Synthesis and application of PLA/PEG block copolymers as anti-cancer drug carriers

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Synthesis and application of PLA/PEG block copolymers as anti-cancer drug carriers

PhD thesis submitted

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Synthesis and application of PLA/PEG block copolymers as anti-cancer drug carriers

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philosophy

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Abstract

In this research project, a novel long-circulating anti-cancer drug delivery system was developed and characterized. This system consists of micelle-like nanoparticles which are made of biodegradable amphiphilic block polymers (PLA/PEG). These amphiphilic block copolymers self-assemble in aqueous solution and form colloidal carriers system for intravenous injection.

This study consists of four main parts: (a) the synthesis and characterization of triblock, diblock and star-shaped PLA/PEG block copolymers; (b) the preparation and characterization of micelle-like nanoparticles (c) the *in vitro* degradation properties of block copolymer in film and nanoparticle forms and (d) the release profiles of 5-FU and paclitaxel from micelle-like nanoparticles.

The triblock, diblock and star-shaped PLA/PEG block copolymers were synthesized by ring opening polymerization using stannous octoate as catalyst. The structural details of the synthesized copolymers were investigated using Fourier Transform Infrared Spectrometer (FTIR) and Nuclear Magnetic Resonance (NMR), molecular weights by Static Light Scattering (SLS) and Gel Permeation Chromatography (GPC) and thermal properties by Differential Scanning Calorimetry (DSC). Copolymers with narrow polydispersity were successfully synthesized. In addition, the thermal properties of copolymers were found to be related to their chain length, composition ratio and molecular structure

The micelle-like nanoparticles were prepared by solvent diffusion method. The morphology and particle size of those nanoparticles were studied by FESEM (Field Emission Scanning Microscopy) / TEM (Transmission Electron Microscopy) /AFM (Atomic Force Microscopy) and DLS (Dynamic Light Scattering) respectively. In addition, the aggregation property, micelle stability in dilution and salt added condition were also characterized.

The surface properties of copolymer in forms of film and nanoparticle were also studied using XPS (X-ray photoelectron spectroscopy). Migration of the hydrophilic segment to the surface was observed in nanoparticle form for some copolymers with certain LA/EG ratio and even in film form for copolymers with long PEG chain. Modification with hydrophilic polymer could improve the surface properties, as characterized by contact angle and Zeta potential measurements.

The *in vitro* degradation profiles of copolymer both in film and particle form were studied. Bimodality in the MWD was noted in some copolymers. The star-shaped copolymer showed some unique degradation characteristics compared to the linear counterparts.

Finally, the *in vitro* release profiles of 5-FU and paclitaxel were compared. It was found that paclitaxel was well entrapped in the core and hence a controlled release profile was obtained. The effects of different drug loading, chain length and copolymer

molecular structure on release rate were also investigated. When using Figuchi model to simulate the data, it was found that not all the release profiles were diffusion controlled, which suggests that degradation of the copolymer might also influence the release rate.

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Contents

1. Introduction	1
1.1 Background	1
1.2 Problems	2
1.3 Objective	3
1.4 Outline of this work	4
2. Literature Review.....	5
2.1 A survey of polymers used in drug delivery	5
2.1.1. Classification of Polymers used as Drug Delivery Carriers	6
2.1.1.1 Biodegradable Polymers	7
2.2 The development of lactic/glycolic acid based polymers as biodegradable systems.....	8
2.3 Copolymers of PEG and PLA or PLGA	8
2.3.1. Synthesis and characterization of block copolymers from PEG and lactones	10
2.3.1.1 Synthesis of block copolymers from PEG and lactones by ring opening polymerization	10
2.3.1.2 Synthesis of block copolymers from PEG and lactones by anionic polymerization	12
2.3.1.3 Synthesis of star-shaped copolymers from PEG and lactones.....	13
2.3.2. Mechanism of copolymerization from PEG and lactones	15
2.3.2.1 Coordination-insertion mechanism	15
2.3.2.2 Anionic mechanism	17
2.3.3. Physical properties of block copolymers from PEG and lactones.....	17
2.3.3.1 Microphase separation and crystallinity	17
2.3.3.2 Hydrophilicity and swelling behaviour.....	19
2.3.4. Degradation characteristics of block copolymers from polyester A-blocks and PEG B-blocks	19
2.3.4.1 Degradation of block copolymers based on PEG and polyesters	20
2.3.4.2 Degradation of star-block copolymers based on PEG and polyesters ..	22
2.3.5. Micellization of block copolymers	23

2.3.5.1	Characterization of block copolymer micelles	23
2.3.6.	Microspheres from ABA triblock polymer as parenteral delivery systems for proteins	26
2.3.7.	Biodegradable long circulating nanoparticulate based on PLA/PEG block copolymers	28
2.3.7.1	Mechanism of MPS (mononuclear phagocyte system)	29
2.3.7.2	Long-circulating drug delivery systems.....	31
2.3.7.3	Parameters may affect the property of long-circulation delivery system	35
2. 4	Nanoparticle and targeted system for cancer therapy	38
2.4.1.	Targeted delivery of nanoparticles.....	39
2.4.1.1	Achieving targeting by avoiding reticuloendothelial system (RES).....	39
2.4.1.2	Targeted delivery through enhanced permeability and retention (EPR effect)	39
2.4.1.3	Tumour-specific targeting.....	40
2.4.1.4	Targeting tumour vasculature	40
2.4.2.	Anti-cancer drugs used for delivery.....	41
2.4.2.1	Paclitaxel.....	41
2.4.2.2	5-Fluorouracil	42
2.4.2.3	Doxorubicin	43
2.4.2.4	Cisplatin	44
2. 5	Research of drug release kinetics-mathematical modelling of controlled drug release	45
2.5.1.	Mathematical modelling of non-degradable system	45
2.5.2.	Mathematical modelling of biodegradable system	46
2.5.2.1	Empirical mathematical biodegradable models	47
2.5.2.2	Diffusion and chemical reaction-base models	48
3.	Materials and Experimental.....	50
3. 1	Materials	50
3. 2	Experimental procedure	50
3.2.1.	Synthesis of the copolymer	50

3.2.2.	Copolymer characterization	51
3.2.3.	Preparation of the micellar aggregates with triblock, diblock and star-shaped block copolymer	52
3.2.4.	Characterization of nanoparticles	52
3.2.4.1	Examination of particle morphology	52
3.2.4.2	Determination of particle size	53
3.2.4.3	Critical Micelle Concentration (CMC) study	54
3.2.4.4	Turbidity Measurements and stability of the micelle to added electrolyte	54
3.2.5.	Determination of surface composition.....	55
3.2.6.	Contact angle study	56
3.2.6.1	Glass substrates cleaning	56
3.2.6.2	Spin coating with PLA/PEG block copolymers on the glass substrates.....	56
3.2.6.3	Dynamic contact angle.....	56
3.2.7.	The surface charge test.....	57
3.2.8.	In <i>vitro</i> degradation of block copolymer in forms of film and nanoparticle	60
3.2.9.	In <i>vitro</i> drug release from nanoparticles (5-FU)	60
3.2.10.	In <i>vitro</i> drug release from nanoparticles (Paclitaxel).....	61
Chapter 4		63
4. Results and discussion.....		63
4. 1	Synthesis of tri, di and star-shaped block copolymers.....	64
4. 2	Characterization of copolymer.....	68
4.2.1.	Copolymer molecular structure characterization	68
4.2.2.	Determination of molecular weight of the copolymers	76
4.2.3.	Thermal Properties of copolymers.....	80
4. 3	Micellization of block copolymer: preparation and characterization	92
4.3.1.	Particle size and conformation of the micellar aggregates	92
4.3.1.1	Particle size study with DLS.....	92
4.3.1.2	Effect of Concentration and Solvent.....	96
4.3.2.	Structure and properties of micellar aggregates.....	99
4.3.2.1	Aggregation number	99

4.3.2.2	Conformation of micellar aggregates.....	101
4.3.3.	Stability of micelle in dilute condition and salt-added condition	104
4.3.3.1	Stability of micelle in dilute condition.....	104
4.3.3.2	Stability of micelle in salt-added condition	107
4.3.4.	Micelle stability in PBS solution	112
4. 4	Surface property of polymer in forms of film and nanoparticles.....	115
4.4.1.	Surface composition of colloidal system and film investigated by XPS	115
4.4.1.1	Surface study of films and nanoparticles made from ABA triblock copolymers PLA-PEG-PLA.....	116
4.4.1.2	Surface study of films and nanoparticles made from AB diblock copolymers PLA-mPEG	121
4.4.1.3	Surface study of films and nanoparticles made from (AB) ₄ star-shaped copolymers (PLA-sPEO) ₄	124
4.4.2.	Copolymer surface characterization with dynamic contact angle	127
4.4.3.	Surface charge of the particle	133
4. 5	Hydrolytic degradation of copolymer in form of film and nanoparticle	136
4.5.1.	Degradation of triblock copolymers	136
4.5.2.	Diblock copolymer.....	146
4.5.3.	Star-shaped block copolymer.....	151
4.5.4.	Comparison of (a) linear and star-shaped copolymer (b) film and nanoparticle form	157
4. 6	In vitro anti-cancer drug release from micellar like nanoparticles	159
4.6.1.	Drug Release Data: 5-FU.....	160
4.6.1.1	Effect of PLA chain length	161
4.6.1.2	Effect of drug loading on profiles of different copolymers	162
4.6.1.3	Effect of different chain type copolymers on drug release profile	163
4.6.2.	Drug Release Data: Paclitaxel	165
4.6.2.1	Drug release profiles of paclitaxel from triblock copolymers	172
4.6.2.2	Drug release profiles of paclitaxel from diblock copolymers.....	174
4.6.2.3	Drug release profiles of paclitaxel from star-shaped block copolymers	176

4.6.2.4	Comparison of all these three types of copolymers	178
4.6.3.	Modelling the drug release.....	179
5.	Conclusions	186
5. 1	Copolymer characterization	186
5.1.1.	Molecular structure	186
5.1.2.	Molecular weight	186
5.1.3.	Thermal property	186
5. 2	Formation and characterization of micellar like nanoparticles.....	187
5.2.1.	Particle size study	187
5.2.2.	Aggregation property	187
5.2.3.	Stability of micelle in dilute condition and salt-added condition	187
5. 3	Surface property of polymer in forms of film and nanoparticles.....	188
5.3.1.	Surface composition of colloidal system and film investigated by XPS	188
5.3.2.	Surface charge of nanoparticle.....	188
5. 4	Hydrolytic degradation of copolymer in form of film and nanoparticle	188
5. 5	In <i>vitro</i> anti-cancer drug release from micelle-like nanoparticles	189
5.5.1.	5-FU	189
5.5.2.	Paclitaxel.....	190
5.5.3.	Drug release modelling	191
5.5.4.	Optimization	191
6.	Suggestions for future work	192
6. 1	The synthesis of 3-arm or 8-arm star-shaped copolymer.....	192
6. 2	Drug release studies	192
6. 3	The biodistribution of nanoparticles	193

Figures

Figure 2-1 Structure of PLA and PLGA	6
Figure 2-2 Core-shell structure of the amphiphilic block copolymer	10
Figure 2-3 The synthesis of 4-arm star-shaped copolymer of PLA/PEG	14
Figure 2-4 Coordination-insertion mechanism of copolymerization of PLA/PEG	16
Figure 2-5 Interactions between a protein and a hydrophobic substrate with attached PEG chains 1: hydrophobic attraction between the protein and the substrate; 2: steric repulsion resulting from PEG chain constriction; 3: van der Waals attraction between the protein and the substrate; 4: van der Waals attraction between the protein and the PEG chains .	33
Figure 2-6 Scheme of EPR effect	40
Figure 3-1 Typical graph of correlation function (a) and Contin distribution (b) of micellar like nanoparticles of PLA/PEG block copolymers	53
Figure 3-2 Small particles with relatively short double layer when $ka \ll 1$	58
Figure 3-3 (a) Typical HPLC graph of paclitaxel in PBS and (b) Calibration curve of Paclitaxel.....	62
Figure 4-1 Synthesis route of (a) triblock, (b) diblock and (c) star-shaped copolymer	66
Figure 4-2 FTIR spectra of PLA-PEG-PLA triblock copolymers	69
Figure 4-3 FTIR spectra for diblock copolymers	70
Figure 4-4 FTIR spectra of star-shaped copolymers.....	70
Figure 4-5 Changes in FTIR spectra of the polymer blend extracted from ethanol	71
Figure 4-6 ^1H -NMR spectrum (CDCl_3) of the copolymer	73
Figure 4-7 ^{13}C -NMR spectrum (CDCl_3) of the triblock copolymer	73
Figure 4-8 ^1H -NMR spectrum of diblock copolymer	74
Figure 4-9 ^1H -NMR spectrum of star-shaped copolymer.....	75
Figure 4-10 A typical zimm plot to get the weight average molecular weight of block copolymer	79
Figure 4-11 Typical DSC thermograms for PLA/PEG block copolymers	82
Figure 4-12 Polarized-light microscopy photo of the crystalline of PLA/PEG block copolymer	84
Figure 4-13 DSC thermograms of PLA/PEG triblock copolymer with homopolymer PLLA (I.V=8.04, PEG 2000, 4000, 6000, 8000) and (b) cooling at $3^\circ\text{C}/\text{min}$	86

Figure 4-14 DSC thermograms of PLA-PEG-PLA triblock copolymers a:(second run after fast cooling at 20°C/min) b :(second run after fast cooling at 3°C/min)	86
Figure 4-15 DSC thermograms of PLA/PEG diblock copolymer with homopolymer mPEG 2k and mPEG 5k: a first heating run, b: cooling run (3°C/min).....	89
Figure 4-16 DSC thermograms of PLA-mPEG diblock copolymers and mPEG 2000, 5000, a:(second run after fast cooling at 20°C/min) b : (second run after fast cooling at 3°C/min).....	89
Figure 4-17 DSC thermograms of PLA-sPEO star-shaped copolymers and sPEO 10,000 : a I(heating at 3°C/min) and b(cooling at 3°C/min)	90
Figure 4-18 DSC thermograms of PLA-sPEO star-shaped copolymers and sPEO 10,000 a:(second run after fast cooling at 20°C/min) b : (second run after fast cooling at 3°C/min).....	90
Figure 4-19 Melting temperature and crystallization temperature of PLA block vs PLA block units with different PEG content and chain structure	91
Figure 4-20 Possible structure of micelle from (a) diblock and (b) triblock copolymer ..	97
Figure 4-21 Plot of hydrodynamic radius (R_{hyd}) versus $N_{PLA}^{0.58}$ for PLA_x -mPEG ₁₁₄ ($50 \leq x \leq 341$) micelle-like aggregates	99
Figure 4-22 AFM graph of diblock and triblock copolymer	103
Figure 4-23 AFM and FESEM graph of star-shaped copolymer micellar aggregates ...	103
Figure 4-24 Possible micellar structure for the star-shaped copolymer with vesicle structure.....	103
Figure 4-25 Determination of CMC of micelles of diblock copolymers.....	106
Figure 4-26 CFPT of (a) triblock, (b) diblock and (c) star-shaped copolymer.....	110
Figure 4-27 Transmittance as a function of temperature during a thermal process at...	111
Figure 4-28 Transmittance as a function of temperature during a thermal process at 1°C /min for star-shaped copolymers colloidal system with 0.1M electrolyte (Na ₂ SO ₄).....	111
Figure 4-29 Transmittance as a function of temperature during a thermal process at 1°C /min for triblock copolymers colloidal system with 0.1M electrolyte (Na ₂ SO ₄).....	112
Figure 4-30 Change of effective diameter of nanoparticles of (a) triblock , (b) diblock and (c)star-shaped copolymers in vitro (pH=7.0 PBS, Merck)	114

Figure 4-31 C 1s regions of XPS data of PLA ₉₀ -PEG ₉₁ -PLA ₉₀ (a) film and (b) nanoparticles	118
Figure 4-32 C 1s regions of XPS data of PLA ₆₁ -PEG ₉₁ -PLA ₆₁ (a) films and (b) nanoparticles	119
Figure 4-33 C 1s regions of XPS data of PLA ₄₃ -PEG ₁₃₆ -PLA ₄₃ (a) films and (b) nanoparticles	120
Figure 4-34 C 1s regions of XPS data of PLA ₂₀₂ -mPEG ₁₁₄	122
Figure 4-35 C 1s regions of XPS data of PLA ₃₄₁ -mPEG ₁₁₄	123
Figure 4-36 C 1s regions of XPS data of PLA _{203*4} -sPEO _{56*4}	125
Figure 4-37 C 1s regions of XPS data of PLA _{74*4} -sPEO _{56*4}	126
Figure 4-38 Definition of contact angle.....	127
Figure 4-39 Definition of advancing contact angle and receding contact angle and hysteresis between them	127
Figure 4-40 (a) Advancing and receding angles of triblock copolymers (b) The effect of copolymer composition ratio on the Hysteresis and reduced hysteresis.....	129
Figure 4-41(a) Advancing and receding angles of diblock copolymers (b) The effect of copolymer composition ratio on the Hysteresis and reduced hysteresis.....	130
Figure 4-42 (a) Advancing and receding angles of star-shaped copolymers (b) The effect of copolymer composition ratio on the Hysteresis and reduced hysteresis	130
Figure 4-43 Molecular weight changes as a function of time of PLA/PEG block copolymer triblock copolymers	137
Figure 4-44 GPC chromatograms of PLA-PEG-PLA triblock copolymers with degradation.....	138
Figure 4-45 Analysis of the triblock copolymer PLA ₉₁ -PEG ₉₀ -PLA ₉₁ components of the two peaks of the whole copolymer molecular weight got from the Gauss model (a) Separation of the two peaks using two peak Gauss model (b) molecular weight change of the two peaks got after the simulation	139
Figure 4-46 Change of film morphology of PLA-PEG-PLA triblock copolymer after 11 days degradation investigated by FESEM	141
Figure 4-47 ¹ H-NMR characterization of select sample PLA ₉₇ -PEG ₄₅ -PLA ₉₇ before and after 20 days in <i>vitro</i> degradation (data for other triblock copolymer not shown)	142

Figure 4-48 FTIR of select sample PLA ₉₇ -PEG ₄₅ -PLA ₉₇ before and after 20 days in <i>vitro</i> degradation (data for other triblock copolymer not shown)	143
Figure 4-49 Melting and crystalline temperature change with the degradation time of selected triblock copolymers.....	144
Figure 4-50A Comparison of degradation data for nanoparticles and film of PLA ₉₀ -PEG ₉₁ -PLA ₉₀ (a) GPC chromatograms of nanoparticles (b) Molecular weight loss	145
Figure 4-51 Molecular weight changes as a function of time of PLA/PEG diblock copolymer	146
Figure 4-52 GPC chromatograms of series of PLA-mPEG diblock copolymers with degradation.....	147
Figure 4-53 Change of film morphology of PLA-mPEG diblock copolymer after 11 days degradation investigated by FESEM	148
Figure 4-54 Melting and crystalline temperature change with the degradation time of selected diblock copolymers.....	150
Figure 4-55 Comparison of degradation data for nanoparticle and film of PLA ₁₅₂ -mPEG ₁₁₄ (a) GPC chromatograms of nanoparticle (b) Molecular weight loss.....	150
Figure 4-56 Molecular weight changes as a function of time of PLA-sPEO star-shaped block copolymer.....	151
Figure 4-57 GPC chromatograms of series of PLA-sPEO star-shaped block copolymers with degradation.....	152
Figure 4-58 Analysis of the components of the two peaks of the whole copolymer molecular weight got from the Gauss model PLA _{74*4} -sPEO _{56*4} (a) Separation of the two peaks using two peak Gauss model (b) molecular weight change of the two peaks obtained after the simulation.....	153
Figure 4-59 Composition of star-block copolymers during in <i>vitro</i> degradation, obtained from ¹ H-NMR	154
Figure 4-60 FTIR of select sample PLA _{74*4} -sPEO _{56*4} before and after 20 days in <i>vitro</i> degradation.....	155
Figure 4-61 Melting and crystalline temperature change with the degradation time of selected star-shaped copolymers.....	156

