Alexandridis, 1994 a]. Therefore, the concentration at the second break point is chosen to be the CMC in this study. The CMC values of PCL4F87, PCL6F87 and PCL11F87 block copolymers are 1.0×10^{-3} , 7.1×10^{-3} , 9.5×10^{-4} wt%, respectively.

Figure 5.16 shows the surface tension of aggregates as a function of $\log C$ of polymer solutions at room temperature for PLA6F87, PLA9F87 and PLA14F87 block copolymers. The CMC values of the three block copolymers are 3.5×10^{-3} , 1.1×10^{-3} , 7.5×10^{-4} wt%, respectively.

It is observed that the CMC values of modified F87 block copolymers with short hydrophobic chains are still much lower than that of F87, indicating that the aggregates formed from modified Pluronic F87 with short hydrophobic chains are very stable in water, which makes them suitable for application in drug delivery systems.

The Γ_{max} and A_{min} of PCL-F87-PCL and PLA-F87-PLA block copolymers at CMC were also calculated and summarized in Table 5.5 and 5.6 using the same methodology described above. For PCL-F87-PCL, it is observed that A_{min} values decrease when the hydrophobic PCL block length is increased, which is consistent with the trend for Pluronic block copolymers. However, the relation between A_{min} and the PLA block length for PLA-F87-PLA is the reverse, which should be related to the complicated chemical structure of these two kinds of copolymers with five blocks. Similarly, the surface areas obtained for both PCL-F87-PCL and PLA-F87-PLA block copolymers are also smaller than anticipated, and further studies are needed to elucidate this observation.

Table 5.5 CMC and interfacial properties of PCL-F87-PCL block copolymers in aqueous solutions at room temperature.

Sample	CMC $\times 10^3$ (wt %)	$d\gamma / d \log C$	$\Gamma_{\max} \times 10^6$ (mol m ⁻²)	$A_{\min}$ (Å ²)
PCL4F87	1.0	-0.022	3.9	43
PCL6F87	7.1	-0.029	5.1	32
PCL11F87	0.95	-0.044	7.8	21

Table 5.6 CMC and interfacial properties of PLA-F87-PLA block copolymers in aqueous solutions at room temperature.

Sample	CMC $\times 10^3$ (wt %)	$d\gamma / d \log C$	$\Gamma_{\max} \times 10^6$ (mol m ⁻²)	$A_{\min}$ (Å ²)
PLA6F87	3.5	-0.030	5.2	32
PLA9F87	1.1	-0.014	2.4	69
PLA14F87	0.75	-0.013	2.2	75

5.2.2 Temperature-sensitivity of modified Pluronic block copolymers with PCL and PLA in aqueous solutions

Since Pluronic block copolymers are thermo-responsive system, the temperature-sensitivity of modified Pluronic block copolymers with hydrophobic PCL and PLA chains was examined by surface tension and rheological measurements.

For PLA-F127-PLA block copolymers with long PLA chains, no CMT was observed, indicating that modified F127 block copolymers with long hydrophobic chains do not exhibit obvious temperature-sensitive property. The reason could be that the hydrophobic interaction between long PLA blocks is much stronger than that between PPO blocks and it becomes the main driving force of micellization.

Interestingly, the PCL-F87-PCL and PLA-F87-PLA block copolymers with short PCL and PLA chains maintain their temperature-sensitive property. The hydrogels formed from PCL-F87-PCL and PLA-F87-PLA also exhibit distinct thermo-reversible sol-gel transition behavior.

5.2.2.1 Critical micellization temperature (CMT) of PCL-F87-PCL and PLA-F87-PLA aqueous solutions

For PCL-F87-PCL block copolymers, CMT of PCL4F87 and PCL6F87 was observed while that of PCL11F87 was not detected. This indicates that the PCL block length in PCL11F87 is sufficiently long to negate the temperature-sensitive property of F87 block copolymer, which is corroborated by the results from DSC in section 4.2.3.2 of chapter 4. There is no crystal structure of PCL blocks formed in PCL4F87 and PCL6F87, while crystalline PCL domain was observed in PCL11F87. The surface tension of PCL4F87 aqueous solution was plotted against the temperature at different concentrations (see Figure 5.17). With increasing temperature, the solubility of polymer decreases, thus polymer chains are partitioned to the air-water interface, resulting in the decrease of surface tension until the CMT is reached. The surface tension should be a constant when the temperature exceeds the CMT due to the formation of polymeric micelles. However, it is known that the surface tension of

Fig. 5.17 Surface tension as a function of temperature for PCL4F87 block copolymer at different concentrations.

Fig. 5.18 Surface tension as a function of temperature for PCL6F87 block copolymer at different concentrations.

Fig. 5.19 Surface tension as a function of temperature for PLA6F87 block copolymer at different concentrations.

Fig. 5.20 Surface tension as a function of temperature for PLA9F87 block copolymer at different concentrations.

water decreases linearly with increasing temperature as shown in Figure 5.17. Therefore, the slower decrease of the surface tension above the CMT shown in Figure 5.17 is probably due to the surface tension change of the solvent with respect to temperature. Figure 5.18 shows the surface tension of PCL6F87 aqueous as a function of temperature at different polymer concentrations. As expected, the CMT of PCL4F87 and PCL6F87 is much lower than that of F87 due to the higher hydrophobicity of the modified Pluronic system, which is favorable for their applications in controlled release systems.

Similarly, for PLA-F87-PLA block copolymers, the CMT of PLA14F87 was not detected due to the relatively longer PLA blocks. The crystalline domain of PLA in PLA14F87 was also observed from DSC measurement. Both PLA6F87 and PLA9F87 exhibit temperature-sensitive property and possess CMT values. Figure 5.19 and 5.20 show the surface tension of PLA6F87 and PLA9F87 aqueous solutions as a function of temperature at different polymer concentrations, respectively, where the CMT of PLA6F87 and PLA9F87 is much lower than that of F87.

The relationship between CMT and polymer concentration for PCL-F87-PCL and PLA-F87-PLA block copolymers is shown in Figure 5.21. For all the polymers, the CMT decreases with the increase of concentration due to the decrease in the polymer solubility, which is consistent with that of Pluronic block copolymers. Compared to Pluronic block copolymers (see Figure 2.2 in chapter 2), the temperature-sensitivity of modified F87 block copolymers is much more dramatic, that is, the CMT decreases much faster with increasing concentration for PCL-F87-PCL and PLA-F87-PLA block copolymers. This finding is consistent with that observed by Bromberg et al. [Bromberg, 1999 e]. The reason could be because PCL and PLA blocks, which do not

Fig. 5.21 The CMT-CCM boundary of PCL-F87-PCL and PLA-F87-PLA block copolymers.

possess temperature-sensitive properties, contribute to the micellization of PCL-F87-PCL and PLA-F87-PLA. Furthermore, the CMT of PCL-F87-PCL decreases faster with an increase of concentration than that of PLA-F87-PLA.

For PCL-F87-PCL and PLA-F87-PLA block copolymers, the standard free energy of micellization (ΔG^0) was calculated from the equation below:

$$\Delta G^0 = R T_{CMT} \ln X \quad (5.6)$$

where R is the gas law constant, T_{CMT} is the absolute critical micellization temperature, and X is the concentration in mole fraction units [Alexandridis, 1995].

Based on the Gibbs-Helmholz equation, the standard enthalpy of micellization (ΔH^0) can be described as follows:

$$\Delta H^0 = -RT^2 \left(\frac{\partial \ln X}{\partial T_{CMT}} \right)_p = R \left[\frac{\partial \ln X}{\partial (1/T_{CMT})} \right]_p \quad (5.7)$$

Thus, ΔH° can be obtained from the slope of the plot of $\ln X$ versus $1/T_{CMT}$. The plot of $\ln X$ as a function of $1/T_{CMT}$ for PCL-F87-PCL and PLA-F87-PLA block copolymers is shown in Figure 5.22.