Figure 4-62 Comparison of degradation data for nanoparticle and film of star-shaped copolymer PLA _{74*4} -SPEO _{56*4} (a) GPC chromatograms of nanoparticles (b) Molecular weight loss	156
Figure 4-63 Comparison of degradation profile of diblock and star-shaped copolymer with approximate LA/EG ratio	158
Figure 4-64 Chemical structure of 5-FU.....	161
Figure 4-65 Release profile of 5-FU from nanoparticles of triblock copolymer PLA-PEG-PLA with different PLA blocks at same drug loading.....	161
Figure 4-66 Release profile of 5-FU from nanoparticles of block copolymer (a) triblock PELA ₆₈ -PEG ₉₁ -PLA ₆₈ (b) diblock PLA ₁₅₂ -mPEG ₁₁₄ and (c) star-shaped PLA _{35*4} -sPEO _{56*4} with different polymer/drug concentration	162
Figure 4-67 Release profile of 5-FU from nanoparticles of triblock PLA ₆₈ -PEG ₉₁ -PLA ₆₈ , diblock PLA ₁₅₂ -mPEG ₁₁₄ and star-shaped block copolymer PLA _{35*4} -sPEO _{56*4} with polymer : drug=10:2	164
Figure 4-68 molecular structure of paclitaxel.....	166
Figure 4-69 TEM graph of paclitaxel loaded micelle-like nanoparticles	168
Figure 4-70 XPS of surface investigation of nanoparticles loaded with Paclitaxel: N 1s regions of star-shaped copolymer PLA _{203*4} -sPEO _{56*4} and PLA _{74*4} -sPEO _{56*4}	170
Figure 4-71 : Release of Paclitaxel from (a) PLA ₉₀ -PEG ₉₁ -PLA ₉₀ and (b) PLA ₉₇ -PEG ₄₅ -PLA ₉₇ micellar nanoparticles.....	173
Figure 4-72 Release of Paclitaxel from (a) PLA ₁₅₂ -mPEG ₁₁₄ (b) PLA ₃₄₁ -mPEG ₁₁₄ micellar like nanoparticles	174
Figure 4-73 Comparison of diblock copolymer with different PLA chain length for higher drug loading	176
Figure 4-74 Release of Paclitaxel from nanoparticles (a) PLA _{203*4} -sPEO _{56*4} (b) PLA _{74*4} -sPEO _{56*4}	177
Figure 4-75 Paclitaxel release from nanoparticle PLA _{74x4} -sPEO _{56x4} at high drug loading	177
Figure 4-76 Paclitaxel release from different type of copolymer micellar like nanoparticles (a) high drug loading and (b) low drug loading.....	178
Figure 4-77 Higuchi model for the drug release study of the PLA ₁₅₂ -mPEG ₁₁₄	180

Figure 4-78 Higuchi model for the drug release study of the PLA ₉₀ -PEG ₉₁ -PLA ₉₀	181
Figure 4-79 Higuchi model for the drug release study of the PLA _{203*4} -sPEO _{56*4}	181

Tables

Table 2-1 Experimental techniques for micelle characterization	24
Table 2-2 Facts might affect the biodegradable drug release system	46
Table 4-1 PLA _x /PEG _y /PLA _x triblock copolymers obtained from polymerization of L-lactide	67
Table 4-2 Light scattering data of the molecular weight compared to the GPC and NMR	79
Table 4-3 Thermal property of triblock copolymers with different PLA/PEG chain length	85
Table 4-4 Thermal property of diblock and star-shaped copolymer	88
Table 4-5 Hydrodynamic diameter and polydispersity of PLA-PEG-PLA micelles	94
Table 4-6 Hydrodynamic diameter, and polydispersity of PLA-mPEG micelle like nanoparticles	95
Table 4-7 Hydrodynamic diameter and polydispersity of PLA-sPEO micelle like nanoparticles	96
Table 4-8 Compare of the micellar aggregation property of different type of copolymer	100
Table 4-9 Conformation of micelle related to the ratio of R _g / R _h	102
Table 4-10 CMC of micellar aggregates prepared with different copolymers	106
Table 4-11 Zeta potential of micellar like nanoparticles	133
Table 4-12 LA/EG ratios of the copolymers at different degradation time	142
Table 4-13 Hydrodynamic diameter, polydispersity of PLA-PEG-PLA nanoparticles w/o drug	160
Table 4-14 Effect of different PLA block chain length on 5-FU drug entrapment	160
Table 4-15 Particle size of drug loaded and blank micelle-like nanoparticle	168
Table 4-16 Drug entrapment efficiency of nanoparticles prepared with triblock, diblock and star-shaped copolymer with different polymer drug feed ratio	172
Table 4-17 Correlation of K vs Sqrt (c)	180
Table 4-18 Correlation of K vs Sqrt (c) for PLA ₉₀ -PEG ₉₁ -PLA ₉₀	182
Table 4-19 Correlation of K vs Sqrt (c) for PLA _{203*4} -sPEO _{56*4}	182

List of Abbreviations

AFM	atomic force microscopy
CFPT	critical flocculation point
CMC	critical micelle concentration
D	polydispersity ($= M_w / M_n$)
DOC	degree of crystallinity
DCA	dynamic contact angle
DDS	drug delivery system
DLS	dynamic light scattering
DMF	dimethyl formamide
DN/DC	differential refractive index increment
DP	degree of polymerization
EE	entrapment efficiency
EPR	enhanced permeation and retention effect
FESEM	field emission electron microscopy
FTIR	Fourier transform infrared spectroscopy
GA	glycolide
GPC	gel permeation chromatograph
ΔH_m	melt enthalpy
HPLC	high performance liquid chromatograph
LA	lactide
MDSC	modulate differential scanning calorimetry
M_n	number average molecular weight
MPS	mononuclear phagocyte system
MS (MP)	microspheres (microparticles)
M_w	weight average molecular weight
NP	nanoparticles
NMR	nuclear magnetic resonance
PBS	Phosphate buffer solution
PCS	photon correlation spectroscopy
PDS	parenteral depot system

PEG	poly (ethylene glycol)
PEO	poly (ethylene oxide)
PLA	poly (lactic acid) (poly (lactide))
PLGA	poly (lactic-co-glycolic acid) (poly (lactide-co-glycolide))
PLA-mPEG	Poly (lactic acid)-block –methoxypoly (ethylene glycol)
PLA-PEG-PLA	poly (lactic acid)-block-poly (ethylene glycol)-block- poly (lactic acid)
PLA-sPEO	poly (lactic acid) -star- poly (ethylene oxide)
ppm	parts per million
RES	reticuloendothelial system
ROP	ring opening polymerization
T _c	crystalline temperature
T _g	glass transition temperature
T _m	melting temperature
SEC	size exclusion chromatograph
SLS	static light scattering
SEM	scanning electron microscopy
TEM	transmission electron microscopy
THF	tetra hydro furan
XPS	X-ray photo electron spectroscopy
ZP	zeta potential
5-FU	5-Fluorouracil

1. Introduction

1.1 Background

Since the time of Erlich, the ultimate goal of drug therapy has been to deliver a drug exclusively to its pharmacological site of action in a controlled and sustained manner. One current approach to achieve site-specific delivery involves the use of a carrier, which should alter the normal physiological distribution of the drug and direct it to its pharmacological sites of action.

Various types of systems (liposomes, emulsions, micelles and nanoparticles) were developed over the last decade in order to achieve controlled intravenous drug delivery or targeting to specific tissues. However, most of these systems, via recognition by the phagocytic cells (mainly the cells of the mononuclear phagocyte system and the polymorphonuclear leukocytes), are detected as foreign bodies and quickly removed from blood circulation. Essentially, macrophages located in the reticuloendothelial system (RES) play a crucial role in phagocytizing injected particles. Short half-lives were observed whatever the chemical composition of the injected particles: albumin ^[1], poly (lactic acid) ^[2,3], poly (lactic acid-co-glycolic acid) ^[4]. Therefore, using these types of systems, it is only possible to target drugs to RES organs, mainly the liver.

Many attempts were made to achieve long blood circulation times by avoiding RES recognition, mainly by chemically attaching or adsorbing appropriate polymers or molecules at the particle surface, which would reduce or minimize the interaction with opsonins. These particles or the associated technology, have been referred to as “Stealth therapeutic systems”. With stealth technology, we can get the distribution of carrier systems to non-MPS sites and the system can survive in the the blood compartment long

enough to improve the immunospecific targeting strategies. Poly (ethylene glycol) (PEG) is widely used in this system. PEG is a hydrophilic polymer with a flexible nature, which can be selected as shell-forming segments. It also assembles into dense clusters of tethered chains to achieve unique properties. The biocompatibility is guaranteed by the dense PEG shell, which endows the micelle with stealth characteristics in the blood compartment, achieving long circulation. ^[5]

Micellar nanoparticles loaded with anticancer drug recently are the hot topics recently for the cancer therapy. Peppas et al. reviewed thoroughly about the nanoparticle and targeting approaches for cancer therapy recently, ^[6] including avoidance of the reticuloendothelial system, utilization of the enhanced permeability and retention effect, and tumor-specific targeting. Several anti-cancer drugs, such as Cisplatin, Doxorubicin, 5-FU and paclitaxel, have been loaded into the polymeric nanoparticles for cancer therapy.

1.2 Problems

PLA/PEG block copolymers are good candidate for the drug delivery carriers. AB and ABA block copolymers and star-shaped copolymers have shown great promise in the area of preparation of micelle-like nanoparticles as long-circulating anti-cancer drug carriers. However, there is still lack of thorough investigation in this field. For linear triblock and diblock copolymer, there are still no systematic investigations of the following effects on the release profiles of the anti-cancer drug:

1. The LA, EG unit length
2. The LA/EG ratio

3. The structural features of the polymer (chain structure, thermal properties, molecular weight...)
4. The features of the micelle-like nanoparticle made from block copolymer (surface, stability, micelle aggregation property and morphology and so on)
5. The *in vitro* degradation property of the block copolymer (in film and nanoparticle form) , especially for short-term *in vitro* degradation study
6. The drug loading property (entrapment efficiency, release profile.....)

For star-shaped copolymers, there are even fewer reported studies to date. The mechanism of the micelle formation of star-shaped copolymer, the drug release profile from the micelle of star-shaped copolymer, the comparison of the branched copolymer to linear copolymer on the drug release profile, have all not been investigated to date.

1.3 Objective

In line with the discussion above, it is deemed meaningful to prepare micelle-like nanoparticles of the ABA triblock copolymer, AB diblock copolymer and star-shaped copolymers. The following effects were investigated in order to find an optimal way to form the carrier of the anti-cancer drug

1. The property of the copolymer
2. The property of the micelle-like nanoparticle
3. The surface property of copolymer in film and nanoparticle form
4. The degradation property of copolymer in film and nanoparticle form
5. The drug release property
6. Find the best candidate for the potential drug delivery carrier

1.4 Outline of this work

To achieve the above objectives, the research work followed the sequence below:

- Synthesize triblock ,diblock and star-shaped copolymers of different composition
- Characterization of the triblock copolymer using ^1H -NMR and ^{13}C -NMR
- Determination of Molecular Weight(M_w) and Molecular Weight Distribution (MWD) using GPC and ^1H -NMR, SLS
- Thermal analysis of the copolymers
- Preparation of nanoparticles using the triblock ,diblcok and star-shaped copolymers
- Morphological study of the nanoparticles using FESEM, TEM and AFM
- Characteristics of the micelle-like nanoparticles (size, stability, form and so on.)
- Surface study of the copolymer in film and nanoparticle form
- In vitro degradation of the copolymer in film and nanoparticle form
- Drug release profile from polymeric micelle (5-FU and Paclitaxel)
- Drug release profile modeling

2. Literature Review

2.1 A survey of polymers used in drug delivery

One of the simplest methods to obtain sustained delivery of a biologically active agent is to physically mix or blend it with a polymer. Because the drug is now encapsulated or trapped within the polymer, it is not easily available to the biological system as when it is used alone. The polymer has to dissolve or disintegrate before the drug can be released or else the drug has to diffuse from within the polymeric matrix. In either case, the release of the drug to the physiological environment is extended over a much greater time than if the drug is used in its native form.

In these types of controlled delivery systems, the drug is not chemically attached to the polymer; it is simply encased within the polymer matrix. Because there are no chemical interactions between the drug and the polymer, the drug remains in a biologically active form, and can exert its effect upon the body as soon as it is released from the polymeric matrix. The polymer is only a vehicle for delivering the drug to the body. Obviously, the major advantage of this type of drug delivery system is that the drug in the polymeric matrix is unchanged; therefore its absorption, distribution, metabolism, and excretion after being released from the polymer is the same as that of the native drug. Moreover, its biological effects after being released from the polymeric matrix is also the same as used alone and can be predicted based upon its release rate.

Because these types of polymeric matrix systems are the simplest to design and the easiest to obtain approval by the Food and Drug Administration, they have been extensively studied in the past two decades. Numerous polymers have been evaluated for these types of drug delivery systems.

2.1.1. Classification of Polymers used as Drug Delivery Carriers

The polymers used as matrices for drug delivery can be classified as three basic types. These are water-soluble polymers, biodegradable polymers and nonbiodegradable polymers. Water-soluble polymers are typically used for short-term (several hours to several days) drug delivery because of their quick dissolution in biological fluids. These polymers do not need to undergo any chemical degradation to become soluble in water.

Biodegradable polymers require chemical modification/alteration to be water-soluble. Polylactides (PLA) and Poly (lactide-co-glycolide) (PLGA) belong to this class of polymers. (Figure 2-1) Because the degradation involves some type of chemical reaction such as hydrolysis, which usually occurs over an extended period, these polymers can be used for longer-term drug delivery. Extensive research has produced biodegradable polymers with degradation times ranging from several days to more than one year; thus these polymers afford a wide range of applications for drug delivery.

Figure 2-1 Structure of PLA and PLGA

The non-biodegradable polymers are essentially inert in the body. They can be used for applications in which extended drug delivery is required. Because the material is

inert, they will remain in the body indefinitely after the drug is depleted, unless they are removed.

The decision to use a polymer from one of these three general classes of polymeric matrices depends upon the route of administration, the type of drug to be used, the amount of drug to be delivered and the duration of release required. For example, if the drug is highly acidic or basic, the polymer has to be selected to be compatible with the drug so that it does not lead to reactions which might alter either the drug or the polymer. This is especially true with biodegradable polymers in which the drug may catalyze the degradation.

The duration of release has a direct effect on the polymer selection. As discussed previously, water-soluble polymers are used mainly for short-term drug delivery, biodegradable ones for intermediate-length of time, and non-biodegradable polymers for the more extended periods.

2.1.1.1 Biodegradable Polymers

Biodegradable polymers are water-insoluble polymers that, by means of a chemical reaction in the body, are converted slowly to water-soluble materials. Of these, the one we focus on is a polymer that contains hydrolyzable functional groups directly in the polymer chain. As the device is incorporated into the body, these groups in the chain are hydrolyzed. The polymer chain is slowly reduced to shorter and shorter chain segments which eventually lead to water-soluble fragments.

The polymer chain of the third category of biodegradable polymers hydrolyzed into shorter and shorter segments have been extensively studied for drug delivery

applications. One of the most widely used synthetic biodegradable polymers is the aliphatic polyesters prepared from lactic and glycolic acids.

2.2 The development of lactic/glycolic acid based polymers as biodegradable systems

The acceptance of lactic/glycolic acid polymers as sutures has made them attractive for a variety of medical and pharmaceutical applications, including wound closure ^[7], dental repairs ^[8], and drug delivery ^[9, 10].

Since the lactic/glycolic acid polymers are important biodegradable materials and have a wide range of applications, it is important to clarify the synthesis, properties, and biodegradation of these polymers. It is also useful to consider experimental approaches that can be used to characterize these polymers.

2.3 Copolymers of PEG and PLA or PLGA

Poly (ethylene glycol) (PEG) presents outstanding physico-chemical and biological properties, including hydrophilicity, solubility in water and in organic solvents. PEG is non toxic and does not show antigenicity and immunogenicity, which makes it an ideal candidate for many biomedical and biotechnological applications. The terminal hydroxyl groups of PEG can readily react with a variety of compounds. Block copolymers have been prepared from PEG and lactides. Copolymerization offers the possibility of varying hydrophilic/hydrophobic and soft/hard segment ratios. PLA/PEG copolymers are of great interest in view of applications in drug delivery systems or as macromonomers for preparation of new generation materials.

PLA/PEG block copolymers have a tendency to self-assemble at surfaces and aggregate into micelles in selective solvents. Block copolymers, made of hydrophilic and hydrophobic segments, show the interesting property of surface segregation of the hydrophilic segment, when at an aqueous interface. In similar fashion, the same block copolymers form micelles with core-shell structure, formed as a result of segregation of insoluble (hydrophobic) segments into the core, and the hydrophilic segments onto the shell. (Figure 2-2). This interfacial activity of amphiphilic block copolymers provides for their wide use in the biomedical field as colloidal dispersants, surface modifiers and drug carriers. PEG chains tethered on a surface or forming the corona of nanometer-scaled micelles exhibit the ability to sterically exclude other macromolecules and particles (steric stabilization) because of their high flexibility and the large exclusion volume of PEG strands in water. It is particularly useful for preventing the adsorption of proteins and adhesion of cells. In this regard, these supramolecular structures involving PEG-based block copolymers should be of substantial importance for the development of blood-contacting biomaterials which are expected to play a key role in such fields as cell and tissue engineering, bio-sensing and drug delivery systems.

Figure 2-2 Core-shell structure of the amphiphilic block copolymer

2.3.1. Synthesis and characterization of block copolymers from PEG and lactones

Homo- and copolymers of lactic acid and glycolic acid are usually synthesized by the ring-opening polymerization of the cyclic monomers, lactide and glycolide. In the last decade, the block copolymers of poly (ethylene oxide) and lactone have been investigated by many research groups. The terminal hydroxyl group of PEG can initiate successfully the polymerization of lactones in the presence of catalysts.

2.3.1.1 Synthesis of block copolymers from PEG and lactones by ring opening polymerization

Synthesis of ABA-triblock copolymers of PEG B-blocks and lactic acid or glycolic acid A-blocks using Sb_2O_3 and phosphoric acid as catalyst was first described by Cohn et al.^[11,12] in 1987. The copolymers were synthesized through the polycondensation of lactic acid or glycolic acid in the presence of PEG under nitrogen flow. They extensively investigated mechanical and thermal behaviour as well as degradation and biocompatibility^[13]. In 1990, Deng et al. used stannous chloride as catalyst to synthesize

successfully ABA triblock copolymers of PEG and PLA ^[14]. The polymerization was carried out in bulk at 170-200°C and yielded ABA block copolymers with a single peak in GPC analysis and narrow polydispersity. Stannous octoate and metal oxides were also used by Kricheldorf et al. as the catalyst to prepare block copolymers of PEG and lactide^[15]. However, with stannous octoate, racemization-free ABA triblock copolymers were obtained with high conversion. Similar results were obtained by others ^[16,17]. Molecular weights of the ABA block copolymers corresponded to the feed ratios of monomer and initiator by an equation based on a simple chain reaction model.

For diblock copolymer, in 1988, Curchill and Hutchinson first synthesized the diblock copolymer PDLLA-mPEG using stannous octoate as catalyst. The reaction proceeded for 3 hour at 160°C ^[18]. Similar reaction conditions have been described for the preparation of other diblock copolymers, with different catalyst concentrations and reaction temperatures being used.^[19,20] This method has also been used to produce diblock copolymers of PLGA and MePEG of much higher molecular weight using low reaction temperature. ^[21] Beletsi et al. studied the effects of different reaction conditions on the preparation of MePEG-PLA diblock copolymers. ^[22] The effect of reaction time, temperature and catalyst concentration was studied in order to find the best reaction condition.

Besides stannous octoate, other catalysts have been used to synthesize the copolymer. Aluminum triisopropoxide was used as the catalyst in the copolymerization of L-lactide or L-lactide/glycolide and PEG ^[23,24]. Alkyl aluminum compound was also used in the copolymerization of PEG and lactide ^[25,26].

Recently, rare earth metal alkoxides were also used to synthesize ABA block copolymers in solution. Yttrium tris(2,6-di-tert-butylphenolates) was used by Feijen et al. for the polymerization of PEG and lactide in dichloromethane at 25°C. This catalyst system is very effective for obtaining narrow Mw distribution product with high conversion^[27].

CaH₂ and Zn metal as well as lithium chloride were used as the heterogeneous catalyst system in the copolymerization of PEG and lactones, and are usually more suitable for synthesizing low M_w block copolymers^[28]. Catalyst-free polymerizations were an ideal goal for polymers used as biomaterials. Cerrai et al. described the synthesis of ABA triblock copolymers of L-lactide and PEG without catalyst^[29]. Their results demonstrated that their method is not competitive with catalyzed reactions, due to a very long reaction time needed to get reasonable conversion of L-lactide.

2.3.1.2 Synthesis of block copolymers from PEG and lactones by anionic polymerization

Anionic polymerization has also been used in the preparation of the block copolymers of PEG and lactone. Jedlinski et al. synthesized block copolymer of PEG and L-lactide through the anionic polymerization of L-lactide in the presence of sodium PEG alkoxide in THF at 25°C^[30], as have Kircheldorf and coworkers^[31].