Accordingly, the standard entropy of micellization (ΔS°) was calculated using the following equation:

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \quad (5.8)$$

The calculated thermodynamic values (ΔG° , ΔH° and ΔS°) of micellization at different concentrations for PCL-F87-PCL and PLA-F87-PLA are summarized in Table 5.7 and 5.8, respectively. All the ΔG° values in Table 5.4 and 5.5 are negative, which means that the micellization of PCL-F87-PCL and PLA-F87-PLA aqueous solution is spontaneous. However, the positive ΔH° values for PCL-F87-PCL and PLA-F87-PLA indicate that the process forming micelles is enthalpically unfavorable. Therefore, the micellization of PCL-F87-PCL and PLA-F87-PLA is entropy-driven, which is consistent with the positive ΔS° values shown in Table 5.7 and 5.8.

Fig. 5.22 The plot of $\ln X$ as a function of $1/T_{CMT}$ for PCL-F87-PCL and PLA-F87-PLA block copolymers.

For 1 wt% Pluronic block copolymer solution at the critical micelle temperature, the reported ΔG° values are in the range -24.5 to -28.8 kJ·mol⁻¹ [Alexandridis, 1995]. Compared to the ΔG° of Pluronics, the values for PCL-F87-PCL and PLA-F87-PLA are smaller at a much lower polymer concentration, which means that PCL-F87-PCL and PLA-F87-PLA block copolymers are easier to self-assemble to form aggregates.

Table 5.7 CMT and thermodynamic properties of micellization for PCL-F87-PCL block copolymers

Sample	$C \times 10^5$ (wt %)	$X \times 10^{10}$ ^a	CMT (°C)	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (kJ/mol)
PCL4F87	3.0	6.27	45	-56	31	0.28
	4.0	8.36	38	-54		0.27
	5.0	1.05	32	-52		0.27
PCL6F87	3.0	5.95	37	-55	30	0.27
	4.0	7.94	33	-53		0.27
	5.0	9.92	25	-51		0.27
	6.0	1.19	20	-50		0.27

a: X is the concentration of polymer in mole fraction units.

Table 5.8 CMT and thermodynamic properties of micellization for PLA-F87-PLA block copolymers

Sample	$C \times 10^5$ (wt%)	$X \times 10^{10}$ ^a	CMT (°C)	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (kJ/mol)
PLA6F87	10	21.0	54	-54	66	0.37
	13	27.3	50	-53		0.37
	20	42.1	46	-51		0.37
	25	52.6	42	-50		0.37
	30	63.1	40	-49		0.37
PLA9F87	5	10	63	-58	92	0.45
	10	20	59	-55		0.44
	15	30	54	-53		0.45
	20	40	52	-52		0.44
	25	50	49	-51		0.45
	30	60	46	-50		0.45

a: X is the concentration of polymer in mole fraction units.

5.2.2.2 Gelation behavior and critical gelation temperature (CGT) of PCL-F87-PCL and PLA-F87-PLA with short hydrophobic chains

The effect of temperature on the gelation properties of modified F87 with short PCL and PLA were studied. Up to now, there are several kinds of definitions for a gel. However, the definition based on rheological observation is believed to be the most reliable one, that is, a gel is formed when the storage modulus (G') is independent of angular frequency (ω), and when the storage modulus (G') is larger than the loss modulus (G'') [Li, 2002; Winter, 1986; Clark, 1989].

To confirm whether modified F87 block copolymers with PCL and PLA are able to form gels, the dynamic viscoelastic properties such as G' , G'' were measured as a function of ω . Figure 5.23 and 5.24 shows the plots of G' and G'' of PCL4F87 (39 wt%) and PLA9F87 (36 wt%) block copolymers versus ω at two temperatures, respectively. For PCL4F87, it is evident from Figure 5.23 that G' is smaller than G'' and not independent of ω at 15 °C, where the system exists as a sol. However, when the temperature was increased to 30 °C, G' increased by three orders of magnitude, and G' is larger than G'' and is almost independent of ω , indicating that a gel was formed. Similarly, PLA9F87 gel was formed when the temperature was increased from 20 to 40 °C because G' is independent of ω and larger than G'' as shown in Figure 5.23.

To study the thermo-reversible properties of gels formed from modified F87 block copolymers with PCL and PLA, the change of G' with temperature for PCL4F87 and PLA9F87 was measured, and the G' of PCL4F87 and PLA9F87 was plotted as a function of temperature at different concentrations shown in Figure 5.25 and 5.26, respectively. It is shown that the G' values for PCL4F87 and PLA9F87

Fig. 5.23 G' and G'' of PCL4F87 (39wt%) as a function of ω at 15 and 30 °C.

Fig. 5.24 G' and G'' of PLA9F87 (36 wt%) as a function of ω at 20 and 40 °C.

Fig. 5.25 G' of PCL4F87 as a function of temperature at different concentrations (35, 39 and 44 wt%).

Fig. 5.26 G' of PLA9F87 as a function of temperature at different concentrations (36 and 40 wt%).

increase by about 1000 times at the sol-gel transition. It is interesting that both PCL4F87 and PLA9F87 exhibit excellent thermo-reversible sol-gel transition above a critical polymer concentration, similar to that observed for Pluronic block copolymers. Such phenomena were also observed for PLA6F87. More interestingly, both sol-gel transition at lower temperature and gel-sol transition at higher temperature were observed for PCL6F87, which is similar to that of F127 gel. For PCL11F87 and PLA14F87, they are able to form gels above a critical polymer concentration, but their gelation behavior is not thermo-reversible. This means the temperature-sensitive property of Pluronic F87 is destroyed when relatively longer hydrophobic chains are attached onto both ends of F87 block copolymer, a result that is consistent with the temperature-sensitive behavior of these two polymers in aqueous solutions.

The relationship between G' and polymer concentration for PCL-F87-PCL and PLA-F87-PLA hydrogels is shown in Figure 5.27. It is shown that G' of gels formed from F87 and modified F87 block copolymers increases with concentration, indicating that PCL-F87-PCL and PLA-F87-PLA hydrogels become stronger when the concentration is higher.

Figure 5.28 shows the CGT values of PCL-F87-PCL and PLA-F87-PLA hydrogels versus the concentrations. High concentration is also required for the gelation of PCL-F87-PCL and PLA-F87-PLA, similar to Pluronics. Therefore, the mechanism of gelation for PCL-F87-PCL and PLA-F87-PLA could be similar to that of Pluronic, that is, micelle close packed mechanism. It was observed that C6F87 sample shows sol-gel transition at lower temperature and gel-sol transition at higher temperature at the same concentration. Similar phenomenon was also found for F127

Fig. 5.27 G' of F87, PCL-F87-PCL and PLA-F87-PLA hydrogels as a function of concentration.

Fig. 5.28 CGT of F87, PCL-F87-PCL and PLA-F87-PLA hydrogels as a function of concentration.

gel as shown in Figure 2.6 of chapter 2. The reason could be that the aggregates in C6F87 gel are able to reorganize to form aggregates with different morphologies with increasing temperature, where the gel structure is destroyed.

Compared with CGT values of F87, those of modified F87 are a little higher at similar polymer concentration. However, the hydrophobicity of modified F87 gels is larger than that of F87 gels, indicating that the former maybe able to deliver more hydrophobic drugs due to the presence of more hydrophobic PCL and PLA domains.

Chapter 6 Hydrolytic Degradation of Modified Pluronics

Block Copolymers with Hydrophobic PLA and PCL Chains

6.1 Introduction

Polyesters such as poly(L-lactic acid) (PLA), polycaprolactone PCL) and poly(glycolic acid) (PGA) have very good biodegradability and biocompatibility. They can be degraded into small molecules through hydrolytic or enzyme degradation, which are then excreted from the human body by renal clearance. Therefore, amphiphilic block copolymers containing hydrophobic polyester segments have attracted increasing attention from the research community [Choi, 2002; Huang, 2003; Wan, 2003; Kim, 2002; Li, 2002 a; Wang, 2001]. The most widely used hydrophilic polymeric backbone for the grafting of degradable polyesters is poly(ethylene oxide) (PEO) because of its water-solubility and excellent biocompatibility. It is well-known that polyesters degrade rather slowly because of their hydrophobic characteristics. However, it was reported that the degradation rate of polyesters increases when they are grafted to flexible hydrophilic PEO backbones [Li, 2002 a; Wang, 2001]. Thus, amphiphilic block copolymers comprising of PEO and polyester segments could be used in various controlled release systems by manipulating the block composition of PEO and polyester.