With anionic polymerization, the great benefit might be the possibility of adding the functional group to the end of the polymer chain. It is very useful for the development of novel micellar carriers for cellular-specific targeting.^[32,33,34,35]

2.3.1.3 Synthesis of star-shaped copolymers from PEG and lactones

Although the PEG-poly(α -hydroxy acids) block copolymers have appropriate swelling properties for diffusion of proteins and polypeptides, PEG is nonbiodegradable and exhibits extraordinarily large hydrodynamic volume. This fact clearly limits the use of high molecular weight analogues of PEG which may not be eliminated in the kidney, even though it is required for the better control of polymer composition governing hydration and mechanical property. Star-shaped polymers exhibit a smaller hydrodynamic radius and lower solution viscosity when compared to linear polymers of the same molecular weight and composition. The smaller hydrodynamic radius of PEG is of importance in its complete renal excretion after degradation of the polyester block. This may allow us to use a relatively high molecular weight multiarm PEG with a hydrodynamic radius small enough for renal excretion, for the synthesis of block copolymers with polyesters such as PLA and PCL.

Star-shaped block copolymers of PEO and lactide were reported by several groups. These copolymers are prepared from multi-arm PEO, i. e. 4-arm or 8-arm PEO [26, 36, 37] (Figure 2-3). Using triethyl aluminum as catalyst, Li et al. have synthesized 4- and 8-arm block copolymers with L-lactide or L-lactide/glycolide. The polymerization was carried out in toluene at 70°C. GPC analysis showed a unimodal GPC trace and light scattering analysis showed a significant increase in M_w , corresponding to the ratio of monomer relative to multi-arm PEO in the feed. Choi et al. reported the synthesis of star-shaped PEO, PLLA and PCL block copolymer by a divergent synthesis method. Multi-arm PEOs with various number of arms with fix PEO segment ($M_w=10,000$) were synthesised by anionic polymerization of ethylene oxide initiated with metalated plurifunctional

initiators and used as a core block. [37] Star-shaped PEO-PLLA and PEO-PCL block copolymers were prepared by ring-opening polymerization of L-lactide and ϵ -caprolactone, which was initiated with multi-arm PEOs in the presence of stannous octoate as a catalyst. Three arms PEO and PAA star-shaped block copolymer was also synthesized by the core-first method. [38]

Other research has focused on another type of star-shaped copolymer, the multi-arm PLLA homopolymers and its copolymer with PEG. [39,40,41]. Interestingly, it was found that these copolymers are sensitive to the temperature change and can form gels at certain temperature. [41,42]

Figure 2-3 The synthesis of 4-arm star-shaped copolymer of PLA/PEG

2.3.2. Mechanism of copolymerization from PEG and lactones

Although the mechanism of the polymerization for lactone with many metal compounds remains somewhat speculative, the coordination-insert mechanism is most generally accepted, especially in the case of metal alkoxides.

2.3.2.1 Coordination-insertion mechanism

In the presence of PEG molecules, the polymerization involves a transfer of metal alkoxide to metal PEGate. The ring-opening polymerization is initiated by this macro-initiator of the metal alkoxide as coordinated-insertion mechanism similar to low M_w metal alkoxide catalysts. Stannous octoate, a salt of 2-ethylhexanoic acid, does not seem to fit into this scheme. Many investigations show that most of the terminal groups of the product of the ring-opening polymerization of lactones initiated by stannous octoate are hydroxyl groups, indicating that hydroxyl groups, e. g. residual water or other impurities, participate in the initiation as a co-catalyst. Zhang et al. studied the effect of hydroxyl and carboxylic acid substances on the polymerization in the presence of stannous octoate [43]. They found both hydroxyl and carboxylic acid functionalities affecting the polymerization of lactide, with the alcohol group increasing the PLLA polymerization rate while the carboxylic acid decreased it.

Du et al. reported their detailed investigation on the mechanism of the copolymerization of PEG and lactones in the presence of stannous octoate [44]. Kinetic measurement and mechanistic studies suggest that the reactivity of the initiator, a hydroxyl group-bearing reagent, is an important parameter on the polymerization mechanism. It was demonstrated that the linking of stannous octoate with the terminated hydroxyl group of PEG formed a metal alkoxide initiator (Fig. 2-9). In situ $^1\text{H-NMR}$ was

used to observe the mechanism of the copolymerization. PEG of M_w 1000 was used for the kinetic study. Their study showed that the hydroxyl terminal group of PEG was esterified and the numbers of the methylene protons were in agreement with those calculated from the M_w 's of the final products, indicating that the lactones were bound to the PEG chains. This evidence suggests that, stannous alkoxide and tin salts of carboxylic acids can initiate the ring opening polymerization, but the stannous alkoxide is more active than the salt. Thus, in the presence of alcohol, the initiation of ring opening polymerization of the lactones will be initiated by stannous alkoxide.

Figure 2-4 Coordination-insertion mechanism of copolymerization of PLA/PEG

In the case of aluminum alkoxide catalyst, i.e. aluminum triisopropoxide, the transfer of the aluminum ion to the terminal hydroxyl group of PEG leads to the evaporation of isopropanol at high temperatures, resulting in the formation of a macroinitiator ^[28].

2.3.2.2 Anionic mechanism

The mechanism of the anionic polymerization of the lactone seems to be clearer than the coordination-insertion mechanism ^[34]. Kircheldorf and coworkers reported in their work that ¹H-NMR shows the existence of more hydroxyl terminated groups than ester groups and 10 to 20% racemization was also observed, indicative of a chain transfer reaction with monomer via deprotonation ^[35].

2.3.3. Physical properties of block copolymers from PEG and lactones

The hydrophilic PEG chain introduced into the hydrophobic polylactones leads to a new family of biomaterials with properties differing from the corresponding homopolymers with respect to their physicochemical and biological behavior. For drug delivery systems, biocompatibility and biodegradability are important prerequisites that are closely linked to their physicochemical properties. There are many reports in the literature on the characterization of the block copolymer of PEG and lactones.

2.3.3.1 Microphase separation and crystallinity

Due to the incompatibility of polyether and polyester blocks, microphase separation in the block copolymers was to be expected depending on their composition. Cohn et al. used differential scanning calorimetry to investigate the thermal property of PLLA-PEG-PLLA triblock copolymers ^[12]. For matrices comprising PEG chains with M_w below 3400, no soft-segment (PEG) crystallinity was detected. When long hard segments (PLLA) were present, essentially monophasic, semicrystalline polymers were obtained

with PLLA blocks melting around 130°C. Polymers with longer soft segments exhibited a two-phase matrix, with both components being able to crystallize. But the thermal history strongly affected the morphology of the block copolymers, especially when both segments are long enough to crystallize.

Deng et al. also found that block copolymers of PEG with $M_w > 4000$ g/mol and D, L – lactide showed fusion endotherms around 35°C which were assigned to the crystallinity of the soft segments, but no glass transition could be detected ^[34].

The thermal property of diblock copolymers were also characterized by DSC and XRD. The thermal transitions and crystalline structures were presented.^[19, 45] Burt et al. assumed that the thermal properties of the diblock copolymers were more similar to the homopolymer MePEG other than to the homopolymer PDLA. ^[46]

Star-shaped block copolymers show significant differences in their physical property in comparison with that of linear block copolymers ^[26]. Due to the three-dimensional branched molecular architecture, the interaction between the molecules of the star block copolymers is reduced. Therefore, it is not surprising that these polymers show lower glass transition and melting temperatures in DSC analysis than their linear counterparts. In the case of the star-block copolymer of 8-arm PEG and L-lactide, nearly no crystallinity of the L-lactide segment was found by DSC analysis.

The particular morphologies of star block copolymers are influenced significantly by incompatibility effects. These star block copolymers offer interesting properties concerning microphase separation when compared to corresponding linear copolymers. Especially, the $(AB)_n$ star-block copolymers composed of asymmetric diblock arms which might lead to hierarchical structure transformation of cubic lattices. Moreover, it is

remarkable that similar phenomena are also observed in structural ordering on multi-armed and gradient-modulus star-shaped polymer solutions. ^[47]

2.3.3.2 Hydrophilicity and swelling behaviour

Hydrophilic PEG segments will change the physicochemical properties of hydrophobic and biodegradable PLA or PLG segments especially with respect to their aqueous swelling. The block copolymers containing hydrophilic microphases are expected to show an increased interaction with water. The introduction of PEG segments is responsible for the significant increase in water uptake depending on the PEG content in the block copolymers.

A biphasic water uptake profile was found by Pitt et al ^[48]. They thought that this biphasic water uptake reflected the contribution of two processes: rapid diffusion of water into the initially miscible PEG and PLA blocks; then a slower rate of hydration possibly due to phase separation and hydrolytic cleavage of the PLA blocks.

2.3.4. Degradation characteristics of block copolymers from polyester A-blocks and PEG B-blocks

Acceleration of degradation for relatively slow degrading polyesters was one of the driving forces to study block copolymers. Although drug delivery systems based on these polymers have attracted considerable interest in the past three decades, their suitability for the application of acid sensitive proteins or peptides is limited due to their degradation behaviour.

An improved degradation pattern has been achieved by inserting hydrophilic, non-degradable poly (oxyethylene) soft segments into a degradable PLA, PLGA or PCL

chain. Degradation of these polymers can proceed either by hydrolytic cleavage of the polyester A-blocks or by enzymatic degradation catalyzed by esters.

2.3.4.1 Degradation of block copolymers based on PEG and polyesters

Possible enzymatic effects on the degradation of PELA (PEG/PLA copolymer) 3400/126 were ruled out by Cohn et al., who observed no acceleration of degradation rates after addition of enzyme ^[49]. Similar to PLGA, random chain cleavages of the A-block segments need to be taken into account.

Compositional parameters, like the ratio of A- and B-blocks and average A- and B-block chain lengths affect physicochemical properties such as crystallinity, hydrophilicity and swelling, which are known to be of importance for the degradation of biomaterials.

Pitt and coworkers investigated relatively short PLA-PEG-PLA copolymers synthesized from D, L-lactic acid and found the rate of chain scission to be independent of the composition of the ABA block copolymers. There was no significant difference detected for the rate of chain scission when compared to PLA homopolymers ^[48]. Thus, they concluded that the shorter induction period prior to the onset of erosion was not due to a higher rate of chain scission but to greater solubility of the PEG-PLA oligomers and a greater rate of diffusional loss in the hydrated copolymer samples. This conclusion in turn shows that the swelling property of the ABA triblock copolymers is a critical factor for their degradation behavior.

Gref et al. have studied the biodegradation of diblock PLA-mPEG copolymers ^[50]. They found the erosion behaviour is similar to that of pure PLA. The crystallinity of the polymer matrices that stems from the Me-PEG blocks was found to change dramatically during erosion. The crystallinity changes are most likely induced by degradation that

causes an increasing mobility of the chains. The loss of swelling causes a drop in the glass transition temperature, increasing the mobility of the polymer chains even further.

Increasing the relative EO content resulted in an increased hydrophilicity facilitating the diffusion of water and thus enhancing erosion of the polymer according to Cerrai et al^[51].

The degradation behaviour of PLLGA-PEG-PLLGA and PLGA has been studied with respect to M_w loss and polymer erosion. Implants were prepared by either compression moulding or extrusion using a laboratory ram extruder. The analysis of the pH inside ABA rods using electron paramagnetic resonance spectroscopy, gave a pH of 5.2 after incubation, with a subsequent increase to pH 6.0 during the first day, approaching the pH of the medium after nearly 33 days. Compared to PLGA rods, acidic degradation products did not accumulate inside the ABA rods, thus making the incorporation of proteins or peptides sensitive to an acidic environment possible^[52].

The postulated degradation mechanism is somewhat controversial, especially for the preferred cleavage site for hydrolysis. Water soluble PEG-PLA degradation products were detected in the buffer medium using ¹H-NMR spectroscopy, suggesting that chain scission occurred statistically in the A blocks, and that EO-LA and LA-LA linkages seem to possess the same hydrolytic liability.

A biphasic degradation profile has been observed for PLLA-PEG-PLLA copolymer films. The pattern has been characterized by a rapid initial loss in number average M_n of the tested copolymers followed by a second phase featuring a slower number average M_n decay. In both stages, the loss of M_n was closely paralleled by polymer mass loss as well. When comparing the mass loss rates of PLLA-PEG-PLLA and PLGA-PEG-PLGA

copolymers, they found the later to erode faster. The mass loss was further accelerated by increasing the molar ratio of glycolic acid. Thus, Kissel and coworkers suggested a different phase model for the swollen PLGA-PEG-PLGA matrix. The PLGA phase was completely in an amorphous rubbery state as compared to the swollen PLLA-PEG-PLLA copolymers, where the PLLA phase is said to consist of a partially crystalline core surround by an amorphous, rubbery shell ^[53].

In principle, three explanations can be put forward to account for the bimodal molecular mass distribution in ABA polymer degradation. Firstly, the degradation rate of the polymer may be faster inside the matrix than at the surface, causing bimodal SEC profiles. Secondly, the degradation of initially crystalline polymers results in bimodal or even multimodal SEC profiles due to the selective degradation in amorphous zones and at the edges of crystalline zones. Thirdly, if the polymer is crystallizable but initially amorphous, the development of crystalline domains may result in a bimodal distribution in the degrading polymer.

As a general rule, an increased degradation rate of PLA/PEG block copolymer can be obtained by either increasing the DP_{PEG}/DP_{PLA} ratio or by introducing randomly copolymerized glycolic acid into PLA A-blocks, which results in a better phase compatibility, less crystallinity and, hence, a more rapid swelling of the resulting copolymer.

2.3.4.2 Degradation of star-block copolymers based on PEG and polyesters

The in vitro degradation properties of star-block PLGA-PEG copolymers differ from those of linear ABA triblock copolymers of PLGA and PEG due to the steric architecture and the shorter PLGA block. While mass loss and molecular weight decay are of

comparable orders in the initial phase, the loss of branched PEG seems to be reduced. These novel biodegradable polyether-polyesters may have potential for parenteral protein delivery systems ^[26].

2.3.5. Micellization of block copolymers

The increasing interest in block copolymers arises mainly from their unique solution and associative properties as a consequence of their molecular structure. In particular their self-associative characteristics leading to micellar systems are directly related to their segmental incompatibility.

The micellization of block copolymers in a selective solvent of one of the blocks is a typical aspect of their colloidal properties. In fact, when a block copolymer is dissolved in a liquid that is a thermodynamically good solvent for one block and a precipitant for the other, the copolymer chains may associate reversibly to form micellar aggregates which resemble in most of their aspects to those obtained with classical low molecular weight surfactants. Recently, many articles discussed the colloidal properties of block copolymers. ^[54,55]

2.3.5.1 Characterization of block copolymer micelles

Most physical methods for the characterization of block copolymer micellar system have been applied in the micellar system for several decades. Chu and Zhou have summarized them and listed them in Table 2-1 ^[56].

Critical micelle concentration

The threshold concentration at which micellization begins is known as the critical micelle concentration. The critical micelle concentration can be determined by various

methods, such as surface tension, fluorescence or dye solubilization technique, conductivity and so on.

Table 2-1 Experimental techniques for micelle characterization

Techniques	Micelle characteristics
TEM, FESEM	Shape , size
SANS, SAXS	Molecular weight (weight average), R_g , R_{core} , macrolattice structures
SLS	Molecular weight (weight average), R_g
DLS	R_h (hydrodynamic radius)
SEC	R_h , dynamic of micellar equilibrium
Ultracentrifugation	Micelle density, molecular weight (Z average)
Fluorescence techniques	Chain dynamics, CMC, hybridization of micelles
Viscometry	R_h , intrinsic viscosity, interparticle interaction
Zeta potential	Surface charge
Stop flow techniques	Kinetics of micelle formation and dissociation

Hagan et al. have determined the CMC of the water dispersible micelle-forming system made from PLA/PEG diblock copolymer. ^[57] Surface tension measurements were measured using the Wilhelmy plate methods. They found that the CMC value for this system is quite low (0.0035% w/v). Liu et al. have also tested the CMC of the triblock PLA-PEG-PLA copolymer micellar formation. ^[58] Pyrene was used as fluorescence probe to determine the critical concentration of the formation of micelle with a fluorescence spectroscopy. They found that the CMC value for PLA-PEG-PLA triblock copolymer changed little with increased PEG chain length in the block copolymers.

Molecular weight of Micelle and aggregation number

The aggregation number of the micelle is obtained from the quotient of the molecular weight of micelle to molecular weight of polymer. The molecular weight of micelle and aggregation number can be determined by several techniques, in particular, by the time-resolved fluorescence quenching (TRFQ) method and by static light scattering. Recently, Vasilescu has discussed the spectroscopic methods to characterize the aggregation number of micelles from block copolymers and surfactants with TRFQ method extensively^[59]. There are more references related to the characterization of aggregation number of block copolymer micelle using static light scattering.^[60, 61] Riley et al. studied the nanoparticles assembled from PLA-mPEG diblock copolymers. They found the aggregation number of the assemblies increased as the molecular weight of PLA block increased^[60]. They also use small-angle neutron scattering (SANS) to study the average aggregation number. It was obtained using the molar volume of the PLA-mPEG chains and numerically integrating over the fitted polydispersity.^[62]

Interparticle interaction

For micelles obtained from diblock copolymer, the classic core-shell model can describe the formation of the micelle. Tadros and collaborators have demonstrated how dynamic rheological measurements on concentrated dispersions can be used to obtain the thickness of the PEG layers (Δ).^[63, 64] Essentially, the storage and loss moduli were measured as a function of the volume fraction of the dispersion. As the surface-to-surface distance between the particles approaches twice the grafted layer thickness, a strong interaction occurs and the system becomes predominantly more elastic than viscous. From knowledge of critical volume fraction at which this occurs, the thickness of the

steric layer can be calculated. ^[65] The rheological properties of concentrated dispersions can also be correlated with interparticle interactions ^[66]. Hagan and Riley used this method to estimate the thickness of the PEG layer and interparticle interaction in micellar-like diblock copolymer PLA-mPEG nanoparticles ^[67, 68].

Surface charge

In aqueous ionic medium, particles are normally charged. The zeta potential is the potential located at the shear plane of the diffuse ionic layer at the particles surface. It is often used to characterize the stability of colloids and can be taken as an indirect measure of the surface charge. Particles with hydrophilic PEG chain as outlayer normally have near neutral zeta potential, if the PEG chain density is sufficiently thick to screen the diffuse ionic layer.

Hawley ^[69] and Tobio ^[70] has found a clear differentiation between the non PEG surface modified and modified nanoparticles, with generally highly negative zeta potential values for non-modified and less negative value for modified nanoparticles. However, sometimes the influence of stabilizer also needs to be considered ^[71].

2.3.6. Microspheres from ABA triblock polymer as parenteral delivery systems for proteins

The application of block copolymers for controlled release of bioactive materials emerged when shortcomings of polyesters, with respect to protein delivery, became apparent and required biodegradable polymers which did not affect protein stability and release properties. The first report on microencapsulation of hydrophilic macromolecules using ABA triblock copolymers consisting of PLGA A-blocks and a hydrophilic PEG B-block appeared in 1994 ^[72].

Several model proteins were then microencapsulated using a modified W/O/W double emulsion method and an ABA triblock copolymer with a M_w of ca. 30,000g/mol and a PEG B-block 10 000 g/mol. Several proteins such as cytochrome c (13kDa), ovalbumin (43kDa), bovine serum albumin (67kDa) and tetanus toxoid (150kDa) could be microencapsulated with very high efficiency and release could be continuous and completed over 20-50 days. Kissel et al. attribute the change in the release behavior compared with PLGA to a combined mechanism of swelling and erosion, leading to a hydrogel-like structure, also observed by SEM ^[73].

Also low-molecular-weight drugs, such as lidocaine and propranol hydrochloride were encapsulated into PLLA-PEG-PDLLA using different microencapsulation techniques. In vitro release rates from microspheres were generally higher for the ABA polymers than the lipophilic lidocaine base at the same drug loading ^[74].

In summary, ABA microspheres as parenteral protein depot systems have clear advantages over PLGA regarding drug loading, control of protein release rate and protein compatibility with the ABA polymer. It seems that quantitative predictions for therapeutically relevant proteins are not yet possible, mainly because of a lack of basic knowledge and models.

2.3.7. Biodegradable long circulating nanoparticulate based on PLA/PEG block copolymers

There has been a growing interest in the development of a colloidal drug carrier, which is small enough for intravenous administration and possesses an adequate circulation half-life in order to enable drug release into a continuous and controlled manner.

There are numerous potential applications for such a system: the protection of sensitive therapeutically active molecules against in vivo degradation, the reduction of toxic side effects which can occur when some highly active drugs like those used for cancer treatment are administered in the form of a solution, the increase of patient comfort by avoiding repetitive bolus injection with the use of perfusion pumps, and the achievement of more favorable drug pharmacokinetics.