The degradation properties of film produced from PLA/PEO, PLGA/PEO and PCL/PEO di-block or tri-block copolymers have been studied by only a few research groups [Kissel, 2002; Anderson, 1997; Witt, 2001]. The techniques that were commonly used to monitor the degradation process are NMR, GPC, FT-IR and

scanning electron microscope (SEM). Vert et al. studied the hydrolytic and enzymatic degradation of physically cross-linked hydrogels prepared from PLA/PEO/PLA block copolymers using NMR, GPC and FT-IR techniques [Li, 2002 b]. All the previous studies focus on the degradation behaviors of polymers in the solid or hydrogel form. For drug delivery applications of PEO/polyester block copolymers, it is more useful to examine the degradation properties of nano-particles in aqueous solution. Up to now, only Wu and co-workers reported the study on the degradation behavior of PCL-PEO-PCL nano-particles in PBS solution using only DLS technique [Nie, 2003; Zhao, 1999].

Compared to the triblock copolymers PCL-PEO-PCL or PLA-PEO-PLA with PEO hydrophilic segment, the synthesized block copolymers PCL-Pluronic-PCL or PLA-Pluronic-PLA with Pluronic block examined in the present study possess several advantages:

1. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer (PEO-PPO-PEO) is a widely used commercial amphiphilic copolymer (Pluronics-BASF) that exhibits thermal-responsive characteristic.
2. The block copolymer is one of the very few synthetic polymeric materials that are approved by the U. S. Food and Drug Administration for use as food additives and pharmaceutical ingredients [Alexandridis, 1995].
3. It was reported that poly(ethylene imine)-Pluronic (PEI-Pluronic) block copolymer exhibited higher transfection efficiency toward plasmid DNA than PEI-PEO copolymer due to the amphiphilic property of Pluronic block copolymer [Nguyen, 2000]. The micelles produced from Pluronic block copolymers have been found to increase the oral and brain bioavailability of drugs [Rosler, 2001].

Therefore, PLA-Pluronic-PLA and PCL-Pluronic-PCL block copolymers are believed to be the better candidates in drug delivery and controlled release systems.

In this chapter, the degradation process of PLA-F127-PLA nano-particles with long PLA chains in PBS solution was examined in details by various physical techniques, such as TEM, DLS, NMR and GPC. We wish to emphasize that TEM has never been used to study the degradation behavior of these degradable polymers before. Thus, it is the first time that the microstructural evolution of the morphologies of the aggregate is examined as the degradation progresses.

The hydrolytic degradation of PCL-F87-PCL and PLA-F87-PLA nano-particles with short hydrophobic chains in PBS solution at 37 °C was also examined by DLS technique respectively.

6.2 Results and Discussion

6.2.1 Hydrolytic degradation of PLA-F127-PLA block copolymers

The hydrolytic degradation of nano-particles formed from PLA-F127-PLA block copolymers in PBS solutions (pH 7.4) at 37 °C were examined in details by DLS, TEM, NMR and GPC over a time period of about two months [Xiong, 2004]. The change in the hydrodynamic radius (R_h) of PLA-F127-PLA nano-particles with degradation was examined by DLS. The concentration used in the laser light scattering experiments is 0.1 wt%, which is in the dilute solution regime.

Figure 6.1 shows the relaxation time distribution functions of PLAF127-23 block copolymer in PBS solution measured at a scattering angle of 90°. Only one single peak was evident before the degradation commences. However, on the 4th day, the size and distribution of particles exhibited an obvious change, where two peaks were

Fig. 6.1 Relaxation time distribution functions of PLAF127-23 block copolymer in aqueous solution (0.1wt%) at 90° with degradation.

detected and they remained unchanged over the remainder for two months. The peak corresponding to the large aggregate became narrower between the 4th to 27th day, and it then became broader until the 55th day. The change in the size (R_h) of nanoparticles as degradation progressed is shown in Figure 6.2. The particle size (R_h) of large aggregate increased dramatically from 50 to 191 nm during the first seven days. It progressively decreased to a minimum on the 20th day where the particle size remained essentially constant ($R_h \sim 100$ nm) for about 20 days. After the 41st day, the particle size (R_h) increased again to a size of ~ 187 nm on the 55th day. However, no significant change in the size of small particles was observed over the two months period.

Fig. 6.2 The change of hydrodynamic radius (R_h) of PLAF127-23 aggregates with the hydrolytic degradation.

In order to understand the changes in the morphologies of PLA-F127-PLA nano-particles with degradation time, samples were removed and examined by TEM. The hydrophobic PLA segments containing double bonds were stained with OsO_4 and the TEM micrographs of PLAF127-23 aggregates as a function of degradation time are shown in Figure 6.3. Before the hydrolytic degradation, PLAF127-23 nano-particles exist as coated micelles with three layers [Xiong, 2003]. On the 7th day of degradation, two kinds of size particle distribution were observed in the TEM micrographs. The morphology of nano-particles became regular large compound vesicles with diameter of around 350 and 60 nm respectively. The morphology of PLAF127-23 aggregates remained unchanged up to the 20th day, but the size of large particles decreased to ~ 200 nm. With further degradation from the 27th to 41st day,

Fig. 6.3 TEM pictures of PLAF127-23 aggregates with the hydrolytic degradation.

The numbers shown at the upper corner of TEM pictures stand for the day on which the degradation happened.

the morphologies of nano-particles became irregular compound vesicles and were rather complicated, where the diameter is about 200 and 60 nm respectively. PLAF127-23 aggregates reassembled into regular large compound vesicles on the 55th day, where the diameter of large particles increased to ~ 350 nm. The morphologies and size of particles determined from TEM are corroborated by the results measured using the DLS technique.

The degradation samples were also examined by NMR and GPC in order to determine the mechanism of degradation by examining the chemical composition and size distribution of the polymer chains. Figure 6.4 shows the NMR spectra of the degradation mixture of PLAF127-23 block copolymer from the 7th to 55th day of degradation. There are two possible degradation sites on the PLA-F127-PLA block copolymers, namely at the middle of PLA block (LA-LA linkage) and at the interface of PLA and F127 blocks (LA-EO linkage) as indicated below:

We believe that the LA-LA bond cleavage occurred first because the EO-LA bond is more stable than LA-LA bond. As shown above, it could be that the EO group (-CH₂CH₂O-) beside the ester group makes the EO-LA bond more stable than the LA group (-CH(CH₃)-C(O)-) in the LA-LA bond. Therefore the LA-LA bond cleaved first. In the degraded samples, the possible compounds that may be present after degradation are PLA-F127-PLA, PLA-F127, F127 and fragmented LA segments (1 ~

Fig. 6.4 ^1H NMR spectrum of PLAF127-23 block copolymer with degradation (CDCl_3).