The successful delivery of macromolecular drugs is often problematic. Protein drugs generally can't be administered orally, since they are hydrolyzed or denatured in the gastrointestinal tract. Parenteral administration also poses some problems, since proteins are usually quickly metabolized and eliminated. The typical pharmacokinetic half-lives of proteins range from 2 to 30 min. The pharmacokinetics of these compounds could be improved by encapsulating them into long-circulating drug delivery devices.

The effective use of pharmacologically active substances in the chemotherapy of cancer, viral infections and many other diseases suffers from non-specific toxicity and poor tissue specificity of drugs. Polymeric nanoparticles are possible carriers for targeting these compounds by the intravenous route in order to increase the compound's effectiveness in the diseased tissue and reduce general toxicity.

Various types of systems (liposomes, emulsions, micelles and nanoparticles) were developed over the last decade in order to achieve controlled intravenous drug delivery or targeting to specific tissues. However, most of these systems, via recognition by the phagocytic cells (mainly the cells of the mononuclear phagocyte system and the polymorphonuclear leukocytes), are detected as foreign products and quickly removed from blood circulation. Essentially, macrophages located in the reticuloendothelial system (RES) (of which the Kupfer cells in the liver comprise 85-95% of the total intravascular phagocytic capacity) play a crucial role to phagocytize injected particles. For example, polystyrene particles as small as 60 nm were found disappeared from blood within minutes. Similar short half-lives were observed whatever the chemical composition of the injected particles: poly (lactic acid), poly (lactic acid-co-glycolic acid), poly (cyanoacrylate) or poly (acryl starch). Therefore, using these types of systems, it is only possible to target drugs to RES organs, mainly the liver.

2.3.7.1 Mechanism of MPS (mononuclear phagocyte system)

Macrophages and closely related cells of the mononuclear phagocyte system, previously known as the reticulo-endothelial system (RES), are strategically placed in the body to recognize, and respond to particulates, as well as macromolecular ligands ^[75]. They are highly efficient phagocytes by virtue of their expression of a wide range of called pattern recognition receptors ^[76]. In addition, the cells are rich in cytoskeletal and other intracellular components that enhance their uptake efficiency. Their interactions with any artificial lipid-enveloped particles (e. g. Liposomes) will be desirable or a nuisance depending on the viewpoint of the investigator, but certainly inevitable, unless steps are taken to interfere with natural recognition mechanisms. The macrophages within

different organs in contact with blood, or at portals of entry such as the airway, gut or skin vary considerably in their potential for recognition and clearance of altered host, or foreign components.

It is generally assumed that the rapid particle phagocytosis is mediated by the adsorption of certain blood components (opsonins) onto the surface of the particles; for example, in vitro experiments showed that serum complement, one of the most important components of the opsonin system of the body and in particular C3, strongly activates phagocytosis by macrophages. Other complements, such as C4, also seem to be involved in the phagocytic process.

The main challenge for administering particulate drug carriers into the vascular compartment is site-selective drug delivery. Such a carrier would avoid indiscriminate interactions with the RES, selectively reach the desired tissue, release the active compound with an optimal rate and finally degrade into non-toxic elements, which can be eliminated from the body and do not cause side-effects.

Various attempts were made to achieve long blood circulation times by avoiding RES recognition, mainly by chemically attaching or adsorbing appropriate polymers or molecules at the particle surface, which would reduce or minimize the interaction with opsonins^[77,78,79,80,81,82,83,84]. They are also called Stealth therapeutic system. With stealth technologies, we can get the distribution of carrier systems to non-MPS sites. The system can act as a drug-reservoir circulating in the blood compartment and improve the immunospecific targeting strategies. PEG is widely used in this system.

2.3.7.2 Long-circulating drug delivery systems

Particle size and shape greatly influence their distribution in the organs. To circulate through the smallest capillaries, the particle size should be less than 5 μm . Moreover, a diameter of less than 200 nm is required to avoid spleen filtering effects. In addition, the surface of the particles should possess RES-avoiding properties (be stealthy with regard to phagocytic cells). Allen wrote “if you want to be invisible, look like water”: indeed, particles with hydrophilic surfaces, upon which water molecules can readily adsorb and have longer blood half-lives.

Particles with neutral surfaces seem the most appropriate with regard to blood persistence. Various attempts were directed towards altering the carriers’ surface properties to reduce their RES clearance. Coated systems and “self-forming” systems are two most important ones. Liposomes and emulsions are also used for this purpose.

Coated systems

Coated systems refer to systems consisting of biodegradable polymeric nanoparticles where the surface is modified by adsorption of PEG containing copolymers.

(a) Coatings obtained by polymer chemical attachment

Polymer chemical attachment was widely used to modify liposomes. PEG coating can decrease RES uptake, increase blood residence time and increase stability to contents leakage, increase flexibility in lipid composition and dose-independent pharmacokinetics [85].

(b) Coatings obtained by polymer adsorption

The polymer adsorption approach was adapted from model PS particles to degradable PLGA particles coated by adsorption of amphiphilic diblock PEG-PLA copolymer,

which advantageously replaced the non-degradable surfactants previously used (Poloxamer and Poloxamine). However, a comparison of the *in vivo* results obtained with the two systems, PLGA/ PEG-PLA and PS/Poloxamer 908, suggested that the adsorbed layers of PEG-PLA might have a lower stability or resistance to protein adsorption than Poloxamer 908 ones.

The adsorbed polymers might desorb *in vivo*, due to replacement by blood compounds with a higher affinity for the particle surface, and this would pose problem for their intravenous administration. To increase the coating stability, polymer chemical attachment is an alternative to polymer adsorption.

(c) Mechanism of action of the PEG coating

PEG-based coatings significantly increase the nanoparticle blood circulation time. Among hydrophilic polymers, PEG has the advantage to be considered non-toxic and was approved by the Food and Drug Administration (FDA) for internal use in humans.

To understand the mechanistic action of hydrophilic and in particular PEG-based coatings would be a step forward in the design of particles with optimized surface properties. A generally assumed mechanism is based upon the formation of a sterically hindered, hydrophilic coating which avoids opsonization by plasma proteins ^[86, 87].

The hydrophilicity was considered a main requirement, but it turned out not to be a sufficient one. Besides hydrophilicity, chain flexibility is another major feature necessary for the coating polymers to provide long-circulating particles. Due to the transient, flexible and rapidly changing structure of PEG, the immune system would have difficulties in modeling an antibody around it ^[88]. The protective layer of PEG is considered as a “cloud” of possible chain conformations, with a density high enough to

prevent the interactions of opsonins with the surface of the particles. If a polymer chain possesses both hydrophilicity and flexibility properties (to enable a high number of possible chain conformations), it can serve as an effective coating protector for particles against opsonization.

The conditions that lead to protein repulsion from hydrophobic plane surfaces to which PEG chains were attached to one chain end in a “brush” configuration were studied.^[89] These authors elaborated a mathematical model taking into account the four types of interactions between a protein and a hydrophobic substrate (Fig 2-5). The best conditions for protein repulsion were the long PEG chain length and high surface density.

It has been suggested that both the reduction in the adsorption of opsonins and the selective adsorption of certain plasma components prevent the recognition and uptake of nanoparticles by macrophages; the competition between these two mechanisms is believed to be the key in controlling the particle uptake by macrophages and their distribution^[90].

Figure 2-5 Interactions between a protein and a hydrophobic substrate with attached PEG chains 1: hydrophobic attraction between the protein and the substrate; 2: steric repulsion resulting from PEG chain constriction; 3: van der Waals attraction between the protein and the substrate; 4: van der Waals attraction between the protein and the PEG chains

Micelles (Self-forming system)

There is a significant interest in self-assemble micelles as drug carriers for drug targeting. Block copolymers in a selective solvent have a tendency to self-assemble at surfaces and into micelles ^[91, 92]. At an aqueous interface, the amphiphilic property of block copolymers composed of hydrophilic and hydrophobic segments can cause the diestral end of the hydrophilic chain to extend into the bulk aqueous solution. Micelles with core-shell structure are formed through the segregation of insoluble blocks into the core, which is surrounded by hydrophilic shell composed of hydrophilic blocks. This interfacial activity of amphiphilic block copolymers provides their high utility in the drug carriers. Multiblock copolymers such as POE-poly(L-lysine), POE-poly(β -benzyl-L-aspartate), POE-poly(ϵ -caprolactone) and poly(acrylic acid)-poly(methyl methacrylate) as well as those that can be used in particle coatings (e. g. PLA-PEG or PLGA-PEG) also self-disperse in water form spherical polymeric micelles with diameter in the size of 15 to 80nm ^[93,94]. Some of these micellar structures have been suggested as promising long-circulating carriers of poorly water soluble and amphiphilic drugs, because of their small size and hydrophilic shell ^[95, 96]. The critical micelle concentration is very important to this system. Upon intravenous injection, micelles are often diluted to less than their minimum micelle concentration and polymer molecules are known to behave in a dramatically different way. Further more, to date little is known with regard to the stability of micellar systems in vasculature and their extent of interaction with blood and cellular components. Micelle stability may be enhanced by cross-linking procedures to produce a solid outer core (e. g., shell-cross –linked kendel-like particles) ^[97, 98].

Liposomes

For incorporation into the liposomal bilayer, numerous lipid derivatives of PEG have been made using lipids ^[99, 100, 101, 102, 103, 104]. Alternatively, activated PEG can be anchored to reactive phospholipid groups of preformed vesicles ^[105]. Another strategy has utilized the transfer of PEG-phospholipid conjugates from the micellar phase in to the lipid bilayer of preformed vesicles ^[106]. The biophysical basis of these observations was realized in 1996 by Bedu-Addo et al. ^[107]. They found that mixtures of PEG-phospholipid conjugates and phospholipids exist in primarily three physically distinct states.

Oil-in-Water Emulsions

Apart from poloxamers and poloxamines, PEG-PE and Tweens have all been used as emulsifiers for the production of long-circulatory oil-in-water emulsions. The blood retention time of such emulsions was shown to be related to PEG chain length and its surface density ^[108, 109].

2.3.7.3 Parameters may affect the property of long-circulation delivery system***The polymer polydispersity***

An important consideration in clinical therapeutics is the ultimate fate of polymeric constituents used in nanoparticle engineering. The fate of any polymer used will be determined by its behaviour on administration into the individual biological system. Synthetic polymers exist as populations with a statistical distribution. The degree of polydispersity will be great if the product has been synthesized by free radical polymerization. Ironically, the future usefulness of these monodisperse systems in medicine remains to be revealed, since in vivo studies so far have indicated their propensity to localize in the liver and spleen ^[110]

There are some preliminary studies on the effect of specific molecular weight fractions present within a polydisperse system to discern individual biological activities in vivo on both short-term and long-term administration^[111, 112]. Variation of in polymer molecular weight fraction has been linked to the tissue toxicity. Limited studies with polydispersed radiolabeled POP/POE copolymers have indicated that the primary route of polymer excretion is renal and the minor route is biliary.^[113, 114] Furthermore, no studies so far have determined to what extent these copolymers are excreted intact or in a modified form from the starting material.

Chemical contamination in polymers

Most polymers are synthesized under relatively hostile conditions (elevated temperature and pressure) and are thus prone to processes of degradation during their formation. To counter the in-process environments processing modifiers (e. g. heat stabilisers and emulsifiers) are frequently used but readily separated from the polymer^[115]. To ensure post-processing polymer stability, chemical modifiers such as antioxidants and ultraviolet stabilisers are added. It must therefore be considered if these or other additives and/or low molecular weight degradative products or monomers are capable of inducing immunological and /or pharmacological events following parenteral administration. There is relatively little information available regarding the biological fate of antioxidants or their break down products. Supercritical fluid fractionation has been used to purify polymers; however, in this case, there was evidence for macromolecular cleavage^[116].

Incomplete polymerization of diblock copolymers may result in homopolymers contamination in the product, which is exceptionally difficult to remove and may explain

the presence of homopolymers in some commercial samples ^[117]. To date, no result shows whether these contaminants are essential for whatever useful activity of the polymer or they have a synergistic role in combination with the copolymer. A possible future method to circumvent the deleterious consequences of hostile synthetic processing conditions and negate the use of specific additives may be achieved through milder microbiological methods. To date, commercially bacterially derived polymers have included polyhydroxyacids and polyester ^[118, 119].

Biodegradable polymers

No study has shown that biodegradable polymers (e. g. PLA or PLGA) will degrade in a uniform manner in vivo. It is known that the in vivo environment will vary depending on the site of parenteral administration, composition of tissue fluids and disease state which further complicates the uniformity of the degradation process. PLGA polymers have been used widely as biomaterials or medical application over the last 30 years and regarded as biocompatible and non-toxic. For example, high molecular PLGA has been used effectively as surgical sutures and bone fixation nails and crews ^[120, 121], while low molecular weight amorphous PLGA has been researched widely for controlled drug delivery application ^[122, 123]. An example of a successful pharmaceutical product for the controlled delivery of lutenizing hormone in the form of injectable depot is Zoladex (Zeneca, UK). However, there is little information about the rate of degradation as well as toxicological problems associated with PLGA and related biodegradable particulate drug delivery systems following parenteral administration. A two fold increase in the rate of degradation of PLA was observed by Mason ^[124]. The specific plasma constituents responsible were not studied. The large surface area of the colloid, which is gradually

presented to opsonic and other immunoregulatory proteins, may potentiate these effects. Researchers also found that pre-degradation of PLA particles can induce cell damage (e.g. swollen mitochondria, widened endoplasmic reticulum, disappearance of membrane ruffling) on Day 2 of experiment with evidence of the necrotic cells and cell debris on Days 3 and 4 ^[125]. It indicated that at least the “bioinert” PLGA can also be cytotoxic to immune cells. Therefore, it is necessary to have a wide range of in vivo studies on biodegradable polymer to determine the nature and fate of the degradation products. The physicochemical nature of particle (size, shape and porosity) will also affect the degradation rate ^[126,127]. The autocatalytic nature of some polymers will further accelerate degradation rates and this may be potentiated when the polymers are isolated or have restricted transport away from a region ^[128].

The precise role of polymer stereochemistry on enzyme degradation and immunological response following parenteral administration has not yet been thoroughly investigated. Li et al have done a little preliminary research on the stereochemical degradation preference for copolymer bonds. ^[129]

2.4 Nanoparticle and targeted system for cancer therapy

In all cases, the most important thing is the ability to target and to kill the cancer cells while affecting as few healthy cells as possible. The degree of change in the patient's life quality is greatly depended on the targeting ability of the treatment. The advances in treatment of cancer are progressing quickly both in the field of new agents against cancer and new ways of delivering therapies.

2.4.1. Targeted delivery of nanoparticles

2.4.1.1 Achieving targeting by avoiding reticuloendothelial system (RES)

As discussed in above context, those delivery systems with Stealth[®] property can avoid the recognition of the MPS system. This allows them to selectively extravasate in pathological sites, like tumors or inflamed regions with a leaky vasculature. As a result, such long-circulating nanoparticles are supposed to be able to directly target most tumors located outside regions ^[130].

2.4.1.2 Targeted delivery through enhanced permeability and retention (EPR effect)

The EPR effect is now recognized as a general characteristic of solid tumor, thus, regarded as a “gold standard” in new anti tumor drug designing. ^[131, 132] Most tumors have unique growth characteristics particularly rapid growth rate and metastatic potential to the distant sites. Enhanced vascular permeability in solid tumor is considered to supply continuously great nutritional demand as well as oxygen. The enhanced vascular permeability of solid tumor is important not only in tumor biology, but more critical in targeted delivery of macromolecular anticancer drugs to tumor. ^[133] Unlike small molecular anticancer drugs used today, which do not discriminate tumor tissue from normal tissue, macromolecular (or polymeric) drugs can target tumor with high selectivity by taking advantage of the enhanced permeability and retention effect (EPR effect) in solid tumors. ^[134, 135] Namely, almost all tumor tissues exhibit the extravasation of macromolecules. However, because of the suppression of lymphatic function in tumor, clearance of macromolecules from tumor is so impaired that they remain in the tumor for a long time. It might result in an important intratumoral drug accumulation which is even

higher than this observed in plasma and other tissues ^[136,137]. This makes them good targets for drug delivery as long as the targets for particular antigens, even on their surface. This also makes them ideal targets for drug delivery as long as the targets for a particular cancer cell type can be identified with confidence and are not expressed in significant quantities anywhere else in the body.

Figure 2-6 Scheme of EPR effect

2.4.1.3 Tumour-specific targeting

Tumour-activated prodrug therapy uses the approach that a drug conjugated to a tumour-specific molecule will remain inactive until it reaches the tumour tissue. ^[138] These systems would ideally be dependent on interactions with cells found specifically on the surface of cancerous cells and not the surface of healthy cells. A lot of research has been done in this field. ^[139, 140]

2.4.1.4 Targeting tumour vasculature

Targeting the tumour vasculature is a strategy that can allow targeted delivery to a wide range of tumour types ^[141, 142, 143] Recent work has also shown that gene delivery

may also be targeted to neovasculature by coupling lipid-based cationic nanoparticles to an integrin $\alpha\beta$ -targeting ligand in tumour-bearing mice ^[144].

2.4.2. Anti-cancer drugs used for delivery

2.4.2.1 Paclitaxel

Paclitaxel is a microtule-stabilizing agent which promotes polymerization of tubulin causing cell death by disruption the dynamics necessary for cell division. Paclitaxel, formulated in a 50:50 mixture of Cremophor[®] EL: absolute ethanol as Taxol[™], is the world's most successful chemotherapeutic drug. In 1971, Drs. Monroe Wall and M.C. Wani of Research Triangle Institute identified compound 17, the active ingredient in Pacific yew tree bark, as paclitaxel. However, development was later suspended because experimental models did not show superior activity and there are problems with producing, extracting, and processing paclitaxel. In 1979, Paclitaxel's unique mechanism of action – tubulin stabilization – was identified by Dr.Susan Horwitz. Paclitaxel prevents cell division by promoting the assembly of and inhibiting the disassembly of microtubules – skeletal structures that assemble and divide throughout the life of a cell. Taxol[™] was approved for use after failure of first-line or subsequent chemotherapy for the treatment of metastatic carcinoma of the ovary in 1992. In 1997, FDA approved semisynthetic Taxol[™] for the second-line treatment of AIDS-related Kaposi's sarcoma. FDA also approved semisynthetic Taxol[™] in combination with cisplatin, for the first-line treatment of advanced carcinoma of the ovary in 1998. In fiscal year 2001, Bristol-Myers Squibb posted nearly \$1.2 billion in sales from Taxol[™]. Now Paclitaxel is one of the best antineoplastic drugs found in nature in the past decades, since it has excellent therapeutic efficacy for a wide spectrum of cancers, especially for ovarian and breast cancers.

Despite these inspiring successes, TaxolTM also brings the side effects such as dyspnea, flushing, rash and urticaria^[145]. This hypersensitivity reaction is believed to be a mast cell degranulation type reaction^[146, 147]. Despite some precautions, minor and severe reactions were found in patients, 44% and 3% respectively^[148, 149].

The hypersensitivity was ascribed to the presence of the Cremophor[®] EL in the TaxolTM. So it is necessary to find an alternative way to replace the solubilizing agent. Liposome^[150], emulsion^[151], nanoparticles^[152, 153]. PLGA has been used to prepare biodegradable nanoparticles for drug delivery^[154, 155]. They have shown comparable activity to traditional formulations and much faster administration. By applying the nanoprecipitation method using acetone and PLGA, very high loading efficiencies can be reached^[153]. In 1996, Zhang et al. first use diblock copolymer PLA/PEG for drug delivery carrier^[19]. Several publications are reported for the development in this drug delivery system, both of the diblock and triblock copolymer were studied^[156, 157]. Very recently, Xu et al. used the PEG-coated biodegradable polycyanoacrylate nanoparticles conjugated to transfer for paclitaxel delivery. This is the first actively targetable nanoparticle carrier for paclitaxel up till now^[158].

2.4.2.2 5-Fluorouracil

5-Fluorouracil (5-fluoro-2, 4-pyrimidinedione) (5-FU) is one of the most effective drugs for the treatment of solid tumours in many organs (colon, liver, breast, ovaries) and is used extensively in combination drug regimens^[159]. It has been used in clinical practice for more than 20 years. One of the problems faced in 5-FU therapy is its host toxicity to the bone marrow and the gastrointestinal tract^[160]. The drug is quite toxic and about 2/3 of the patients showing signs of toxicity. In the case of 5-FU, it is also rapidly

absorbed through blood capillaries into systemic circulation. These results were in relatively low levels of drug near the site of action with the subsequent loss of efficacy and increased risk of systemic toxicity ^[161]. It has been recognized that, optimally, this drug should be dosed once or twice a week, preferably as a long-acting injectable and the drug can be delivered to the desired target site ^[162, 163, 164]. Based on the fact the local sustained delivery may enhance therapeutic effects, a pilot study involving eight patients with newly diagnosed glioblastoma was conducted in which 5-FU in PLGA microspheres were delivered locally after surgical resection. Promising results were reported by this study with 2/8 of the patients achieving disease remission ^[165]. 5-FU can also be used with Antisense oligonucleotides (AODNs). The combination therapy of AODNs and 5-FU using biodegradable polymeric delivery system (PLGA) microspheres was studied by Hussain et al. ^[166]. They found that the separate PLGA sustained release formulations of AODNs and 5-FU appears worthy of further investigation in vivo for potential combination therapy in cancer. Dendrimers of poly (amidoamine) modified with mPEG-500 was also used to load 5-FU. Due to its slight solubility in water, the 5-FU allowed it to complex with the dendrimers after simply incubating the polymers with the drug ^[167].

2.4.2.3 Doxorubicin

Doxorubicin is one of the most potent and widely used anticancer drugs which work by inhibiting the synthesis of nucleic acids within cancer cells ^[168]. Various researchers have studied ways to target doxorubicin delivery to cancer tissues or at least to diminish its side effects. Conjugates of dextran and doxorubicin have been encapsulated in chitosan nanoparticles of ~100nm diameter. It was found that mice injected intravenously with both dextran-doxorubicin conjugates and the conjugates encapsulated in chitosan

nanoparticles showed a decrease in the tumour volume. Treatment with doxorubicin alone did not decrease the tumour volume^[169]. One group has conjugated doxorubicin to PLGA and formed nanoparticles from these conjugates. Their result showed that a single injection of doxorubicin-PLGA conjugate nanoparticles could suppress tumour growth for up to 12 days^[168].

2.4.2.4 Cisplatin

Cisplatin is one of the most potent anticancer agents.^[170] However, its use is associated with serious side effects, including renal and auditory toxicity, nausea and vomiting. The selective delivery (targeting) of cisplatin to tumour would significantly reduce drug toxicity, improving its therapeutic index. A number of strategies have been developed for a more selective cisplatin delivery. One such strategy involved the local (adjacent to the tumour cells) administration of cisplatin to easily accessible tumours, either as a water-soluble drug-polymer complex^[171] or entrapped in sustain release poly (lactide-co-glycolide) microspheres^[172, 173], by using poly (ethylene glycol)-poly (aspartic acid) copolymer^[174] or pegylated liposomes.^[175] as drug delivery carriers, the tumor targeting can also be improved. Recently, Cisplatin was also loaded into PLGA-mPEG diblock copolymer nanoparticles. The in vitro nanoparticle degradation, in vitro drug release and in vivo drug residence in blood properties of PLGA-mPEG were investigated. The nanoparticles exhibited rapid degradation and sustained drug release properties. In addition, prolonged drug residence in blood was observed when PLGA-mPEG nanoparticles of cisplatin were administered intravenously to mice^[176].

2.5 Research of drug release kinetics-mathematical modelling of controlled drug release

Mathematical modeling of controlled drug delivery can help provide a scientific knowledge base concerning the mass transport mechanisms which are involved in the control of drug release. In addition, if an adequate mathematical theory is identified for a particular pharmaceutical system it can be used to simulate the effect of the device design parameters (e. g. geometry and composition) on the resulting drug release kinetics. In an ideal case, the system formulation parameter required to achieve a certain desired drug release profile can be predicted. Thus mathematical modeling can significantly facilitate the optimization for existing and the development of new pharmaceutical products. There is no overall mathematical model covering all the possible chemical and physical process that can occur. Thus it is crucial to identify or develop an adequate mathematical theory for a specific drug delivery system.

2.5.1. Mathematical modelling of non-degradable system

Lots of research has already been done in this field and many models have been developed which can be applied to many kinds of non-degradable system. In 1963, Higuchi described the release of drugs from an insoluble matrix as square root of the time-dependent process on the basis of Fickian diffusion ^[177]. The Hixson-Crowell cube root law can well describe the drug release from systems in which there is a change in surface area and the diameter of the particles present in the tablet ^[178]. The Weibull distribution can also be applied to simulate the drug release kinetics ^[179]. The dissolution data can also be analysed by the Korsmeyer and Peppas equation, with two parameters k

and n . K is the kinetic constant incorporating structural and geometric characteristics of the tablets, and n is the diffusional exponent indicative of the release mechanism. ^[180]

2.5.2. Mathematical modelling of biodegradable system

There are relative few documents about the mathematical drug release modeling of biodegradable delivery systems such as the modeling of diffusion or swelling-controlled devices, since it is very complicated ^[181]. Besides physical mass transport phenomena, chemical reactions such as polymer chain cleavage have to be considered in biodegradable systems. These reactions change the conditions for mass transfer processes continuously, thus making the mathematical treatment of biodegradable-controlled drug release rather difficult ^[182,183].

There are two major categories to depict the biodegradable-controlled drug release. First are empirical models that usually assume a single zero-order process to control drug release rate. Second are models considering physicochemical phenomena such as diffusional mass transfer or chemical reaction process.

Depending on the property of biodegradable device and geometry (size and shaped), many mass transport phenomena and chemical reaction phenomena effect might be considered:

Table 2-2 Factors that might affect the biodegradable drug release system

Water intrusion	Drug dissolution	Polymer degradation	Formation of aqueous pores	Diffusion of drug
Polymer degradation	Crystalline of polymer degradation	pH changes	Diffusion of the drug	Autocatalytic effects
Diffusion of H^+ or OH^-	Osmotic effects	Adsorption/desorption process	Convection process	Polymer swelling

It is impossible to take all of these parameters into modeling calculation. What is important is to identify the dominating physical and chemical process and to take only these into account. The physico-chemical properties of drugs and polymers, the dominating chemical reaction will greatly affect the drug release profile.

2.5.2.1 Empirical mathematical biodegradable models

Compared to mechanistic mathematical models, empirical models quantifying drug release from biodegradable delivery systems are not based on the real physical or chemical process. Empirical models describe the apparent drug release rate. It is simple and easy to use. Hopfenberg assumed that the rate of drug release from biodegradable system is proportional to the surface area of the device which is allowed to change with time. All mass transfers are assumed to add up to single zero-order process confined to the surface area of the system. He derived the following equation, which is valid for sphere, cylinders and slabs. ^[184]

$$\frac{M_t}{M_\infty} = 1 - \left(1 - \frac{k_0 * t}{c_0 * a}\right)^n \quad \text{Equation 2-1}$$

M_t and M_∞ are the cumulative amounts of the drug released at time t and at infinite time, respectively; C_0 denotes the uniform initial drug concentration with the system; a is the radius of cylinder or sphere; n is “shape factor”, representing spherical ($n=3$), cylindrical ($n=2$) or slab geometry ($n=1$). This model is good for predicting the surface degradation matrix system.

A more detailed analysis for spheres and cylinders undergoing heterogeneous biodegradation was researched by Cooney. ^[185] His model is also based on the assumption which Hopfenberg has been made.

2.5.2.2 Diffusion and chemical reaction-base models

Non-Monte Carlo based model

Heller and Baker first developed a mathematical model to describe the drug release from biodegradable polymers. They assumed that the polymer matrix underwent bulk degradation and can be described with first-order kinetics. PLA and PLGA are examples of this system. ^[186] Lee also gave an approximate model for drug release from thin surface eroding polymer films. It is valid for different ratios of drug loading to drug solubility. ^[187]

In 2000, Charlier and coworkers developed a mathematical model to quantify drug release from bulk eroding films, considering polymer degradation and drug diffusion simultaneously ^[188]. They used a pseudo-steady state approach, for initial drug loadings well above the solubility of the drug within the matrix. They also assumed the first-order degradation of polymers. The drug diffusion coefficient, D , increase exponentially to t

$$D = D_0 \cdot \exp(k \cdot t) \quad \text{Equation 2-2}$$

D_0 is the diffusion coefficient of the drug at $t=0$; k is the polymer degradation rate constant. So the cumulative absolute amount of drug released, Q can be obtained

$$Q = S \sqrt{\frac{2 \cdot C_0 \cdot C_s \cdot D_0 \cdot [\exp(k \cdot t) - 1]}{k}} \quad \text{Equation 2-3}$$

S is the surface area of the film exposed to the release medium; and C_0 and C_s are the initial drug concentration and solubility of the drug in the system, respectively.

Based on the Fick's second law of diffusion and monolithic dispersion, N. Fainst recently developed a new and simple model to quantify the drug release from the biodegradable PLGA microparticles ^[189]. 5-FU was used as the model drug. Based on the approach developed by Koizumi ^[190], which did not take into account the change of the diffusion

coefficient during the drug release process, they correlated the diffusion coefficient to the molecular weight loss.

$$D(M_w) = D_0 + k / M_w \quad \text{Equation 2-4}$$

$$M_t = 4 * \pi * r^2 \left[\sqrt{2 * (C_0 - C_s) * C_s * D * t} + \frac{4 * C_s * D * t}{9 * r} * \left(\frac{C_s}{2 * C_0 - C_s} - 3 \right) \right] \quad \text{Equation 2-5}$$

D_0 is the diffusion coefficient of the drug in the non-degradable polymer and k is a constant. The initial drug concentration within the system, C_0 , should be much greater than the solubility of the drug ($C_0 \gg C_s$). M_t is the cumulative absolute amount of drug released at time t ; r represents the radius of the spherical device; C_0 and C_s are the initial drug concentration and the solubility of the drug within the system, respectively; and D denotes the constant diffusion coefficient of the drug.

Monte Carlo-based models

Zygourakis first applied the Monte Carlo-based models for the simulation of drug release from surface eroding polymer matrices in the early 90's^[191,192]. The major idea of this method is to represent the polymer matrix cross-sections by 2-dimensional grids. Later, Göpferich et al. developed their models, taking both Monte Carlo simulation-based polymer degradation and diffusional mass transport process into consideration^[193, 194]. These are not only applied to surface eroding polymers, but also to bulk eroding systems^[195], three dimensional rotationally symmetric matrices^[196] and composite matrices made of bulk and surface eroding polymer^[197].

3. Materials and Experimental

3.1 Materials

L-Lactide, purchased from PURAC Far East Pte Ltd, was recrystallized three times from dry ethyl acetate and dried in vacuo at 40 °C for 12 hrs. Stannous octoate was purchased from Sigma and used without further purification. The Poly (ethylene glycol) (M_n =2000, 4000, 6000, 8000), Methoxy poly (ethylene glycol) (M_n =2000, 5000 Sigma) and 4-arm PEG (M_w =10k, Nektar) were dried at 120 °C for 6 hrs in vacuo, prior to use. Toluene was distilled over sodium and benzophenone was used as indicator. It was used until the color turned into purple. Ethyl acetate was distilled over P_4O_{10} . Other chemicals were used as received. Phosphate Buffered Saline (PBS) (pH=7.4, 10mM) was obtained from Sigma-Aldrich.

3.2 Experimental procedure

3.2.1. Synthesis of the copolymer

The L-lactide was first recrystallized from ethyl acetate, at least three times, to remove the residual impurities in the monomer. The recrystallized lactide and dried m-PEG or s-PEO with different feed ratios (details can see Table 4-1) were transferred into a 250 ml pre-dried three-neck flask which was purged with dry nitrogen. Dry toluene was added into the three-neck flask followed by stannous octoate (0.05% w/w, in toluene solution). The mixture was heated to 110 °C and allowed to reflux for 24 hrs. The mixture was stirred over night. All the experiments were strictly under no oxygen and no water environment. Toluene was removed under vacuum after completion of the reaction and the product was purified by repeated solution into dichloromethane and precipitation

from cold ethyl ether and ethanol. The isolated polymer was dried at 40 °C under vacuum before further tests.

3.2.2. Copolymer characterization

The copolymers were characterized by FTIR and NMR. A Perkin Elmer system 2000 Fourier Transform Infrared Spectrometer was used to characterize the polymer. The NMR studies were carried out on a Bruker 400 MHz instrument, operating at 400 MHz (^1H) and 100 MHz (^{13}C). CDCl_3 was used as solvent and TMS as internal reference.

Molecular weight (M_w) and molecular weight distribution (MWD) of the polymers were determined using an Agilent series 1100 gel permeation chromatography. A mixed bed column (PLGel Mixed Bed C, 5 μ , 300 x 7.5 mm, Polymer Laboratories), along with a guard column (50 x 7.5 mm, Polymer Laboratories), was employed. The mobile phase was a mixture of tetrahydrofuran (THF) and dichloromethane (DCM) in a volume ratio of 80:20. Molecular weights of samples were obtained relative to polystyrene standards. The flow rate was 1 ml/min and the temperature (both the column compartment and the flow cell of the refractive index detector) was 35 ± 0.1 °C.

The thermal properties of the polymer were studied using a modulated DSC (DSC 2920, TA Instruments, USA), equipped with a refrigerated cooling system (RCS), under a constant flow of nitrogen (50 ml/min). The copolymers (ca. 5mg) were sealed into aluminum pans under dry conditions. In a typical DSC run, the sample was cooled down to -60 °C, then heated to 200 °C at 3 °C min⁻¹. Apparent melting temperature was obtained from the endothermic peak and the enthalpy of fusion, from the area of the melting peak. Glass transition temperature (T_g) was determined from the second run.

3.2.3. Preparation of the micellar aggregates with triblock, diblock and star-shaped block copolymer

10 to 40mg of the copolymer dissolved in 1 ml of organic solvent (acetone, THF, DMF (Dimethyl formamide) and DMAC (Dimethyl acetamide)) was dropwisely added into 20 ml of distilled water, under mild magnetic stirring. The solution was stirred overnight at 400 rpm/min to evaporate the organic solvent for acetone and THF, while, for DMF and DMAC, the dialysis method was used to remove the organic solvent (COMW 12~14K, Medicell Inter. Ltd.). The micelle like nanoparticles were obtained by freeze drying and kept in desiccators.

3.2.4. Characterization of nanoparticles

3.2.4.1 Examination of particle morphology

Surface morphology of nanoparticles was observed using a JEOL JSM-6340F field emission scanning electron microscope (FESEM) at 5.0 KV. The samples were mounted on a metal holder using double-sided copper tape, then coated with gold using a pulse plasma system before observation.

Transmission Electron Microscopy (TEM) photographs were obtained using transmission microscope at an accelerating voltage of 120 kV. Typically, a sample was prepared by wetting a carbon-coated grid with a small drip of the dilute latex solution. Upon drying, it was stained with 2% phosphotungstic acid (PTA) for several minutes and dried at room temperature before analysis.

AFM images were taken with a Dimension 3100 atomic force microscope under ambient conditions. The atomic force microscope was operated in the tapping mode with an optical readout using Si cantilevers.

3.2.4.2 Determination of particle size

The effective diameters of micelle like nanoparticles in aqueous solution were measured by dynamic light scattering (DLS, Brookhaven Instruments BI-200SM) system. The samples were prepared by the methods motioned before (section 3.2.3) (freshly made without freeze dry) and were filtered with different pore size filters (0.2, 0.45 or 0.8 μm) according to their particle sizes respectively before the light scattering test. The light source is a 35mW Helium Neon Laser with a wavelength at 632.8 nm. The temperature was controlled at 25 ± 0.1 °C. To minimize the effect of stray light, a 90° angle was chosen for measurement. The digital autocorreleator BI9000AT was used for data analysis. The correlation decay functions from DLS were analyzed by the cumulants method to obtain the effective diameter and the polydispersities. The constrained regularized CONTIN method was applied to obtain the particle size distributions.

Figure 3-1 Typical graph of correlation function (a) and Contin distribution (b) of micellar like nanoparticles of PLA/PEG block copolymers

3.2.4.3 Critical Micelle Concentration (CMC) study

The critical micelle concentration (CMC) of the copolymers was determined by fluorescence spectroscopy using pyrene as fluorescence probe. Upon formation of micelles, pyrene would move into the core of the micelles from the aqueous phase which results in an alteration in the intensity ratio (I_1 / I_3) of pyrene fluorescence bands I (371 nm) and III (383 nm).

A series of concentrations of PLA-PEG-PLA triblock, PLA-mPEG diblock and PLA-sPEO star-shaped copolymer solution were prepared as follows: The deionized water was replaced by the saturated pyrene solution (6×10^{-7} M). This stock solution was diluted to different concentrations with pyrene saturated water. The diluted stock solutions were put into 65 °C water bath to equilibrate the pyrene and the micelles and subsequently kept at room temperature overnight for equilibration. A Shimadzu RF-5301PC fluorescence spectroscopy was used for this measurement with excitation wavelength being 339 nm and emission wavelength ranging from 350 nm to 450 nm.

3.2.4.4 Turbidity Measurements and stability of the micelle to added electrolyte

Turbidity measurements were performed using a UV/visible spectroscopy system (Hewlett-Packard 89090A) equipped with a temperature controller. This system allows one to measure the transmittance of a liquid sample in a standard cuvette through a thermal cycle programmed from heating to cooling. In this investigation, a visible wavelength of 500 nm was used for the turbidity measurements. The cloud point (T_c) was defined as the temperature at which the transmission has dramatically dropped to 90%.

Riley ^[198] reported the effect of the added electrolyte to the aggregation behavior and we followed this method. It was mainly applied to simulate the salt-added stability of micelle-like nanoparticles when they were i. v administered. Namely adding 0.5 ml of

each colloidal dispersion to 2.5 ml of Na_2SO_4 solutions of various concentration (0-0.8 M), and measuring the turbidity at a wavelength of 500 nm after 15 min. The critical flocculation point (CFPT) was taken as the electrolyte concentration at which a dramatic increase in the turbidity was first detected. The reversibility of this process was assessed by diluting the flocculated dispersion with water and observing if the flocs could be redispersed with agitation.

3.2.5. Determination of surface composition

XPS measurements were carried out on a VG Scientific ESCA LAB Mk II spectrometer equipped with a Mg Ka X ray source (1235.6 eV photons) and a hemispherical energy analyzer. The copolymer samples were analyzed in film form. They were first dissolved in DCM (dichloromethane) and then spin coated on pre-cleaned glass slides. After removing the solvent in vacuum, they were tested by XPS. After freeze drying, the prepared nanoparticles were mounted on the glass slide with the help of double-sided adhesive tape. The tape was fully covered by the freeze dried nanoparticles.

Series of survey spectra were recorded over a binding energy range of 0 to 1000 eV using passive energy 20 eV. In all cases the survey spectra recorded the presence of only oxygen (O1s 533 eV) and carbon (C 1s 285 eV) on the surface. Detailed analysis of C1s regions of each sample was recorded over a binding energy range of 280 to 300 eV. Charge referencing was performed using the C-H peak (285.0 eV) for PLA-PEG-PLA, PLA-mPEG and PLA-sPEO samples.

The curve fitting was performed by the computer program Kratos provided by the equipment manufacturer. A Gaussian-Lorentzian function was applied to all data. No asymmetry component was introduced.

3.2.6. Contact angle study

PLA-PEG-PLA, PLA-mPEG and PLA-sPEO block copolymer was dissolved in DCM at a concentration of 40% w/v and a film was cast onto a clean glass slide. The film cast from a solution of PLLA (I.V. = 1.04) homopolymer was used for comparison. The contact angle was measured by a dynamic contact angle system (FTÅ200) with the μ -tip® needle offered by FTA.

3.2.6.1 Glass substrates cleaning

The glass substrates (26*76mm) were subsequently cleaned ultrasonically for 10 min in water, acetone, hexane and then subjected to a piranha etch. A piranha etch was used to remove organic residues from substrates. The most common one was the acid piranha: a 3:1 boiling mixture of concentrated sulfuric acid (H_2SO_4) with hydrogen peroxide (H_2O_2). These glass substrates were sustained in the piranha solution for 60 mins and then thoroughly rinsed in water. The clean substrates were stored under vacuum..

3.2.6.2 Spin coating with PLA/PEG block copolymers on the glass substrates

Those copolymers were spin coated on the glass substrates with a 40 mg/ml DCM solution. The spin rate is 2000 rpm for 20 seconds. After that, those glass substrates were put into the vacuum oven dried at room temperature until no weight change. This method was also applied to the XPS study for the surface composition of copolymer films.

3.2.6.3 Dynamic contact angle

The captive needle approach offers the possibility of advancing and receding angle measurements in the same experiment. The pump can be programmed to both dispense and pick up. To get good result, two salient conditions must be met

1. The needle tip must be small compared to the drop so fluid adhesion does not distort the drop shape. So we order the glass needles from the FTA μ -tip® needle (ID= 5 μ m,

OD = 1 mm). With these glass needles of small tips, reliable result can be obtained from the DCA analysis.

2. The expansion or contraction of volume must be slow enough that equilibrium conditions prevail. The pump was programmed at 0.1 $\mu\text{L}/\text{sec}$.