2 repeating units of LA). The insoluble fragments of degraded LA segments have been removed by filtration. The results obtained from NMR are summarized in Table 6.1. The small peak “c” at δ of 4.2 - 4.3 ppm belongs to the methylene protons of PLA-CO-OCH₂-CH₂-O-F127- segment. The peak “a” and “d” belong to the methine proton of PLA blocks (O-CH(CH₃)-CO-: $\delta = 5.15 - 5.19$ ppm) and the

methyl protons of F127 block ($-\text{OCH}_2-\text{CH}_2-$ and $-\text{OCH}_2-\text{CH}(\text{CH}_3)-$: $\delta = 3.35\text{--}3.75$ ppm). From the integration ratio of peak “c” and “d”, the approximate molar content of PLA-F127-PLA (N) on the different degradation time was calculated. Before the degradation, the integration ratio of peak “c” and “d” is 4 : 995. As degradation progressed, the integration value of peak “c” decreased to 3.8 on the 7th day. Therefore, the approximate molar content of PLA-F127-PLA (N) present in the degradation mixture is 95% ($= 3.8/4$). The averaged $\bar{M}_n$ of PLA blocks in PLA-F127-PLA block copolymers was derived from the integration ratio of peak “a”, “c” and “d”. It should be pointed out that the segment length of PLA blocks on both ends of PLAF127-23 copolymer may be different after the hydrolytic degradation as one would not expect identical scission of the LA segments during the degradation process. Thus, the $\bar{M}_n$ of PLA blocks shown in Table 6.1 is the mean value of PLA blocks at both ends of the Pluronic polymer chains. From the NMR results in Table 6.1, the degradation occurred mainly at the LA-LA linkage on the 7th day of degradation where the $\bar{M}_n$ of PLA blocks decreased rapidly from 10200 to 4800 Da. The initial rapid degradation of PLA blocks was also reported by Li et al for thin slabs of PLA-PEO-PLA block copolymers [Li, 1998]. On the 20th day, the degradation occurred mainly at the LA-LA linkage and less than 5% of PLAF127-23 degraded at the EO-LA linkage. Beyond the 20th day, the degradation rate was reduced and the degradation mainly occurred at the EO-LA linkage. About 38% of PLA-F127-PLA block copolymer had degraded on the 55th day due to the cleavage of EO-LA linkage. The $\bar{M}_n$ of PLA blocks in PLA-F127-PLA block copolymer remained unchanged beyond the 20th day. The degradation percentage of PLA blocks in PLAF127-23 block copolymer over a period of two months is shown in Figure 6.5.

Table 6.1 NMR results of PLAF127-23 copolymer with the hydrolysis degradation.

Degradation time (days)	Integration ratio of peaks d : c : a	Approximate molar content of PLA-F127-PLA (N) ^a	Approximate $\bar{M}_n$ (PLA) on both ends ^b
0	995 : 4 : 142	100%	10 200
7	995 : 4 : 66	100 %	4 800
20	995 : 3.8 : 55	95 %	4 200
55	995 : 2.5 : 38	62 %	4 400

a: $N = (\text{integration value of peak c}) / 4$

b: $\bar{M}_n(\text{PLA}) = 72 \times (\text{Integration value of peak a}) / N$

Fig.6.5 The degradation percentage of PLA blocks in PLAF127-23 block copolymer with time.

The degraded samples of PLAF127-23 block copolymer were analyzed using the GPC. Figure 6.6 shows the GPC traces of PLAF127-23 during the hydrolytic degradation process. On the 20th day, the GPC curve exhibited four different peaks (peak 1, 2, 3 and 4 as marked) when compared to a single peak observed for the virgin PLAF127-23 solution. The main distribution peak [1] obviously shifted to the right side on the 20th day, confirming the scission of the PLAF127-23 chains. Three peaks [2, 3, 4] corresponding to smaller molecular weight fragments were also observed, and the retention time of these three peaks remained unchanged with degradation proceeding. However, the intensity of the peak increased with degradation time. The GPC curve did not change much on the 55th day while the intensity of peak [1] decreased slightly and intensity of peaks [2, 3, 4] increased. This indicates that the molecular weight of PLA-F127-PLA copolymer remained unchanged while the weight content of PLA-F127-PLA copolymer decreased for the degradation from the 20th to 55th day. Such trend corroborated with our earlier finding from NMR that the primary degradation occurred at the interface of Pluronic and PLA blocks. Accordingly the content of compounds with smaller molecular weight fragments increased. The GPC curves in Figure 6.6 were analyzed by Origin software and the four peaks were fitted by Lorentzian formulation, where the curves were deconvoluted into separate curves with identifiable peak. The fractional areas of the four peaks at different degradation times are summarized in Table 6.2. The area of peak [1] decreased from 47% on the 20th day to 35% on the 55th day while the area of peak [2] increased from 35% to 46%. These results are in agreement with that determined from NMR.

Fig.6.6 The GPC traces of PLAF127-23 block copolymer with the hydrolytic degradation (RI signal).

Table 6.2 The fractional areas of the four peaks shown in Figure 6.6 at different degradation time.

Peak area (%)	[1]	[2]	[3]	[4]
Time (days)				
20 th day	47	35	16	2
55 th day	35	46	18	1

Based on the results obtained from DLS, TEM, NMR and GPC, a probable degradation mechanism of PLAF127-23 block copolymer is given below and the pictorial representation is shown in Figure 6.7.

1. In the process step “ a ” (from 0th to 7th day), the particle size and morphology of PLAF127-23 block copolymer rapidly change due to the high degradation rate of PLA blocks (LA-LA linkage). Because the dramatic reduction in the length of PLA blocks, hydrophobic interaction of PLA segments is significantly reduced and is not strong enough to produce regular coated micelles. Therefore, polymer chains again self-assemble to form more complicated large compound vesicles.
2. With further degradation (process step “ b ”, from 7th to 20th day), the cleavage rate of LA-LA linkage decreases significantly and the degradation at the interface of PLA and F127 blocks (EO-LA linkage) dominates. The morphology of nano-particles remains unchanged due to the slow degradation rate of both LA-LA and EO-LA linkages. However, the particle size exhibits a large decrease from the 7th to 20th day. We believed that the polymer chains reorganize to form more stable and uniform aggregates containing lower aggregation number because of the cleavage of EO-LA linkage.
3. In step “ c ” (from 20th to 41st day), the cleavage rate of EO-LA linkage increases and large compound vesicles are destroyed to produce irregular shaped compound vesicles.
4. With further degradation (step “ d ”, from 41st to 55th day), the hydrophobic interaction of PLA blocks is further weaken, thus more polymer chains aggregate together to form spherical large compound vesicles, resulting in an increase in the particle size.

Fig. 6.7 The schematic picture of the mechanism of the hydrolytic degradation of PLAF127-23 block copolymer in vitro.

The degradation behaviors of PLAF127-29 and PLAF127-48 block copolymers were also examined by TEM (see Figure 6.8). Large compound vesicles were also observed after the 3rd day for both polymers, which is consistent with the results observed from PLAF127-23 block copolymer.

Fig. 6.8 TEM pictures of PLAF127-29 (A) and PLAF127-48 (B) nanoparticles on the 3rd day of hydrolytic degradation.

6.2.2 Hydrolytic degradation of PCL-F87-PCL and PLA-F87-PLA with short hydrophobic chains

The hydrolytic degradation of nano-particles formed from PLA-F87-PLA and PCL-F87-PCL block copolymers in PBS solutions (pH 7.4) at 37 °C were studied by DLS over a period of one month, respectively.

The change in the hydrodynamic radius (R_h) of PLA9F87 particles with degradation is shown in Figure 6.9. For comparison, the R_h change of PLAF127-23 large nano-particles with degradation is also shown in Figure 6.9. Before hydrolytic degradation, the aggregates formed from PLA9F87 nano-particles are rather complicated, and this was described in details in section 5.2.1.1.2 of chapter 5. There are three kinds of aggregates detected by DLS. R_h of these aggregates is 10, 77 and 409 nm, respectively. However, on the 4th day, there is only one kind of particles

Fig. 6.9 The change of hydrodynamic radius (R_h) of PLA9F87 and PLAF127-23 aggregates with the hydrolytic degradation.

observed and R_h of the particles is 86 nm. The reason should be similar to that of PLAF127-23 block copolymer due to the similar chemical structure of these two copolymers. PLA9F87 aggregates were destroyed by the initial rapid LA-LA bonding cleavage and reorganized to form more stable particles. It is noticed that the particle size of PLA9F87 aggregates (86 nm) on the 4th day is relatively close to that of PLA9F87 mid-size aggregates (77 nm) before degradation, which may suggest that the small (10 nm) and large (409 nm) PLA9F87 aggregates are less stable than the mid-size aggregates before the degradation occurs. With further degradation, the size of PLA9F87 particles continued to increase until the peak value (243 nm) was reached on the 7th day. This initial largest particles for PLA9F87 with short PLA chains appeared at the same time as those for PLAF127-23 with long PLA chains. With further degradation, the particle size of PLA9F87 aggregates decreased to 125 nm on the 10th day and finally reached an equilibrium size of 107 nm on the 20th day.