The sessile contact angle as a function of time, i. e., the spontaneous relaxation of the water-vapor interface in contact with block copolymer surface was measured. The needle of the syringe containing the liquid was fixed just above the substrate, so that the falling drop immediately touched the solid surface and began to spread. The spreading of the drops was monitored, from the moment they detached from the needle, by a video recorder connected to a CCD camera and a microscope. The drops were back-lighted. The advancing contact angle, (θ_{adv}) and receding contact angle (θ_{rec}) were measured. A sequence of pictures of the droplet was monitored by a CCD camera. The hysteresis was calculated as the difference between θ_{adv} and θ_{rec} .

3.2.7. The surface charge test

The zeta potential is defined as the electrical potential as measured at the shear plane of the electrical double layer of a surface or particle. Due to the surface charge, particles will move when placed in an electric field, E . It is called electrophoresis. Specifically, the particles move with an average drift velocity, V_s towards the electrode of proportional to the electric field

$$V_s = \mu_e E \quad \text{Equation 3-1}$$

Experimentally the velocity of the particle is measured and then the mobility is obtained from the measured velocity. Since there is no independent method for determining, so in microelectrophoresis the zeta potential is always calculated from a

measured mobility. The relationship between zeta potential and mobility depends on the theoretical model chosen. There is neither a method of calculating the zeta potential independent of the mobility measurement nor a standard substance with a certified zeta potential. There are two classic models that result in two classic limits: the Smoluchowski and the Hückel equations. They have a common root as the magnitude of the dimensionless product κa , where a is the radius of the kinetic unit.

$$\text{Hückel Limit } \mu_e = (2\varepsilon\xi) / (3\eta) \text{ for } \kappa a \ll 1 \quad \text{Equation 3-2}$$

μ_e is the particle mobility, η is the viscosity of suspending liquid, ε is the permittivity of liquid and ξ is the zeta potential.

Figure 3-2 Small particles with relatively short double layer when $\kappa a \ll 1$

In the opposite limit, κa is very large,

$$\text{Smoluchowski Limit } \mu_e = \varepsilon\xi/\eta \text{ for } \kappa a \gg 1 \quad \text{Equation 3-3}$$

For most colloidal particles of interest dispersed in water or highly polar liquids, it is neither possible to satisfy the Hückel limit nor easy to completely satisfy the Smoluchowski limit. In general, mobility is related to zeta potential by the following equations:

$F(\kappa a, \xi)$ is a model-dependent function. In the simplest Henry model, for 1:1 electrolytes,

$$f(\kappa a, \xi) = f(\kappa a) = 3/2 - 4.5/(\kappa a) + 37.5/(\kappa a)^2 - 330/(\kappa a)^3 \quad \kappa a > 1 \quad \text{Equation 3-4}$$

Henry's equations are strictly valid only for $\xi \ll 25.7 \text{ mV}$.

Since for typical colloids and salt concentrations, it is easier to arrange $\kappa a > 1$ than for $\kappa a < 1$, therefore, the Smoluchowski limit is more suitable than the Hückel limit for measurements of colloids. So in the measurement of zeta potential, the default calculation employs the Smoluchowski limit. In the practical measurement, we apply the photoelectron spectroscopy for zeta potential test. It is ideal for small particles in dilute solution and nearly monodispersity system. In the testing, charged particles in a dilute suspension move between two oppositely charged electrodes. Due to the motion of the charged particles a frequency shift, called the Doppler shift, ω_s , occurs in light scattered by the particles moving with velocity, V_s in an electric field, E is given by the dot product of vectors:

$$\omega_s = q \bullet V_s = q V_s \cos \phi \quad \text{Equation 3-5}$$

$$q = \left(\frac{4\pi n}{\lambda_o} \right) \sin(\theta / 2) \quad \text{Equation 3-6}$$

$$V_s = \mu_e E \quad \text{Equation 3-7}$$

ϕ is the angle between the vectors, q is the magnitude of scattering wave vector, n is the refractive index of the liquid and λ_o is the wavelength of the laser in vacuum.

The zeta potential of the micelle-like nanoparticles (preparation method related in sec 3.2.3) was tested with the Zetaplus (Brookhaven instruments) with platinum electrodes at 25 degree. The laser is a 15 mW solid state laser with the wavelength of 675 nm. The medium for test is pH 7.4 PBS with $I = 0.01 \text{ M}$ (sigma). 0.5 ml micelle suspension was

mixed with 2.5ml PBS medium and then transferred to the testing cuvette. Each measurement represents the mean of three observations.

3.2.8. In *vitro* degradation of block copolymer in forms of film and nanoparticle

Polymer films cast from 5% (w/v) DCM solutions on Teflon[®] plates were allowed to dry for 72 hours. Residual solvents were then removed in vacuum at room temperature for 2 days. Films were cut into 2*2 cm slabs with a thickness of around 50 μm . The samples were totally immersed in 30 ml of isotonic phosphate buffered saline solution (PBS, pH 7.4) in sealed bottle and equilibrated in a water bath at 37 $^{\circ}\text{C}$. At certain time intervals, samples were withdrawn and dried in vacuum until constant weight was reached. Changes of the molecular weights (GPC), film morphology (FESEM), thermal properties (MDSC) and chemical structure (NMR) were investigated. For selected copolymers, micelle-like nanoparticles were also degraded for comparison with films.

3.2.9. In *vitro* drug release from nanoparticles (5-FU)

Copolymer 10mg and 5-FU with certain ratios (10:1, 10:2 and 10:5) were both dissolved in 1ml DMF. This solution was added dropwise into 15 ml of deionized water under moderate magnetic stirring (similar procedure as nanoparticles preparation mentioned in sec 3.2.3). After dialysis procedure to remove DMF (against 1L water), the suspension was centrifuged to remove the supernatant. The medium removed from the dialysis and centrifuge was carefully collected and the free drug amount was obtained from the concentration of the 5-FU in the medium. Thus the drug entrapped inside the nanoparticles was calculated as the subtract the free drug in the medium from the total loaded drug. 1 ml PBS was added into the remaining residue and the colloidal solution was redispersed and placed into dialysis membrane again. The membrane was placed in

bottles with 10 ml PBS (pH = 7.4) in an incubator at 37 °C. At a fixed time interval, the release medium was replaced by 10 ml fresh PBS. The concentration of released 5-FU was determined by its absorbance at 266 nm (see Figure 3-3). The cumulative released amount of 5-FU was calculated by the concentration and volume of the solution medium according to the calibration curve. The experiment data was obtained from the average of three parallel tests.

Figure 3-3 UV spectrum of 5-FU in PBS

3.2.10. *In vitro* drug release from nanoparticles (Paclitaxel)

PLA/PEG block copolymer and paclitaxel with certain ratio (Polymer/drug= 100:1, 50:1, 20:1, respectively) were dissolved in 1ml DMF. After the polymer thoroughly dissolved in DMF, the clear solution was dropwisely added into 10ml deionized water with mild magnetic stirring. After about half hour stirring, the colloidal solution was subjected to the centrifugation at (50000g X 2hr, 4°C) to remove the supernatant containing with free drug and organic solvent. The residue was redispersed by adding to 4 ml deionized water and divided into four equal portions. 1ml solution was added into a centrifuge tube containing 9 ml PBS (pH=7.4) and put into 37 °C water bath. The release

medium was replaced at certain time interval and was under HPLC test after extraction with DCM to get the amount of the drug release.

Reverse-phase HPLC was used to quantify the amount of paclitaxel released from the copolymer micelle. The chromatographic analysis was performed on an Agilent 1100 Series instrument equipped with a VW detector and a ZORBAX 300SB-C18 column. The operating conditions were as follows: sample volume 20 μ l, mobile phase flow-rate 1.0 ml/min, $\lambda = 227$ nm. 50 / 50 v/v acetonitrile / water were used as mobile phase. Calibration curve was run with paclitaxel / acetonitrile solution with different paclitaxel concentrations. (As shown in Figure 3-4)

Figure 3-4 (a) Typical HPLC graph of paclitaxel in PBS and (b) Calibration curve of Paclitaxel

Chapter 4

4. Results and discussion

The main focus of this study was the evaluation of PLA/PEG block copolymers as drug delivery carrier for anticancer drugs. These amphiphilic block copolymers can self-assemble in the aqueous solution into micellar nanoaggregates. Due to its “Stealth” properties, it was a good candidate for anti-cancer drug carrier, which can deliver the drug to the target sites without being taken up by the MPS system.

In our research, we tried to answer the following questions

1. Can we synthesize a series of block copolymers to fulfill some of the requirements of drug carriers such as surface hydrophilicity and reasonable (2-3 weeks) periods of degradation?
2. What are the major parameters that affect the particle size, stability and other properties of the micellar nanoparticles?
3. What is the surface property of these micellar nanoparticles?
4. What is the degradation behavior of those copolymers in form of films and micelle-like nanoparticles?
5. What kinds of drugs can these micelles carry? What is the release profile of these drugs? How do the branched star-shaped copolymers compare to the linear ones?
6. What micelle properties are relevant for “stealth” characteristics (i.e, avoiding the reticulo-endothelial system) and what properties are useful for controlling drug release? Can some of these block copolymers exhibit both properties?

4.1 Synthesis of tri, di and star-shaped block copolymers

A series of PLA-PEG-PLA, PLA-mPEG and PLA-sPEO block copolymers have been synthesized by ring opening polymerization of PEG (mostly, we use PEG or PEO of different molecular weight (conventionally, the term PEG is used for low molecular weight and PEO for high molecular weight polymers of the same repeat structure) and L-lactide. (Here we shall use the letter “m” to describe linear diblock copolymers, and the letter “s” to denote 4-armed star-branched copolymers). Different LA/EG or LA/EO feeding ratios were applied. (See Table 4-1) Stannous octoate was used as the catalyst with concentration at 0.05% wt. The lactide was recrystallized at least three times to remove the residual impurities using the ethylacetate as solvent and the PEG was heated at 120°C for at least 5hrs under vacuum to remove the residue water. The purity of the monomer has great influence on the polymerization. Only low molecular weight copolymer could be obtained when the purity of the monomer was not high enough. The reaction lasted 24 hours at 110°C with the reflux of toluene. The resulting copolymer was precipitated in cold ethyl acetate and ethanol to remove the unreacted PEG and L-lactide. The absence of detectable homopolymer and monomer demonstrate the completeness of this polymerization reaction.

Compared to the synthesis of linear block copolymer, the star-shaped copolymer will be more difficult to obtain because of the special topology of star-shaped PEO. Li et al. first used $\text{Al}(\text{Et})_3$ as the catalyst to get this star-shaped copolymer^[26], which was a very active catalyst and very flammable. Choi et al. used stannous octoate as catalyst to prepare the star-shaped copolymer^[37]. The reaction was conducted in bulk polymerization at 130 °C for 10hr. They obtained good conversion ratio (over 95%).

However, as we know, the disadvantage of bulk polymerization is the difficulty for heat transfer and diffusion. So it is not suitable for large-scale product preparation. Hence in this study, we used the stannous octoate as catalyst and solution polymerization was used to get the star-shaped copolymers. Toluene was used as solvent and the reaction was kept at the boiling temperature of toluene for 12 hours. Although our conversion ratio is not as high as Choi's, it is an alternative way to get the star-shaped copolymer in a large batch. Another advantage of this polymerization method is that a copolymer with relative low polydispersity copolymer is obtainable. As to Choi's result, we find that their four-arm products have polydispersity near 2 (2.07). This broad distribution may be disadvantageous to applications involving control of drug release via biodegradation. While applying the solution method, we can get the products with narrower molecular weight distribution. This would enable us to use them for biomedical applications.

Figure 4-1 Synthesis route of (a) triblock, (b) diblock and (c) star-shaped copolymer

Table 4-1 PLA/PEG block copolymers obtained from polymerization of L-lactide

Copolymer	$^1\text{H-NMR}$		GPC			
	LA/EG		DP_{PLA}	M_n	M_n^b	M_w / M_n
	feed	product				
PLA ₁₇ -PEG ₉₁ -PLA ₁₇	0.50	0.37	17	6480	7940	1.07
PLA ₆₁ -PEG ₉₁ -PLA ₆₁	1.52	1.34	61	12710	13280	1.10
PLA ₆₈ -PEG ₉₁ -PLA ₆₈	2.09	1.47	68	13710	14680	1.11
PLA ₉₀ -PEG ₉₁ -PLA ₉₀	2.49	1.98	90	17940	17310	1.12
PLA ₄₁ -PEG ₄₅ -PLA ₄₁	2.05	1.82	41	7950	10520	1.11
PLA ₉₇ -PEG ₄₅ -PLA ₉₇	4.52	4.26	97	15940	20900	1.19
PLA ₆₂ -PEG ₁₈₂ -PLA ₆₂	0.95	0.68	62	16900	15690	1.22
PLA ₅₀ -mPEG ₁₁₄	0.5	0.44	50	8600	8360	1.16
PLA ₁₂₁ -mPEG ₁₁₄	1.0	1.10	121	13750	10100	1.15
PLA ₁₅₂ -mPEG ₁₁₄	1.5	1.30	152	15960	13830	1.20
PLA ₂₀₂ -mPEG ₁₁₄	2.0	1.90	202	19640	16900	1.25
PLA ₃₄₁ -mPEG ₁₁₄	3.0	3.00	341	29540	25500	1.25
PLA ₅₄ -mPEG ₄₅	1.0	1.20	54	5930	6980	1.16
PLA ₁₅₉ -mPEG ₄₅	3.0	3.50	159	13460	12350	1.14
PLA _{35*4} -sPEO _{56*4}	1.0	0.60	35	16760	14500	1.19
PLA _{74*4} -sPEO _{56*4}	2.0	1.30	74	25800	17000	1.36
PLA _{203*4} -sPEO _{56*4}	4.0	3.60	203	57600	33660	1.26

^aLA/EG in product and DP_{PLA} are determined from the integration ratio of resonance due to PEG blocks at 3.64ppm (-O-CH₂-CH₂-) and to the PLA blocks at 5.17 ppm (Me-CH*-) in the $^1\text{H-NMR}$., ^b based on polystyrene standards

From Table 4-1, it can be seen that with the increase of the LA/EG in feed ratio, the molecular weight of the copolymers increased as expected. It can also be seen that the molecular weight obtained from H-NMR calculation was similar to that obtained from GPC for linear block copolymer. However, for star-shaped copolymer, these values do not agree as well. This could be due to the inapplicability of the polystyrene-based calibration to the GPC analysis of star-shaped or highly-branched polymers. So static light scattering (SLS) was used to obtain the absolute molecular weight of these star-shaped copolymers

4.2 Characterization of copolymer

4.2.1. Copolymer molecular structure characterization

Infrared spectroscopy (IR) is one of the convenient ways to characterize the polymer structure. They are sensitive to the molecular environment, chain conformations and morphology and so afford a useful method for polymer analysis. When used either alone or in combination with other physicochemical techniques, IR spectroscopy is capable of providing detailed information on polymer structure.

The IR spectra of the different composition block copolymers such as PLA-PEG-PLA, PLA-mPEG and PLA-sPEO star-shaped copolymer are presented in Figure 4-2 to Figure 4-4. The LA carbonyl peak was at 1757 cm^{-1} . The EG-related C-H stretching band is centered at 2884 cm^{-1} . The IR bands observed from 1427 to 1573 cm^{-1} originated in the methylene groups in the PEG segments. Further information can be obtained by exploring in more detail the 1000 - 700 cm^{-1} spectral region, by comparing the 755 cm^{-1} (C) and 871 cm^{-1} (A) peaks, which were assigned to crystalline and amorphous respectively and the peak of 950 cm^{-1} assigned to the EG group.

As we know, from the famous Beer-Lambert Law, the absorbance is proportional to the concentration and the cell thickness,

$$A = \varepsilon CL \quad \text{Equation 4-1}$$

C is the concentration, L is the cell thickness and ε is the frequency dependent extinction coefficient. Some researchers do use this law to calculate the LA/EG ratio in the copolymer, since we know the characteristic peaks at 1760cm^{-1} and 950cm^{-1} related to LA and EG respectively.^[11] However, this is only a rough calculation, due to the difficulty of obtaining the accurate result from the deconvolution of characteristic peaks in the FTIR spectrum. More accurate LA/EG data can be obtained from the H-NMR spectrum

Figure 4-2 FTIR spectra of PLA-PEG-PLA triblock copolymers

The question of whether the materials obtained were copolymers with the two components alternating along the chain and covalently bound together, or mere blends where the two homopolymers are physically mixed, was thoroughly investigated. Physical mixtures of PLA and PEG were prepared at room temperature, by dissolving and casting it from 20 wt% chloroform solution. Mixing by mutual dissolution was preferred to melt blending because of the danger that some reactions could take place when the mixture was heated to high temperature. The blend was characterized before and after the extraction procedure, which typically consists of ethanol extraction at room temperature.

IR spectra of a PEG4000/ PLA blend, before and after extraction in ethanol (Figure 4-5), showed the almost complete disappearance of the PEG characteristic adsorption bands (2884cm^{-1} , 951cm^{-1}) after the ethanol extraction. Furthermore, the spectrum of the remaining polymer is very much like the spectra of PLA. These findings strongly indicate that when there is no covalent bond connecting the PEG and PLA chains, both components of the system can be easily separated by a rather mild, selective solubilization procedure.

Figure 4-5 Changes in FTIR spectra of the polymer blend extracted from ethanol

From this result, it can also be concluded that there is covalent bond in the copolymer PLA-PEG-PLA. Although the copolymer was extracted twice from ethanol, the PEG related peak could clearly be seen, which means that the PEG has already been chemically connected to the PLA segments.

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most useful instrumental techniques for characterizing polymeric materials, much information such as chain structure of polymer and copolymer, molecular motions and transitions in the solid state can be determined. Being more sensitive than FTIR, the ^1H -NMR can also be used to determine the accurate composition ratio of the copolymer. The LA/EG or LA/EO ratios can be obtained from the integration ratio of resonances arising from protons due to PEG (PEO) blocks ($-\text{O}-\text{CH}_2-\text{CH}_2-$) and PLA blocks (CH_3) and ($-\text{CH}$). Therefore, it can also be used to determine the number average molecular weight of the copolymer. All the LA/EG (EO) ratios and the molecular weights calculated from ^1H -NMR were tabulated in Table 4-1. In these ^1H -NMR and ^{13}C -NMR results for linear block copolymers, no detectable homopolymers can be found from the spectrum. Typical ^1H -NMR spectra for triblock and diblock copolymer are shown in Figure 4-6 to Figure 4-8 respectively, which are very similar. The presence of methine (CH) and methyl (CH_3) protons in PLA was observed at around 5.18 ppm (c) and 1.56 ppm (a) respectively. Tetralet and doublet split peaks were observed. The methene protons in CH_2 group of PEG were around 3.63 ppm. The ^{13}C -NMR spectra of the triblock copolymers are presented as Figure 4-7. The presence of the $\text{C}=\text{O}$, CH and CH_3 groups in the PLA block was detected at 170.01(d), 69.44(c) and 17.05(a) ppm in the ^{13}C -NMR spectra, respectively. It further verifies the success of target copolymer synthesis.

Figure 4-6 ^1H -NMR spectrum (CDCl₃) of the copolymer

Figure 4-7 ^{13}C -NMR spectrum (CDCl₃) of the triblock copolymer

Figure 4-8 $^1\text{H-NMR}$ spectrum of diblock copolymer

The NMR spectrum of branched block copolymer is shown in Figure 4-9, which is also similar to that of the linear block copolymer. Further evidence for the molecular architectures of star-block copolymers is supported by the analysis of the $^1\text{H-NMR}$ spectra of the 4-arm star-shaped block copolymer PLA-sPEO. The chemical shifts of CH_3 and CH of the polylactide block main chain were found at 1.53 and 5.16 ppm, respectively and the methylene of PEO main chain was at 3.63 ppm. With the enlarged $^1\text{H-NMR}$ spectrum of star-block copolymer, the α -methylene protons of PLA-connecting EO units (PLA-COO-CH_2) appeared at 4.35 ppm (a), while the β -methylene groups remained unchanged at 3.82 ppm (b). This shows the existence of the esterification reaction and that all the hydroxyl end groups of 4-arm PEO are esterified by L-lactide. There were no signals found at 4.0, or 4.9-5.0 ppm, which are the characteristic peaks for

carboxyl end groups of lactic acid and free lactic acid methane protons. This shows that there is no homopolymerization of the L-lactide as side reaction.

a b

Figure 4-9 ^1H -NMR spectrum of star-shaped copolymer

4.2.2. Determination of molecular weight of the copolymers

The molecular weights of the copolymers were mostly determined by the GPC. The data are shown in Table 4-1. From the GPC data, we find that using ring opening polymerization in solution; we can get narrow polydispersity for both linear and block copolymers.