After the 20th day, PLA9F87 particles increased slightly to 148 nm on the 34th day. Compared to PLAF127-23, it was observed that PLA9F87 exhibited the similar trend for the change of particle size during the hydrolytic degradation process. Also, the chemical structure of PLA9F87 is similar to that of PLAF127-23. Therefore, the degradation mechanism for PLA9F87 with short PLA chains should be similar to that for PLAF127-23 with long PLA chains.

The hydrolytic degradation of PCL6F87 block copolymer in PBS solution was also examined by DLS. Figure 6.10 shows the change of R_h of PCL6F87 aggregates with degradation. Before the hydrolytic degradation, two kinds of PCL6F87 aggregates were detected, whose size are 156 and 7.3 nm, respectively. After the degradation progressed for one day, only one peak appeared where the R_h of PCL6F87 particles is 181 nm. As the degradation proceeds, R_h of PCL6F87 increased rapidly from 181 to 320 nm on the 3rd day and then decreased to 124 nm on 16th day. As the degradation progressed further, the particle size of PCL6F87 nanoparticles increased marginally to 177 nm on the 30th day. The trend for the change in R_h for PCL6F87 particles is similar to that of PLA9F87 and PLAF127-23 aggregates. However, in contrast to PLAF127-23 (with longer PLA chains), whose size peaked on the 7th day, the peak value of R_h for PCL6F87 aggregates appeared on the 3rd day, a likely explanation is due to the shorter PCL chain length. However, further studies using NMR, GPC and TEM techniques are needed to confirm whether other factors such as the degradation rate of PCL blocks, block composition, and morphologies of aggregates may have contributed to the degradation process.

Fig. 6.10 The change of hydrodynamic radius (R_h) of PCL6F87 aggregates with the hydrolytic degradation.

Chapter 7   Drug Release of Modified Pluronic Block Copolymers Containing PLA and PCL Segments

7.1 Introduction

Polymeric drug delivery systems have attracted increasing attention during the last two decades. Amphiphilic block copolymers have been widely studied due to their potential application in drug delivery systems as they are capable of forming aggregates in aqueous solutions. These aggregates comprise of a hydrophobic core and hydrophilic shell. They are good vehicles for delivering hydrophobic drugs, since the drugs are protected from possible degradation by enzymes [Gan, 1999; Rosler, 2001]. The morphology of nano-particles produced from amphiphilic block copolymers can be varied by changing the composition of hydrophobic and hydrophilic blocks on the polymer chains. Various forms of morphologies have been reported, such as sphere, vesicles, rods, lamellas, tubes, large compound micelles, and large compound vesicles. Some of these structures are good candidates for drug delivery applications [Discher, 2002; Yu, 1998]. Compared to the normal core-shell micelles, vesicles with a hydrophilic core and hydrophobic layers are better for their application as drug delivery carriers because they can deliver not only hydrophobic but also hydrophilic drugs. It was proven in clinical studies that vesicles can improve the treatment efficacy of anticancer drugs, especially for hydrophilic anticancer drugs such as doxorubicin, due to the enhanced permeability and retention properties [Harrington, 2000; Massing, 2000].

The block copolymers comprising of commercial Pluronic systems and biodegradable polyesters (PLA or PCL) have been synthesized for the present study. They are believed to be excellent candidates for application in the biomedical field such as drug delivery system, and controlled release system due to their excellent biocompatibility.

Interestingly, the morphologies of nano-particles formed from PLA-F127-PLA with long hydrophobic PLA blocks in aqueous solutions are found to be bi-layer and even coated micelles. Vesicles are excellent candidates as drug delivery vehicles compared to other morphologies because they can deliver both hydrophobic and hydrophilic drugs. Thus, the drug release behavior of PLA-F127-PLA aggregates loaded with hydrophobic drug model, MAMA and hydrophilic drug model, PrHy in PBS (pH 7.4, 0.01M) aqueous solution was studied by UV spectrophotometer respectively.

For the synthesized PLA-F87-PLA and PCL-F87-PCL block copolymers with short hydrophobic chains, the important property for such system is the observed thermo-reversible gelation behavior in aqueous environment. Since the temperature-sensitive sol-gel transition of Pluronic block copolymers has attracted much attention due to its potential in controlled release applications. It was reported that no burst release was observed for drugs released from Pluronic gels, that is, the loaded drug is released initially at a constant rate [Moore, 2000; Anderson, 2001]. This is a very important behavior because the burst release is very common for drug release from most polymeric nano-particles in aqueous solutions. Drug release at a constant rate will be able to maintain the concentration of drugs in human body, thus avoiding the frequent injection of drugs. In this chapter, the drug release behavior of hydrogels formed from PCL-F87-PCL and PLA-F87-PLA block copolymers loaded with

hydrophobic drug model MAMA and hydrophilic drug model PrHy in PBS (pH 7.4, 0.01M) solutions were studied. Compared to the Pluronic gels, it is believed that the hydrogels formed from PCL-F87-PCL and PLA-F87-PLA will have a higher drug loading capacity for hydrophobic drugs because of the presence of additional hydrophobic PCL and PLA domains.

The drug release behavior of PLA-F87-PLA and PCL-F87-PCL nano-particles loaded with hydrophobic drug model MAMA and hydrophilic drug model PrHy in PBS (pH 7.4, 0.01M) aqueous solution was also monitored by UV spectrophotometer respectively.

7.2 Results and Discussion

7.2.1 Drug release behavior of PLA-F127-PLA Block Copolymers in PBS aqueous solutions

It was found in previous studies that the morphologies of PLAF127-29 and PLAF127-48 block copolymers are that of vesicles and the morphologies of PLAF127-23 consist of interesting coated micelles with three layers. Thus, PLA-F127-PLA nano-particles are expected to have potential applications in drug delivery systems. The PLA-F127-PLA aggregates should be able to deliver both hydrophobic and hydrophilic drugs due to the vesicular structure. Thus, the hydrophobic drug model MAMA and hydrophilic drug model PrHy were loaded into the PLA-F127-PLA aggregates in PBS (pH 7.4, 0.01M) solutions respectively.

7.2.1.1 Drug release kinetics of hydrophobic drug model MAMA

The release behaviors of MAMA loaded PLAF127-23 and PLAF127-29 aggregates in PBS solution at 37 °C were examined. The effect of block composition and concentration on the loading capacity of MAMA in the polymeric nano-particles was studied. The loading capacity of MAMA into PLAF127-23 (0.08wt%), PLAF127-23 (0.02wt%) and PLAF127-29 (0.08wt%) aqueous solutions is 58, 53 and 49 mg/g polymer, respectively. For the same polymer, it is shown that the drug loading capacity increases marginally with polymer concentration. For the relationship between loading capacity and polymer concentration, two types of trends were observed, i.e. (1) the loading capacity increases with polymer concentration; and (2) the loading capacity is independent of polymer concentration [Xing, 1997; Gadelle, 1995]. We observed that the loading capacity of MAMA in PLAF127-23 nano-particles is slightly larger than that of PLAF127-29 nano-particles at the same polymer concentration. For amphiphilic block copolymers with similar hydrophilic block length, it was reported that the loading capacity of hydrophobic drug increases with the hydrophobic block length due to the increase of the size of hydrophobic core of micelles [Xing, 1997]. However, our finding revealed trends that are in contradiction to the published results, and we believe that this is related to the difference in the morphologies of the two polymeric systems. As discussed in Chapter 5, the morphologies of PLAF127-29 nano-particles are that of normal bi-layer vesicles, while the morphologies of PLAF127-23 nano-particles are coated micelles containing three layers (see Figure 5.4 A and B), where the most inner layer of PLAF127-23 nano-particles comprised of hydrophobic PLA core. The size of PLAF127-23 and PLAF127-29 nano-particles is fairly identical, therefore, PLAF127-

Fig. 7.1 The released behaviors of MAMA loaded PLA-F127-PLA aggregates in PBS (pH 7.4, 0.01M) solutions at 37 °C.

23 nano-particles possess a larger hydrophobic domain, where larger amounts of hydrophobic drug model MAMA can be incorporated.

The released behavior of MAMA loaded PLAF127-23 and PLAF127-29 nano-particles in PBS solution at 37 °C is shown in Figure 7.1, where the common burst release of drug was observed. The release behavior of free MAMA from dialysis membrane was also examined as shown in Figure 7.1 to check whether there is interaction between MAMA and dialysis membrane. It is shown that all the free MAMA was released from the dialysis membrane 2 hours later while only 57% of MAMA was released from PLAF127-29 (0.08 wt%) aggregates within 2 hours. The results indicate that the dialysis membrane does not play an important role in the “Fickian release” of MAMA from PLA-F127-PLA aggregates.

Generally, the release kinetics of drug from polymeric micelles are affected by several factors, such as particle size, block composition, molecular weight,

concentration, degradation rate, etc [Kim, 2000]. The hydrophobic PLA blocks in PLA-F127-PLA are biodegradable and the hydrolytic degradation of the polymers has been studied as reported in section 6.2.1 of chapter 6. Therefore, the release kinetics of drugs from PLA-F127-PLA nano-particles could be affected by both diffusion and degradation of polymers. Normally, the drug release from polymeric systems can be described by a simple, semi-empirical equation, which has a power law relationship:

$$\frac{M_t}{M_\infty} = kt^n \quad (7.1)$$

where M_t and M_∞ are the absolute cumulative amounts of drug released at time t and infinite time, respectively; k is the release constant; exponent n describes the kinetic and thus release mechanism, which depends on the geometry of the system. For diffusion-degradation controlled drug release system, n for spherical particles is usually between 0.43 and 0.85 [Siepmann, 2001; Zuleger, 2001]. When n is close to 0.43, diffusion is the major driving force, and this is normally called the “Fickian diffusion”. When n is close to 0.85, the drug release is mainly controlled by degradation [Siepmann, 2001; Zuleger, 2001]. It is noted that the power law (equation 7.1) is not valid for all the drug release stages, in particular the later release stage. In order to obtain a linear fitting for the drug release data, equation 7.1 was described in another form as shown below:

$$\log(\text{released}\%) = \log\left(\frac{M_t}{M_\infty}\right) = n \log t + \log k \quad (7.2)$$

From the above equation, n can be obtained from the slope of the plot $\log(\text{released}\%)$ versus $\log t$ by linear fitting.

The relationship of between $\log(\text{released}\%)$ and $\log t$ for MAMA release from PLAF127-23 nano-particles (0.02 wt%) is shown in Figure 7.2. It is obvious that the

Fig. 7.2 The calculated experimental data based on the power law model for MAMA release from PLAF127-23 nano-particles (0.02 wt%): (A): fitting to 100% MAMA release: (B): fitting to 80% MAMA release.

relation between $\log(\text{released}\%)$ and $\log t$ is not linear in all the release stages (Figure 7.2 A). However, the experimental data satisfy the linear fitting up to 80 percent MAMA release (Figure 7.2 B). Also, n value obtained from the fitting result is 0.45, which is very close to 0.43. Thus, for the majority of MAMA release (about 80%) from PLA-F127-PLA nano-particles, the release mechanism is mainly dominated by the diffusion control mechanism. This is reasonable because the degradation of PLA blocks should not significantly affect the release behavior of drugs from PLA-F127-PLA nano-particles due to the relatively slow degradation rate. The degradation rate of PLA block is shown in Figure 6.5 in chapter 6 (Page 137). Although the $\bar{M}_n$ of PLA blocks in PLA-F127-PLA block copolymers decreased by 50% on the 7th day of degradation, 75% of MAMA has already been released from PLAF127-23 nano-particles after 7 hours. Thus, compared to the release rate of

MAMA, the hydrolytic degradation of PLA-F127-PLA block copolymers is much slower.

Compared to PLAF127-29 nano-particles, MAMA loaded in PLAF127-23 nano-particles is released at a lower rate. For example, 75% of MAMA was released from PLAF127-23 nano-particles after 7 hours, while 95% of MAMA loaded in PLAF127-29 nano-particles had already been released after 7 hrs. The reason for this is probably related to the morphologies of these two kinds of nano-particles. MAMA loaded in PLAF127-29 nano-particles is partitioned to the hydrophobic domain of bi-layer

Fig. 7.3 The schematic pictures of diffusion process of loaded MAMA from PLAF127-23 (A) and PLAF127-29 (B) aggregates in PBS.

vesicles (Figure 7.3 B), while the MAMA loaded in PLAF127-23 nano-particles is partitioned to the outer hydrophobic layer of coated micelles and to the inner hydrophobic core of the nano-particles (Figure 7.3 A). Thus, MAMA that is partitioned to the hydrophobic core of PLAF127-23 nano-particles will be released at a slower rate.

The concentration of polymer solution also has an impact on the release rate of MAMA drug model. For the PLAF127-23 nano-particles, we observed that the release rate of MAMA is reduced at higher polymer concentration. Kim et al reported similar findings on the effect of polymer concentration on the release rate of hydrophobic drug (indomethacin) from PCL-*b*-PEO nano-particles [Kim et al., 1998]. This could be related to the concentration gradient of MAMA within and outside PLAF127-23 nano-particles. For PLAF127-23 nano-particles of two different polymer concentrations (0.08 and 0.02 wt%), only minor difference in the loading capacity was observed, which suggests that the concentration of MAMA in the hydrophobic domain of nano-particles is fairly similar. However, the concentration of MAMA outside the nano-particles for the higher concentration PLAF127-23 solution (0.08 wt%) is higher than that for the lower concentration PLAF127-23 solution (0.02 wt%). Thus, the concentration gradient of MAMA for the former is lower, leading to a slow release rate.

7.2.1.2 Drug release kinetics of hydrophilic drug model PrHy

The release behaviors of model hydrophilic drug (PrHy) loaded in PLAF127-23 and PLAF127-29 aggregates were examined in PBS solution at 37 °C.

The loading capacity of PrHy in PLAF127-23 (0.08wt%) and PLAF127-29 (0.08wt%) nano-particles is 160 and 210 mg/g polymer, respectively. Compared to

hydrophobic MAMA drug, the loading capacity of hydrophilic PrHy is much larger due to larger volume of hydrophilic domain in PLAF127-23 and PLAF127-29 nanoparticles within the vesicular structure. The high loading capacity of hydrophilic drug also indicates that considerable amounts of drug is dissolved in the inner hydrophilic domain of PLA-F127-PLA nano-particles, thus confirming that PLA-F127-PLA vesicles are more effective for delivering hydrophilic drug systems. Because the PLAF127-23 nano-particles with coated micelles have a lower fraction of hydrophilic domain, the amounts of loaded PrHy in PLAF127-23 nano-particles is thus smaller than in PLAF127-29 nano-particles.

Figure 7.4 shows the release behaviors of PrHy drug loaded in PLAF127-23 and in PLAF127-29 nano-particles in PBS solution at 37 °C, where an initial burst release was observed. According to equation 7.2, the calculated experimental data satisfy the linear fitting up to around 90 percent PrHy release and n is equal to 0.43 from the fitting result (Figure 7.5). Thus, for a majority of PrHy release from PLA-F127-PLA nano-particles, the release mechanism is also dominated by diffusion control mechanism.

7.2.2 Drug release behavior of PCL-F87-PCL and PLA-F87-PLA Block

Copolymers in PBS aqueous solutions

7.2.2.1 Drug release behavior of PCL-F87-PCL and PLA-F87-PLA nano-particles

The aggregation behavior of PCL-F87-PCL and PLA-F87-PLA block copolymers with short hydrophobic chains in water was discussed in chapter 5. One to three types of aggregates with size range from 7.3 to 454 nm were detected by DLS, suggesting

Fig. 7.4 The release behaviors of PrHy loaded PLA-F127-PLA aggregates in PBS (pH 7.4, 0.01M) solutions at 37 °C.

Fig. 7.5 The linear fitting of calculated experimental data based on the power law model for PrHy release from PLAF127-23 nano-particles: (0.08 wt%).