The gel permeation chromatography (GPC) (or called size exclusion chromatography (SEC)) is one of the most useful methods for routine determination of average molecular masses and molecular mass distributions of polymers. Separation of the molecules occurs by preferential penetration of the different sized molecules into the pores; small molecules are able to permeate more easily through the pores compared to the larger sized molecules so that their rate of passage through the column is slower.

However, there is also a drawback of the GPC test, especially for the block or graft polymer, as the data obtained from GPC is relative. It was calibrated with linear poly (styrene) standards. The assumption that the $[\eta]M$ vs M relationship is similar to that of polystyrene may not hold for block and star-branched polymers. The three-dimensional architecture of star-shaped copolymers, in particular, can yield smaller hydrodynamic volumes in solution and cause a underestimation of absolute molecular weights^[199]. Due to this limitation, GPC may not yield accurate MWs for the block copolymers. As indicated in Table 4-1, for star-shaped copolymers, there is a difference between experimental data obtained from GPC and calculated from $^1\text{H-NMR}$. So we used static light scattering method to get the absolute molecular weights of the branched star-shaped copolymers.

If the value of dn/dc (differential refractive index increment) is known, the light scattering method could provide an absolute molecular weight. In the limit of low concentration and scattering angle the data obtained from SLS measurements for a dilute solution homopolymer in a single solvent can be written as:

$$Kc/\Delta R(\theta, c) = \frac{1}{M_w} \left(1 + \frac{R_g^2 q^2}{3} \right) + 2A_2c$$

$$Kc/\Delta R(\theta, c \rightarrow 0) = \frac{1}{M_w} + \frac{R_g^2 q^2}{3M_w} ; \quad Kc/\Delta R(\theta \rightarrow 0, c) = \frac{1}{M_w} + 2A_2c ;$$

$$K = 4\pi^2 n_o^2 (dn/dc)^2 / N_{avo} \lambda_o^4 ; \quad q = [4\pi n_o \sin(\theta/2)] / \lambda_o$$

where n_o is the refractive index of the solvent; dn/dc is the specific refractive index increment of the solution; c is the polymer concentration; N_o is the Avogadro's number; λ_o is the wavelength in vacuum of the laser; θ is the scattering angle in degrees, ΔR is the excess Rayleigh ration, M_w is the weight-average molecular weight of polymer ; $P_z(q)$ is the z-average particle scattering function or structure function; q is the magnitude of the scattering wave vector and A_2 is the second virial coefficient. Usually we use the Zimm plot to get the molecular weight.

Radius of gyration (R_g) is a physical property depending only on molecular architecture and molecular weight, following the equation $R_g = A * M_w^\alpha$, in which A is a constant and α is correlated to the polymeric structure. Stiff macromolecular chains show a more rod-like structure, leading to large radii of gyration and α value. A random coil like structure, leads to smaller R_g as well as α , but still larger than the values of molecules with a spherical structure in solution

Block copolymers are different from homopolymers, when the molar mass of the macromolecule and the conformational properties are considered. The refractive index increment may be different for different blocks, and the spatial conformation of each block in solution may also differ. Thus, with copolymers only an apparent weight average M_{LS} can be determined. However, with suitable choice of the solvent the difference between the apparent M_{LS} and true weight average molar mass M_w can be minimized. The difference between M_w and M_{LS} in the two values results from the optical contrast of the monomeric units over the solvent as determined by the square of refractive index increment $(dn/dc)^2$. As shown by Benoit ^[200],

$$\frac{dn}{dc} = m_A \left(\frac{\partial n}{\partial c} \right)_A + m_B \left(\frac{\partial n}{\partial c} \right)_B$$

$(\partial n/\partial c)_A$ refers to the refractive index increment of monomer A and the other to monomer B; m_A and m_B stand for the mass fractions.

In our experiment, THF was chosen as the solvent as it is a good one for both blocks. The molecular data and dn/dc of the copolymer are tabulated in Table 4-2. The data we obtained from light scattering are close to the GPC data. Thus, M_w values obtained by either method are usable for further comparisons; the M_n data from NMR may be suspect, for sensitivity reasons. This must necessarily imply that, the block copolymers exhibit an $[\eta].M$ vs. M relationship similar to that of polystyrene. We also found, compared to the linear counterparts, the star-shaped copolymer has smaller radii of gyration, which is a further evidence for their star-shaped architecture. With the increase of PLA chain length of the star-shaped copolymer, the radius of gyration also increased as expected.

Figure 4-10 A typical zimm plot to get the weight average molecular weight of block copolymer

Table 4-2 Light scattering data of the molecular weight compared to the GPC and NMR

copolymer	LA/EG		HNMR M_n	GPC		SLS		
	feed	product ^a		M_n^b	M_w	M_w	dn/dc	R_g
PLA ₉₇ - PEG ₄₅ -PLA ₉₇	4.5	4.3	15940	22898	24813	25160	0.0477	20.4
PLA _{74*4} - sPEO _{56*4}	2.0	1.30	25800	17000	23815	24800	0.0512	9.9
PLA _{203*4} - sPEO _{56*4}	4.0	3.60	57600	33660	42410	42800	0.0580	17.4

a: obtained from H-NMR calculation; b obtained from GPC result

4.2.3. Thermal Properties of copolymers

Modulated DSC is a technique that measures the difference in heat flow between a sample and an inert reference as a function of time and temperature. For a simple first order thermodynamic transition, the form of the curve, its magnitude and location are the same for both cooling and heating cycles. The system is therefore thermodynamically reversible. However, for a number of processes such as the glass transition and polymerization of a thermoset resin the heat capacity can be thermodynamically irreversible. The glass transition is normally considered to be a uniquely defined temperature; however, in practice the precise value does reflect the effects of disorder which may be frozen as a consequence of rapid cooling of the sample. The effect of irreversible processes on the measured DSC trace is that the first measured curve is often different from the second and subsequent traces obtained by cooling and re-heating the sample. The modulated technique has been devised to allow separation of reversible and irreversible components to the total measured heat capacity. Superposition of an oscillatory heating and cooling cycle on the linear ramp allows separation of these components. The sample is heated to a temperature T_1' that is above the linear ramp value T_1 and then cooled to T_1'' which is below the linear ramp value. The period of the oscillation is adjusted to increase the sensitivity of the experiment

The net effect of imposing this more complex heating profile on the sample is the same as if two experiments were run simultaneously on the material, one experiment at the traditional linear (average) heating rate and one at a sinusoidal (instantaneous) heating rate. The general equation that describes the resultant heat flow at any point in a DSC or MDSC experiment is:

$$\frac{dQ}{dt} = C_p \beta + f(T, t)$$

dQ/dt is the total heat flow, C_p is the heat capacity, β is the heating rate and $f(T, t)$ is the heat flow from kinetic (absolute temperature and time-dependent) processes. The total heat flow (dQ/dt), which is the only heat flow measured by conventional DSC, is composed of two components. One component is a function of the sample's heat capacity and rate of temperature change, and the other is a function of absolute temperature and time. These components are generally referred to as the “reversing” and “nonreversing” heat flows respectively. Fourier Transformation analysis can be used to separate the resulting raw experimental heat flow into the heat capacity related (reversible) and kinetic (non-reversible) heat flows. Typical reversing events are glass transitions and crystalline melting and examples of non-reversing events are cold crystallization, evaporation, thermoset cure and decomposition.

In Figure 4-11, there is a typical MDSC graph for block copolymer. As we know for most block copolymers, if the two components of the block copolymer are immiscible and crystallizable, there might be two melting peaks. In contrast, if they are miscible components, there would be only one melting peak. The T_m of PEG homopolymer is about 60 °C for PEG 4k and 8k and 50 °C for PEG 2k, while T_m of PLA homopolymer is about 178 °C. Since the PLA and PEG are both crystallisable and immiscible, we find two melting peaks, indicating good phase separation.

Figure 4-11 Typical DSC thermograms for PLA/PEG block copolymers

Thermal characteristics of $\text{PLA}_x\text{-PEG}_y\text{PLA}_x$ triblock copolymers were investigated by MDSC. Homopolymer PLLA (I. V. =1.04) and PEG 2k, 4k, 8k were also studied for comparison. Thermograms data are shown in Figure 4-13 and Table 4-3. The copolymer shows two distinct melting peaks for the two different chain segments of PLA and PEG, wherever phase separation is extensive.

The presence of the PLA sequences attached to PEG blocks decreased the melting temperature of both the PEG block and PLA block. It was also found that with a relative short PEG chain ($\text{PLA}_{41}\text{-PEG}_{45}\text{-PLA}_{41}$, $\text{PLA}_{97}\text{-PEG}_{45}\text{-PLA}_{91}$), the PEG chain did not crystallize, which agrees with the results of Li's [201, 202]. So it might imply that the short PEG chain could cause insufficient phase separation which leads to the elimination of the endothermic peak of the soft block. However, for triblock copolymer with long PEG chain ($\text{PLA}_x\text{-PEG}_{91}\text{-PLA}_x$), it was found that with the increase of PLA chain length, the PEG endothermic peaks change accordingly. Even when the PEG chain is long enough, the melting peak of PEG block disappeared when the EG ratio lower than ~40% in the copolymer. We also found that during the cooling run, there was no exothermal peak for

PEG crystalline (Figure 4-14b). So it might imply that not only the PEG chain length but also the PEG composition affect the crystallization of PEG chain; higher PLA compositions in the copolymer lead to the incomplete PEG phase separation, presumably due to inter-chain or intra-chain entanglements that preclude closeness of approach of the PEG segments. It has been reported that the PLA segments have higher tendency in retaining their crystalline array than the PEO segments.^[11] The PLA hard segments are the first to solidify upon cooling, hindering the mobility of soft PEO segments. As a result, the crystallization of the PEO segments is hampered, especially in a block copolymer with small content of PEO.

As to the melting point of PLA blocks, it was found that for copolymer PLA₁₇-PEG₉₁-PLA₁₇, no melting peak existed for the PLA block, as its PLA chain was too short. So for same PEG₉₁ chain length, with the increase of the PLA chain length, the melting peak of PLA block became more obvious and the T_m and DOC (degree of crystallinity) also increased correspondingly. Therefore, DSC thermograms of PLA_x-PEG-PLA_x triblock copolymers reflected an increasing crystallizability of the PLA blocks with increasing chain length. Interestingly, it was found in the second heating run, there was some difference for the PLA melting peak. For fast cooled samples, the thermograph of the second heating run was much similar to first run (except the disappearance of the cold crystalline peak). However, for the slower-cooled sample (3°C/min), we found a small melting peak of PLA (shoulder peak), before the onset of the main endothermal peak. This small melting peak might be attributed to the PLA crystalline region being interpenetrated by the PEG chain. If cooled fast enough, this peak disappears. This peak may be due to another crystalline phase.

Another direct way to study the crystalline behaviour of this copolymer is using polarizing microscopy. The triblock copolymers were also heated over its melting point and cooled down. The whole procedure was observed by the polarizing microscopy (Nikon Labophot 2-POL). It was found that when the copolymer was heated over the melting temperature, and cooled down, structures with Maltese Cross were easily found, indicating spherulite formation for this block copolymer.

Figure 4-12 Polarized-light microscopy photo of the crystalline of PLA/PEG block copolymer

It should be noted that PLA has a T_g just at about 50 °C. It is known that a block copolymer of two immiscible segments exhibit two T_g 's (each for one block), regardless of the composition. In contrast, only one glass transition would be observed in the case of two miscible segments. However, in this case, as pointed out in the literature ^[13, 203], it would be difficult to evaluate the miscibility of PLA and PEG or of the sequences within PLA/PEG block copolymers from T_g measurements, because T_g of PLA and T_m of PEG

overlap. The T_g of the PEG block is around 0 °C, which shows little affect from the PLA chain length

Table 4-3 Thermal property of triblock copolymers with different PLA/PEG chain length

<i>Sample</i>	<i>LA/EG ratio</i>	<i>T_m (PEG)</i> (°C)	<i>T_m (PLA)</i> (°C)	<i>ΔH_m</i> (J/g)	<i>T_c (PLA)</i> (°C)	<i>ΔH_c</i> (J/g)	<i>DOC</i> (%)	<i>T_g</i> (°C)
PLLA	-		177.5					54
PLA ₁₇ -PEG ₉₁ - PLA ₁₇	0.4	37.3	-	-	-	-	-	-
PLA ₆₁ -PEG ₉₁ - PLA ₆₁	1.3	42.5	141.4	34.9	-	-	37.3	-3
PLA ₆₇ -PEG ₉₁ - PLA ₆₇	1.5	-	148.7	45.7	95.7	11.1	37.0	-1
PLA ₉₀ -PEG ₉₁ - PLA ₉₀	2.0	-	153.8	47.9	102.2	8.3	42.3	-1
PLA ₄₁ -PEG ₄₅ - PLA ₄₁	1.8	-	146.5	43.4	93.6	1.7	46.3	0
PLA ₉₇ -PEG ₄₅ - PLA ₉₇	4.3	-	162.3	61.1	108.3	11.5	53.4	-3
PLA ₆₂ -PEG ₁₈₂ - PLA ₆₂	0.7	48.5	150.3	23.6	88.6	5.8	19	-1
PEG (2000)		50.4						-
PEG (4000)		59.6						-1
PEG(6000)		60.4						
PEG (8000)		60.1						4

DOC = Degree of crystallinity of PLA block, rating it to the reference 100% crystalline L-PLA (93.6 J/g) ^[204]

Figure 4-13 DSC thermograms of PLA/PEG triblock copolymer with homopolymer PLLA (I.V=8.04, PEG 2000, 4000, 6000, 8000) and (b) cooling at 3°C/min

A series of diblock copolymer and star-shaped copolymer were also synthesized and their thermal properties measured, together with the homopolymer mPEG 5k, 2k and 4-arm star-shaped PEG 10k.

Similar to the results for triblock copolymers, the results show that, with the increase of PLA block content, the melting and crystallization temperatures of PLA also increased, accompanied by the increase of crystalline degree of PLA block. Compared to the triblock copolymer, the PEG block in the diblock copolymer PLA₅₅-mPEG₄₅ does exhibit a melting peak. Similar to the triblock copolymers, the diblocks and the star-branched copolymers also show a shoulder in the PLA melting peak, which was more pronounced in star-shaped copolymers than in diblock copolymers.

Some research groups have also studied the thermal transitions and crystalline structure of the diblock copolymer. Burt et al. showed that the diblock copolymer PLA-mPEG had thermal properties similar to the homopolymer mPEG, but different from the homopolymer PLLA [46,19]. However, Kubies' s result for triblock copolymer PLA-PEG-PLA is different [205]. They found that two major factors would affect the thermal property of the material, copolymer composition and stereo regularity. Our results show that the similarity of the thermal property of block copolymer to homopolymer is more dependent on the ratio of the two blocks. This means with the increase of LA composition in the block copolymer, the thermal property of block copolymer would be more similar to PLA homopolymer; similarly with PEG content.

The steric architecture of star-shaped copolymers leads to their lowered crystalline temperature (T_c), the degree of crystallinity (DOC) and melting temperature (T_m) compared to linear PLA/PEG block copolymer. For branched star-shaped copolymer, the

T_m , T_c and DOC are all lower than their linear counterparts with similar PLA chain length. Physico-chemical properties of all these polyesters are also summarized in

Table 4-4

Table 4-4 Thermal property of diblock and star-shaped copolymer

	product ratio	T_m (PLA) (°C)	T_m (PEG) (°C)	T_g (PEG) (°C)	T_c (°C)	H_c (J/g)	H_m (PLA) (J/g)	DOC
MPEG5k	-	-	62.4	-	-	-	-	-
PLA ₅₀ - mPEG ₁₁₄	0.44	N/A	52.9	-4	N/A	N/A	N/A	N/A
PLA ₁₂₁ - mPEG ₁₁₄	1.26	151.9	45.1	-3	90.9	3.2	41.46	0.41
PLA ₁₅₂ - mPEG ₁₁₄	1.34	158.0	43.8	n/a	99.1	3.2	46.24	0.46
PLA ₂₀₂ - mPEG ₁₁₄	1.79	159.0	56.9	-3	98.9	5.6	48.96	0.46
PLA ₃₄₁ - mPEG ₁₁₄	3.00	170.9	54.0	N/A	112.0	6.8	55.00	0.51
MPEG2K	-	-	51.4	-	-	-	-	-
PLA ₅₅ - mPEG ₄₅	1.20	147.2	30.4	6	93.2	3.5	35.95	0.35
PLA ₁₅₉ - mPEG ₄₅	3.50	160.6	N/A	3	105.4	6.6	60.07	0.57
S-PEO10k	-	-	57.8	-	-	-	-	-
PLA _{35x4} - SPEO _{56x4}	0.62	125.0	37.3	N/A	N/A	N/A	8.42	0.09
PLA _{74x4} - SPEO _{56x4}	1.29	153.4	56.9	N/A	99.3	12.7	33.50	0.22
PLA _{203x4} - SPEO _{56x4}	3.63	167.6	N/A	-6	111.8	13.0	51.36	0.41

DOC = Degree of crystallinity of PLA block, rating it to the reference 100% crystalline

L-PLA (93.6J/g) ^[204]

Diblock copolymer

Figure 4-15 DSC thermograms of PLA/PEG diblock copolymer with homopolymer mPEG 2k and mPEG 5k: a first heating run, b: cooling run (3°C/min)

Figure 4-16 DSC thermograms of PLA-mPEG diblock copolymers and mPEG 2000, 5000, a:(second run after fast cooling at 20°C/min) b : (second run after fast cooling at 3°C/min)

Star-shaped copolymer

Figure 4-17 DSC thermograms of PLA-sPEO star-shaped copolymers and sPEO 10,000 :

a (heating at 3°C/min) and b(cooling at 3°C/min)

Figure 4-18 DSC thermograms of PLA-sPEO star-shaped copolymers and sPEO 10,000

a:(second run after fast cooling at 20°C/min) b : (second run after fast cooling at 3°C/min)

Figure 4-19 Melting temperature and crystallization temperature of PLA block vs PLA block units with different PEG content and chain structure

Finally, we compared the melting temperature and crystallization temperatures of these three types of copolymers. From Figure 4-19, a lot of information could be obtained. First, it could be seen that with similar PLA block lengths in the block copolymer, the melting temperature of this branched copolymer was obviously lower than in the linear polymers. The chain structure of the star-shaped copolymer made the PLA block difficult to crystallise which leads to the low melting point. Both the linear diblock and triblock copolymer showed the same trend, the one with short PEG chain (PEG 2000, mPEG 2000) had higher melting temperature of the PLA block than the one attached to the long PEG chain (PEG 4000, mPEG 5000) with same length of PLA chain. So we can conclude that the shorter PEG chain has less effect on the PLA crystalline region than the longer PEG chain, namely the phase separation of the two blocks with shorter PEG chain is more perfect than longer PEG chain. The same trend was also seen from the T_c of the PLA block in Figure 4-19.

4.3 Micellization of block copolymer: preparation and characterization

4.3.1. Particle size and conformation of the micellar aggregates

4.3.1.1 Particle size study with DLS

The hydrodynamic diameter of particles is determined by dynamic light scattering (DLS). DLS measures the time-dependent intensity of scattering light, which gives information on hydrodynamic diameter of a particle, a transport property depending on the Brownian motion of particles coming from the frictional forces between particles and solvents. The Brownian motion is a function of the size of the particles, the viscosity of the fluid and temperature.

$$g^{(2)}(\tau) = 1 + \beta \left| g^{(1)}(\tau) \right|^2 \quad \tau \neq 0 \quad \text{Equation 4-2}$$

$g^{(2)}(\tau)$ is the normalized second-order correlation function, β is a parameter of the optical systems. $\beta = f(A) (\sim A_{\text{coh}} / A)$ is related to the number of spatial modes collected by the detector covering a photocathode surface A . Equation 1 forms the basis of the self-beating technique. $g^{(1)}(\tau)$ is the normalized first-order correlation function and τ is the delay time. For small monodispersed particles and homogeneous spheres of any size the normalized scattered electric field autocorrelation function, $g^{(1)}(\tau)$ can be expressed as the following equations:

$$g^{(1)}(\tau) = \exp(-\Gamma \tau) \quad \text{Equation 4-3}$$

$$\Gamma = q^2 D_T \quad \text{Equation 4-4}$$

$$q = (4\pi n / \lambda) \sin(\theta / 2) \quad \text{Equation 4-5}$$

Γ is the line width, D is the Z-averaged diffusion coefficient, n is the refractive index of the solvent, λ is the wave length and θ is the scattering angle. For a continuous polydisperse sample, $g^{(1)}(\tau)$ is described by the following decay rate distribution:

$$g^{(1)}(\tau) = \int_0^{\infty} G(\Gamma) \exp(-\Gamma \tau) d\Gamma \quad \text{Equation 4-6}$$

$G(\Gamma)$ is the G distribution function and can be evaluated by inverse Laplace transform techniques, as $g^{(1)}(t)$ is the Laplace transform of $G(\Gamma)$

By the cumulant expansion, Eq. 4-6 can be expanded as the following Eq. 4-7 using the average characteristic line width, $\bar{\Gamma}$,

$$\ln|g^{(1)}(\tau)| = -\bar{\Gamma}\tau + (\mu_2 / \tau^2) / 2! - (\mu_3 / \tau^3) / 3! + \dots \quad \text{Equation 4-7}$$

Where

$$\bar{\Gamma} = \int_0^{\infty} \Gamma G(\Gamma) d\Gamma, \quad \text{Equation 4-8}$$

$$\mu_i = \int_0^{\infty} (\Gamma - \bar{\Gamma})^i G(\Gamma) d\Gamma, \quad \text{Equation 4-9}$$

yielding polydispersity index, $\mu_2 / \bar{\Gamma}^2$. Also the hydrodynamic diameter, d_h , can be

obtained from $\bar{\Gamma}$ using Eq. 4-4 and the following Eq. 4-10

$$d_h = kT / 3\pi\eta D_T \text{ (Stokes- Einstein equation)} \quad \text{Equation 4-10}$$

k is the Boltzmann's constant, T is the absolute temperature and η is the viscosity index of the solvent, D_T is the translational diffusion constant and d_h is the hydrodynamic diameter.