Fig. 7.6 The release behavior of MAMA loaded in PLA6F87 aggregates (0.08 wt%) in PBS aqueous solutions at 37 °C.

Fig. 7.7 The linear fitting of calculated experimental data based on the power law model for MAMA release from PLA6F87 nano-particles (0.08 wt%).

Fig. 7.8 The release behavior of PrHy loaded in PCL4F87 aggregates (0.08 wt%) in PBS aqueous solutions at 37 °C.

Fig. 7.9 The linear fitting of calculated experimental data based on the power law model for PrHy release from PCL4F87 nano-particles (0.08 wt%).

that the microstructure is rather complicated. The morphologies of these aggregates should be studied further. The drug release behavior of PCL-F87-PCL and PLA-F87-PLA aggregates loaded with hydrophilic and hydrophobic drug models in PBS (pH 7.4, 0.01M) aqueous solution was monitored by UV spectrophotometer.

The release behavior of MAMA loaded in PLA6F87 aggregates (0.08wt%) is shown in Figure 7.6. The loading capacity of MAMA in PLA6F87 nano-particles is 100 mg/g polymer, where a less obvious burst release was observed. However, over 80% of MAMA is released after one day. The release mechanism was also studied by a mathematical model. The calculated experimental data based on the power law (equation 7.2) satisfy the linear fitting up to around 60 percent MAMA release (Figure 7.7). From the fitting result, n is equal to 0.37, which is close to 0.43. Thus, for a majority of MAMA release from PLA6F87 nano-particles, diffusion control kinetic should play an important role. We observed that the degradation rate of PLA-F87-PLA aggregates with short PLA segments is almost identical to that of PLA-F127-PLA with long PLA segments. Therefore, it is reasonable that the release of MAMA from PLA6F87 aggregates is controlled by the concentration driving force.

The release behavior of PrHy loaded in PCL4F87 aggregates (0.08 wt%) is shown in Figure 7.8. The load capacity of PrHy in PCL4F87 nano-particles is 15mg/g polymer, where burst release was evident. The fitting of the experimental data to the power law diffusion model is shown in Figure 7.9. Good linear fitting was obtained for about 85% PrHy release and n is equal to 0.43, indicating that the release mechanism is mainly diffusion controlled.

7.2.2.2 In-vitro drug release behavior of PCL-F87-PCL and PLA-F87-PLA hydrogels

The drug release behavior of PCL-F87-PCL and PLA-F87-PLA hydrogels loaded with hydrophobic and hydrophilic drug models in PBS (pH 7.4, 0.01M) aqueous solution was monitored by UV spectrophotometer. In order to study the effect of temperature on the drug release behavior of PCL-F87-PCL and PLA-F87-PLA hydrogels, the release study in the early stages was examined at two temperatures, 25 and 37 °C.

The release behavior of MAMA (0.004 wt%) loaded in PCL4F87 (35 wt%) is shown in Figure 7.10. The initial MAMA release was examined at two temperatures (25 and 37 °C) shown in Figure 7.10 (A). The purpose in preparing the PCL4F87 sample at 35 wt% is to make sure that the PCL4F87 is a gel at 37 °C, and a sol at 25 °C. Thus, the effect of sol-gel transition on the drug release behavior can be studied. It is interesting that no burst release was observed for MAMA in PCL4F87 sample. The release rate is almost constant at the initial release stage, which is similar to Pluronic and other types of gels [Moore, 2000; Anderson, 2001]. Only 25% of MAMA was released after 6 hours, while over 70% of MAMA was released from PLA-F127-PLA nano-particles after 6 hours. This finding indicates that PCL4F87 hydrogel could be an attractive system for controlled release applications. After 6 hours, the MAMA release process was examined only at 37 °C and the release result are shown in Figure 7.10 (B).

The release mechanism was examined according to the power law release model (equation 7.2). It was discussed earlier that the exponent “n” is related to the shape of release matrix. The shape of PCL4F87 hydrogel in this study is irregular, but is fairly similar to a cylinder. Thus, n values for a cylinder were used in the linear fitting, that is, n is 0.45 (diffusion control) or 0.89 (degradation control). The calculated experimental data satisfy the linear fitting up to over 90% as shown in Figure 7.11.

Fig. 7.10 The release behavior of MAMA (0.003 wt%) loaded in PCL4F87 (35wt%) samples in PBS aqueous solutions at 25 and 37°C: (A) is the initial part of release curve shown in (B), where the release rate of MAMA is constant.

Fig. 7.11 The linear fitting of calculated experimental data based on the power law model for MAMA release from PCL4F87 hydrogel (35 wt%).

From the fitting result, n is equal to 0.63, which is between 0.45 and 0.89. This indicates that the MAMA release from PCL4F87 hydrogel is controlled by both degradation and diffusion.

It is shown in Figure 7.10 (A) that there is no much difference between the release rate of MAMA from PCL4F87 sol at 25 and PCL4F87 gel at 37 °C. This indicates that the diffusivity of hydrophobic drug from gels is not a strong function of the viscosity since the viscosity of PCL4F87 gel is much larger than that of PCL4F87 sol, which is in agreement with the results for F127 gel obtained by Moore et al [Moore, 2000]. The reason could be that the diffusion rate of drug is mainly influenced by the microscopic viscosity of hydrogel instead of the macroscopic viscosity.

Similarly, the release behavior of PrHy (0.08 wt%) loaded in PLA6F87 (45 wt%) is also shown in Figure 7.12. The loaded hydrophilic drug model PrHy was found to increase the CGT of PLA6F87 hydrogel in this research. Ricci et al. also reported that the addition of hydrophilic drug may have an impact on the gel properties at the sol-gel transition, since the drug located outside the polymer micelles of gel can interfere with the gelation process [Racci, 2002]. PrHy is also released from PLA6F87 sample at a constant rate at 25 and 37 °C, respectively. We noted that the temperature has an effect on the release rate of PrHy, where PrHy is released faster from PLA6F87 gel at 37 °C than from PLA6F87 sol at 25 °C. The experimental data up to around 50% PrHy release at 37 °C was analyzed by the power law shown in Figure 7.13. The linear fitting was satisfied and n is 0.84, confirming that the initial release mechanism is mainly degradation controlled.

Compared to the release of hydrophobic MAMA from PCL4F87 hydrogel, the release rate of hydrophilic PrHy from PLA6F87 hydrogel is much higher. The reason

Fig. 7.12 The release behavior of PrHy (0.09wt%) for loaded PLA6F87 (45wt%) samples in PBS aqueous solutions at 25 and 37°C.

Fig. 7.13 The linear fitting of calculated experimental data based on the power law model for PrHy release from PLA6F87 hydrogel (45wt%).

is likely to be attributed to the hydrophobic MAMA, which is dissolved in the micellar core of PCL4F87 hydrogel, while hydrophilic PrHy drug is only partitioned at the corona of the micelles of PLA6F87 hydrogel. Therefore, compared to hydrophilic PrHy, the diffusion resistance for hydrophobic MAMA is greater, resulting in a corresponding reduction in the diffusion. Jeong also found that hydrophilic drug, Ketoprofen was released much faster from PEG-PLGA-PEG gels than hydrophobic drug, spironolactone [Jeong, 1999 b].

Due to the time constraints, the drug release behavior of drug models from modified F87 hydrogels with PLA and PCL segments was only examined briefly, and there is scope for more work in the future. One of the important studies will be to explore the effect of hydrolytic degradation of PLA and PCL on the release kinetics of drugs. It was previously reported that the release rate of drug from PLA-PEG-PLA hydrogels increased with the degradation of PLA block [Mason, 2001]. In addition, the effect of the block composition, concentration and other factors of block copolymers on the release kinetics of drugs should be also studied in future.

Chapter 8   Conclusions,   Contributions   and Recommendations for Future Work

8.1   Modified Pluronic F127 with Long Hydrophobic PLA Chains

PLA-F127-PLA block copolymers with long hydrophobic chains have been successfully synthesized. The structure of the copolymers was confirmed by NMR and GPC techniques. PLA-F127-PLA block copolymers with different PLA contents were obtained by changing the monomer L-Lactide contents in the feed.

The important findings for this type of copolymers are their interesting aggregate morphologies in aqueous solutions, consisting of bilayer and in some cases, interesting onion-like vesicles. As drug delivery carriers, vesicles are better candidate than normal core-shell micelles because they can accommodate not only hydrophobic but also hydrophilic drugs due to the existence of both hydrophobic shell and hydrophilic core.

The aggregation behavior of PLA-F127-PLA block copolymers in water was studied in details by LLS. The aggregates produced from PLA-F127-PLA (R_h 50-56 nm) are much larger than those from Pluronic F127 (R_h 2-15 nm). This is because the hydrophobicity of modified Pluronic F127 block copolymers is strengthened. Very importantly, it is found that the morphologies of PLAF127-29 and PLAF127-48 aggregates are bilayer vesicles with hydrophilic core surrounded by hydrophobic layers and that of PLAF127-23 aggregates is coated micelles with three layers, as confirmed by TEM results. The vesicular structure of PLA-F127-PLA nano-particles

is favorable for their application in drug delivery systems because they can deliver both hydrophobic and hydrophilic drugs.

The CMC of modified Pluronic block copolymers with long hydrophobic chains is much lower than that of Pluronics, indicating that the PLA-F127-PLA aggregates are much more stable than F127 aggregates.

The hydrolytic degradation behavior of PLAF127-23 block copolymer in PBS (pH 7.4) solution at 37 °C was studied by DLS, TEM, NMR and GPC techniques. The degradation mechanism of PLAF127-23 block copolymer is proposed. The initial rapid degradation of LA-LA linkage results in the dramatic change in the morphologies and particle size of PLAF127-23 nano-particles. The morphologies transform from regular onion-like vesicles to large compound vesicles and accordingly the particle size (R_h) increases from 50 to 191 nm. With further degradation, the cleavage of EO-LA linkage dominates. The regular larger compound vesicles formed from PLAF127-23 are destroyed and become irregular compound vesicles. With the continuous degradation at the interface of PLA and F127 blocks, the hydrophobic interaction of PLA blocks is weakened. Therefore irregular vesicles aggregate together to form regular large compound vesicles and the particle size increases correspondingly. The initial change in the morphologies with the degradation for PLAF127-29 and PLAF127-48 block copolymers is in agreement with that observed for PLAF127-23 block copolymer.

The release behaviors of both hydrophobic and hydrophilic drug models from PLA-F127-PLA nano-particles in PBS solution at 37 °C were examined by UV, respectively. According to the mathematical fitting of the power law, it is believed that the release of drugs from PLA-F127-PLA nano-particles is mainly controlled by concentration gradient due to the slow hydrolytic degradation of PLA segments. It

was found that the release rate of drug from PLA-F127-PLA nano-particles is affected by the block composition and concentration of polymers and also by the physico-chemical properties of the drugs.

8.2 Modified Pluronics with Short Hydrophobic Chains

PCL-F87-PCL and PLA-F87-PLA block copolymers with short hydrophobic chains have also been successfully synthesized. Similarly, the structure of the copolymers was confirmed by NMR technique. PCL-F87-PCL and PLA-F87-PLA block copolymers with different PCL and PLA contents were obtained by changing the monomer ϵ -CL and L-Lactide contents in the feed. The important contribution for these two kinds of copolymers is their thermo-reversible sol-gel transition, thus leading to potential applications in controlled release systems.

The aggregation behavior of PCL-F87-PCL and PLA-F87-PLA block copolymers in water was studied by LLS technique. The aggregation behavior of modified Pluronic block copolymers with short hydrophobic blocks is complex. Several kinds of particles (micelles, large aggregates and larger aggregates) are known to form in such systems. Furthermore, the size of these particles is very broad (ranging from 6.8 to 454 nm for PCL-F87-PCL and from 10 to 409 nm for PLA-F87-PLA). The reason that modified Pluronic copolymers exhibit such complicated aggregation behavior is largely related to the complex structure of the copolymers. In these block copolymers, there are five segments, including two kinds of hydrophobic blocks. Although the hydrophobicity of PCL and PLA is stronger than PPO, the block length of PCL and PLA is much shorter than that of PPO. Thus the hydrophobicity of PPO competes with PCL and PLA blocks during the aggregation of modified Pluronic

block copolymers with short hydrophobic chains, resulting in the complicated aggregation behavior of the copolymers.

The CMC of modified Pluronic block copolymers with short hydrophobic blocks is much lower than that of Pluronics, indicating that the stability of the aggregates in these copolymers is enhanced. This property is very attractive for applications in drug delivery system.

More importantly, the modified Pluronic copolymers with short hydrophobic chains retain the temperature-sensitive property of Pluronics, as confirmed by surface tension and rheological measurements. However, the temperature-sensitive property of these copolymers is not apparent for C11F87 and PLA14F87 with relatively longer hydrophobic PCL or PLA blocks. Compared to Pluronics, the CMT of modified Pluronic copolymers decreases largely due to the increase in the hydrophobic character of the modified polymers. A reversible sol-gel transition was detected for hydrogels formed from PCL-F87-PCL and PLA-F87-PLA block copolymers.

The hydrolytic degradation of nano-particles formed from PCL-F87-PCL and PLA-F87-PLA block copolymers in PBS solutions at 37 °C were studied by DLS, respectively. The change in the hydrodynamic radius (R_h) of C6F87 and PLA9F87 nano-particles with degradation is similar to that of PLA-F127-PLA nano-particles.

The release behaviors of drug model from PCL-F87-PCL and PLA-F87-PLA hydrogels in PBS solution were examined by UV-Vis spectrometer. Interestingly, it was found that there is no burst release for hydrophobic MAMA from C4F87 hydrogel and the initial release rate is almost constant. The degradation control kinetic should play an important role on the release process according to the fitting of experimental data based on the power law model. Compared to PLA-F127-PLA block copolymers, the absence of burst release indicates that PCL-F87-PCL and PLA-F87-

PLA hydrogels may be better systems for controlled release application. The hydrophilic drug model PrHy is released faster from the hydrogel than MAMA, due to the different partition coefficients of these two drugs in hydrogels. Also, the hydrophilic PrHy loaded in PLA6F87 hydrogel was found to increase the CGT of the polymer.

8.3 Recommendation for Future Work

The aggregation behavior of PCL-F87-PCL and PLA-F87-PLA block copolymers with short hydrophobic chains in water was reported in chapter 5. There are one to three kinds of aggregates in the range 7.3 - 454 nm detected by DLS for these copolymers due to the complicated chemical structure. The morphologies of these aggregates are not very clearly yet and would require further study by TEM and other techniques.

The hydrolytic degradation of PCL-F87-PCL block copolymers with short PCL was only studied by DLS technique. It was found that the maximum particles for PCL-F87-PCL with the degradation process appeared earlier than that of PLA-F87-PLA and PLA-F127-PLA. Therefore, the hydrolytic degradation of PCL-F87-PCL block copolymers would require further verification by other techniques such as TEM, NMR and GPC.

Due to the time constraints, the application of synthesized block copolymers as drug delivery systems was only briefly studied. For PLA-F127-PLA block copolymers, the interesting vesicular nano-particles are believed to have great potential for the application as drug delivery carriers. Therefore, the release mechanism of drugs from these nano-particles should be studied in greater depth and details. For example, the effect of the physico-chemical properties of drugs,

localization of drugs in particles, the diffusion coefficient of drugs etc on the release behavior of drugs should be examined. Since vesicles were found to improve the treatment efficacy of anticancer drugs, clinical anticancer drug such as doxorubicin should be loaded in PLA-F127-PLA vesicles and the release kinetics can be examined.

Furthermore, the release rate of hydrophobic drug from modified F87 hydrogels with short PLA and PCL is rather slow, which is a good phenomenon for controlled release systems. Thus, more work on this system will be conducted in future. One of the important studies will be to explore the effect of the hydrolytic degradation of PLA and PCL on the release kinetics of model drugs. In addition, the effect of the block composition, concentration and other factors of copolymers on the release kinetics of drugs from hydrogels should be examined.

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