In our experiments, what we obtained from the instrument is the effective diameter. This effective diameter is an average diameter defined as follows:

$$\bar{d}_{eff} = \sum n_i d_i^6 P_i / \sum n_i d_i^5 P_i \quad \text{Equation 4-11}$$

P_i is the single-particle scattering factor, for sufficiently small spherical particles.

From this equation, we can find that the effective diameter is very sensitive to large particles. The trace dust might affect the final results greatly.

The most common routine used to perform the inverse Laplace Transform (ILT) is CONTIN.

The effective diameter and polydispersity of particulate dispersions are presented in Table 4-5 to Table 4-7

Table 4-5 Hydrodynamic diameter and polydispersity of PLA-PEG-PLA micelles

	Particle size mean $\pm$ SD (nm)	Polydispersity (mean $\pm$ SD)
PLA ₆₁ -PEG ₉₁ -PLA ₆₁ (20mg/ml,in DMF)	63.8 $\pm$ 3.0	0.31 $\pm$ 0.03
PLA ₆₈ -PEG ₉₁ -PLA ₆₈ (20mg/ml,in DMF)	124.0 $\pm$ 0.5	0.21 $\pm$ 0.01
PLA ₉₀ -PEG ₉₁ -PLA ₉₀ (20mg/ml,in DMF)	167.7 $\pm$ 0.5	0.25 $\pm$ 0.01
PLA ₆₂ -PEG ₁₈₂ -PLA ₆₂ (20mg/ml,in DMF)	168.8 $\pm$ 1.3	0.26 $\pm$ 0.01
PLA ₄₁ -PEG ₄₅ -PLA ₄₁ (20mg/ml,in DMF)	136.3 $\pm$ 1.3	0.19 $\pm$ 0.08
PLA ₉₇ -PEG ₄₅ -PLA ₉₇ (20mg/ml,in DMF)	147.8 $\pm$ 0.3	0.20 $\pm$ 0.01

Table 4-6 Hydrodynamic diameter, and polydispersity of PLA-mPEG micelle like nanoparticles

	Particle size mean $\pm$ SD (nm)	Polydispersity (mean $\pm$ SD)
PLA ₅₀ -mPEG ₁₁₄ (20mg/ml,in DMF)	70 $\pm$ 8	1.12 $\pm$ 0.1
PLA ₁₂₁ -mPEG ₁₁₄ (20mg/ml,in DMF)	103 $\pm$ 3	0.32 $\pm$ 0.01
PLA ₁₅₂ -mPEG ₁₁₄ (20mg/ml,in DMF)	116 $\pm$ 0.3	0.12 $\pm$ 0.04
PLA ₂₀₂ -mPEG ₁₁₄ (20mg/ml,in DMF)	154 $\pm$ 1	0.19 $\pm$ 0.01
PLA ₃₄₁ -mPEG ₁₁₄ (20mg/ml,in DMF)	197 $\pm$ 1	0.02 $\pm$ 0.03
PLA ₅₄ -mPEG ₄₅ (20mg/ml,in DMF)	80 $\pm$ 1	0.21 $\pm$ 0.01
PLA ₁₅₉ -mPEG ₄₅ (20mg/ml,in DMF)	89 $\pm$ 1	0.14 $\pm$ 0.03
PLA ₁₅₂ -mPEG ₁₁₄ (5 mg/ml,in DMF)	59 $\pm$ 0.3	0.14 $\pm$ 0.03
PLA ₁₅₂ -mPEG ₁₁₄ (10 mg/ml,in DMF)	83 $\pm$ 0.2	0.15 $\pm$ 0.02
PLA ₁₅₂ -mPEG ₁₁₄ (20mg/ml,in DMF)	116 $\pm$ 0.3	0.12 $\pm$ 0.04
PLA ₁₅₂ -mPEG ₁₁₄ (30mg/ml,in DMF)	132 $\pm$ 2.1	0.31 $\pm$ 0.03
PLA ₁₅₂ -mPEG ₁₁₄ (20mg/ml,in DMF)	116 $\pm$ 0.3	0.12 $\pm$ 0.04
PLA ₁₅₂ -mPEG ₁₁₄ (20mg/ml,in DMAc)	174 $\pm$ 2.3	0.32 $\pm$ 0.01
PLA ₁₅₂ -mPEG ₁₁₄ (20mg/ml,in Acetone)	244 $\pm$ 17	0.36 $\pm$ 0.10
PLA ₁₅₂ -mPEG ₁₁₄ (20mg/ml,in THF)	269 $\pm$ 13	0.30 $\pm$ 0.02

Table 4-7 Hydrodynamic diameter and polydispersity of PLA-sPEO micelle like nanoparticles

	Particle size mean $\pm$ SD (nm)	Polydispersity (mean $\pm$ SD)
PLA _{35*4} -sPEO _{56*4} (20mg/ml,in DMF)	126 $\pm$ 1	0.19 $\pm$ 0.01
PLA _{74*4} -sPEO _{56*4} (20mg/ml,in DMF)	140 $\pm$ 1	0.21 $\pm$ 0.01
PLA _{203*4} -sPEO _{56*4} (20mg/ml,in DMF)	129 $\pm$ 1	0.21 $\pm$ 0.01
PLA _{74*4} -sPEO _{56*4} (20mg/ml,in DMF)	140 $\pm$ 1	0.21 $\pm$ 0.01
PLA _{74*4} -sPEO _{56*4} (30mg/ml,in DMF)	176 $\pm$ 2	0.26 $\pm$ 0.01
PLA _{74*4} -sPEO _{56*4} (40mg/ml,in DMF)	226 $\pm$ 1	0.26 $\pm$ 0.01
PLA _{74*4} -sPEO _{56*4} (20mg/ml,in DMF)	140 $\pm$ 1	0.21 $\pm$ 0.01
PLA _{74*4} -sPEO _{56*4} (20mg/ml,in DMAc)	145 $\pm$ 1	0.21 $\pm$ 0.02
PLA _{74*4} -sPEO _{56*4} (20mg/ml,in Acetone)	356 $\pm$ 7	0.11 $\pm$ 0.04
PLA _{74*4} -sPEO _{56*4} (20mg/ml,in THF)	551 $\pm$ 4	0.11 $\pm$ 0.01

4.3.1.2 Effect of Concentration and Solvent

The effects of the concentration and different organic solvents on particle size of triblock, diblock and star-shaped polymer were investigated. It was found that micellization from DMAc and DMF resulted in nanoparticles with smaller size than those from acetone and THF. This means that the miscibility of the organic solvent with water could affect the particle size of the micelle.

The concentration of the copolymer also affected the particle size of the nanoparticles. It was found that the particle size increased with the increase of the concentration of the copolymer. This could be due to micelle formation with larger aggregation number during the diffusion procedure.

The effect of the different PLA and PEG segments length on the particle sizes was also investigated. DMF was used as organic solvent and the concentration was kept at 20mg/ml for organic solution. It was found that for linear tri and diblock copolymer, with the increase of the PLA segments length, the particle sizes increased. However, it seemed to have less impact on star-shaped copolymers. With the increase of PLA block, the particle sizes of star-shaped copolymers changed very little. This is to be expected, as star-shaped polymers form a different type of micelle, compared to di- and tri-block copolymers

Figure 4-20 Possible structure of micelle from (a) diblock and (b) triblock copolymer

The possible micelle-aggregation forms of diblock and triblock copolymer are shown in Figure 4-20. For diblock copolymers, a power law-type equation can be used to express the dependence of hydrodynamic radius on the number of chain units in the core-forming (N_A) and shell-forming (N_B) blocks. There are two limiting cases, namely as “crew cut” or “star” micelle. In the large core limit, $N_A \gg N_B$, the volume fraction of B

segments in the shell is independent to the distance from the core. In this model, R_{hyd} can be scaled as:

$$R_{hyd} \propto N_A^{2/3}$$

On the contrary, when $N_B \gg N_A$, the micelle has a small core while a large shell.

Harperin has proposed the “star” model and obtained the following scaling relationship:

$$R_{hyd} \propto N_A^{4/25} N_B^{3/5} \text{ [206,207,208]}$$

In our research, the scaling exponent governing the increase in hydrodynamic radius with the length of the core-forming block was between these two extremes. (See Figure 4-21), but closer to 2/3, indicating a dominance of the core-former in determining micelle size.

However these relationships do not hold for star-shaped copolymers. Our result shows that there was little impact of core-forming block length on the hydrodynamic radius. Up to now, very little work has been reported relating to the micelle-like aggregates from star-shaped block copolymer. Since the unique steric architecture of the star-block copolymers makes it difficult to form the classical core shell structure, the power law is not applicable. The static light scattering was used to characterize the possible micellar aggregates form of star-shaped copolymer, together with the transmission electron microscopy.

Figure 4-21 Plot of hydrodynamic radius (R_{hyd}) versus $N_{PLA}^{0.58}$ for PLA_x -mPEG₁₁₄ ($50 \leq x \leq 341$) micelle-like aggregates

4.3.2. Structure and properties of micellar aggregates

4.3.2.1 Aggregation number

Knowledge of aggregation number can greatly help us to understand the structure of micelle aggregates. There are a range of methods, which can be used to determine the aggregation number. Among them two are the most widely used: static light scattering and fluorescent probes. In our experiment, we chose the static light scattering to measure the aggregation number of the micellar aggregates.

In the above text, we have discussed the use of static light scattering to get the weight average molecular weight of the copolymer. It can also be applied to the micellar system to get the aggregation number with a little modification:

$$k(C - CMC) / \Delta R_{\theta} = 1 / M_w + 2A_2(C - CMC) \quad \text{Equation 4-12}$$

$$K = 4\pi^2 n_o^2 (dn/dc)^2 / N_{avo} \lambda_o^4 \quad \text{Equation 4-13}$$

n_o is the refractive index of the solvent; dn/dc is the specific refractive index increment of the solution; C is the polymer concentration; N_{avo} is the avogadro's number; λ_o is the

wavelength in vacuum of the laser; θ is the scattering angle in degrees, ΔR is the excess Rayleigh ration, M_w is the weight-average molecular weight of polymer.

The reason for this modification is that, in micellar system, when c is below CMC, there would be a great change in the intensity of the light scattering. So (C-CMC) is used to replace C .

If the CMC is sufficiently low compared with the concentration of the concentration ranged studied, so that (C-CMC) is $\sim C$, the equation can be simplified to the following one

$$kC / \Delta R_{\theta} = 1 / M_w + 2A_2C \quad \text{Equation 4-14}$$

Table 4-8 Comparison of the micellar aggregation of different type of copolymer

copolymer	R_{hyd}	M_w (polymer)	M_w (micelle)	R_g	A_2	N_{agg}	R_g/R_h	Dn/dc
PLA _{35*4} -sPEO _{56*4}	42	18868 ^{GPC}	1.187e7	43	5.6e-5	629	1.02	0.122
PLA _{74*4} -sPEO _{56*4}	43	24800 ^{SLS}	2.195e7	36	1.5e-5	885	0.84	0.118
PLA _{203*4} -sPEO _{56*4}	36	42800 ^{SLS}	2.964e7	31	5.4e-6	693	0.86	0.113
PLA ₉₇ -PEG ₄₅ -PLA ₉₇	74	25160 ^{SLS}	6.05e8	110	4.2e-7	24046	1.48	0.108
PLA ₄₁ -PEG ₄₅ -PLA ₄₁	68	11704 ^{GPC}	2.353e8	79	1.2e-6	20104	1.16	0.111
PLA ₆₈ -PEG ₉₀ -PLA ₆₈	62	16296 ^{GPC}	1.39e8	117	6.8e-5	8230	1.89	0.118
PLA ₉₁ -PEG ₉₀ -PLA ₉₁	84	17655 ^{GPC}	3.960e8	114	1.8e-6	22430	1.36	0.119
PLA ₁₅₂ -mPEG ₁₁₄	58	16577 ^{GPC}	2.58e7	48	5.6e-5	1556	0.83	0.120
PLA ₃₄₁ -mPEG ₁₁₄	98	31800 ^{GPC}	7.69e7	73	4.9e-6	2418	0.74	0.113

The R_h here is obtained from the DLS measurement, from the angular dependence of the scattering at any concentration. With the help of the software, we can get the dilute diffusion coefficients for each concentration. For slightly concentrated systems, the measured and infinitely dilute diffusion coefficients are expressed as:

$$\overline{D_T} = \overline{D_0} * (1 + k * c + \dots) \quad \text{Equation 4-15}$$

So we can plot the D_T versus the concentration and get the infinitely dilute diffusion coefficients. With the value of D_0 , we can calculate the values for the $R_h (=d_h/2)$ with

$$d_h = kT / 3\pi\eta D_0 \quad \text{Equation 4-16}$$

Furthermore, the aggregation number (the number of molecules in each “aggregate” or micelle) can be calculated from the M_w measured at a given concentration ($C > \text{CMC}$) and the “infinite dilution” M_w obtained from the classical Zimm plot, as follows:

$$N_{agg} = M_w(\text{micelle}) / M_w(\text{polymer})$$

From the results tabulated in Table 4-8, it can be noted that there is no direct dependence of the PLA chain length to the aggregation number. However, it can be concluded that all the triblock copolymers have very large aggregation number while the star-shaped copolymers have relatively small aggregation numbers. Compared to their linear counterparts, the radius of gyration of star-shaped copolymer micelles are also smaller.

4.3.2.2 Conformation of micellar aggregates

As we know, the ratio of R_g/R_h is related to the conformation of the micelle (see Table 4-9). Therefore, with the R_g/R_h value we obtained, we might be able to better understand the morphology of the micelles.

First, we find that the triblock copolymer shows a larger R_g/R_h ratio (value > 1) than the other two types of copolymers. Coupled with the large N_{agg} values for the triblocks, we believe that the triblock copolymer forms a vesicle-type aggregate rather than a core-shell structure. This was verified with the AFM photographs (Figure 4-23). In these AFM graphs, we clearly find the aggregates are made from many small particles and these particles assembled to the rod like form.

For diblock and star-shaped copolymers, these two copolymers will form more spherical aggregates. The R_g/R_h for both of these two copolymer micellar aggregates are smaller than 1. This is also verified by the AFM graphs. For diblock copolymer micelle, we can see clearly the spherical particles.

Table 4-9 Conformation of micelle related to the ratio of R_g / R_h ^[209,210]

R_g/R_h	<0.6	~ 0.774	~ 1	$1.5\sim 2$	>2
Conformation	Core-shell	Hard sphere	Vesicle	Random coil	Long rod

Figure 4-22 AFM graph of diblock and triblock copolymer

Figure 4-23 AFM and FESEM graph of star-shaped copolymer micellar aggregates

Figure 4-24 Possible micellar structure for the star-shaped copolymer with vesicle structure

For star-shaped copolymer, from the SLS, TEM and AFM results, we infer that this star-shaped copolymer might have a vesicle structure. Xiong et al. have investigated the vesicle structure of the PLA modified Pluronic F127 ^[211], which is similar to the star-shaped copolymer. From the TEM photograph, we can clearly see the inner core of the hydrophobic PLA, and the grey ring of hydrophilic PEO. So when this copolymer is used as hydrophobic drug carrier, the drug might be dispersed in the inner PLA core and also the outer PLA ring.

4.3.3. Stability of micelle in dilute condition and salt-added condition

Critical micelle concentration (CMC), the critical flocculation point (CFPT) and cloud temperature (T_c) are important parameters to indicate the stability of the micelle.

4.3.3.1 Stability of micelle in dilute condition

The CMC is defined as the copolymer concentration when the micelles first appear. Below CMC all the copolymers molecules exist as a single chain; above CMC the concentration of single chains increases only very slightly while the majority of additional copolymer molecules assemble into micellar structure. Possible micelle dissociation upon dilution certainly has an important influence on the drug-delivering capacity of polymer micelles composed of amphiphilic block copolymers. A low CMC is favorable for drug retention in the micelle in vivo under considerable dilution. So it is necessary to determine the CMC for the micelle.

PLA/PEG block copolymers are amphiphilic in nature as water is a poor solvent for hydrophobic PLA block while it is a good solvent for hydrophilic PEG blocks. So with the increase of concentration, the block copolymer may form multi-molecular micelles and micelle aggregates.

The critical micelle concentration (CMC) of the copolymers was determined by fluorescence spectroscopy using pyrene as fluorescence probe. Upon formation of micelles, pyrene would move into the inside of the micelles from the aqueous phase which results in an alteration in the intensity ratio (I_1 / I_3) of pyrene fluorescence bands I (371 nm) and III (383 nm).

Plots of I_1/I_3 band intensity ratio of pyrene monomer as a function of PLA / PEG copolymer concentration are shown in Figure 4-25. Below certain concentration, the I_1/I_3 value was near constant. Above this concentration, this value decreased with increasing concentration, which indicated partitioning of this hydrophobic probe between the hydrophobic core of micelle and aqueous phase.

From the result, it can conclude that the CMC is very low which is favorable for drug delivery carriers. Since our micelle is non-ionic, it is reasonable as the CMC is so low, since the free energy cost of bringing the polar heads together is much smaller when the head group is uncharged than when charged. For small molecular surfactants, the CMC depends more strongly on the alkyl chain length. The head group repulsion varies substantially in magnitude between different uncharged surfactants.

However, when it comes to our micellar aggregates system, there is little difference among the CMC values for triblock copolymer. It was found that, with the increase of the PLA chain length, the CMC also increased slightly. This implies that the PLA chain length has little effect on the CMC in our research range. For the dib lock and star-shaped copolymers, the micelle-like aggregates behave in a manner similar to the small-molecule surfactant.

Figure 4-25 Determination of CMC of micelles of diblock copolymers

PLA₃₄₁-mPEG₁₁₄

Table 4-10 CMC of micellar aggregates prepared with different copolymers

Chain structure	Copolymer	LA length	EG length	CMC(mg/ml)
Triblock	PLA ₆₁ -PEG ₉₁ - PLA ₆₁	122	91	0.002
	PLA ₆₈ -PEG ₉₁ - PLA ₆₈	136	91	0.004
	PLA ₉₀ -PEG ₉₁ - PLA ₉₀	180	91	0.005
	PLA ₆₂ -PEG ₁₈₂ - PLA ₆₂	124	182	0.007
Diblock	PLA ₅₀ - mPEG ₁₁₄	50	114	0.023
	PLA ₁₅₂ - mPEG ₁₁₄	152	114	0.003
	PLA ₂₀₂ - mPEG ₁₁₄	202	114	0.002
	PLA ₃₄₁ - mPEG ₁₁₄	341	114	0.002
Star-shaped	PLA _{35*4} - sPEO _{56*4}	140	224	0.011
	PLA _{74*4} - sPEO _{56*4}	296	224	0.003

4.3.3.2 Stability of micelle in salt-added condition

When two colloidal particles undergoing Brownian motion approach each other, they experience two types of interaction: the static forces and the hydrodynamic force mediated by solvent molecules. The hydrodynamic interaction influences the dynamic equilibrium properties of colloidal systems.

The DLVO (Derjaguin, Landau, Verwey and Overbeek) theory provides a basic framework for investigating the interactions between the colloidal particles. It assumes that the more long-range interparticle interactions mainly control the colloidal stability. One is the long range van der Waals force existing irrespective of the chemical nature of the particles or the medium. For similar particles, the forces are always attractive. The other is the double-layer force. Most colloidal particles acquire a charge either from surface charge groups or by specific ion adsorption from the solution. For similar particles, this charge leads to a repulsive double-layer force.

$$V(h) = V_A(h) + V_R(h)$$

The attractive term $V_A(h)$ dominates the repulsive term $V_R(h)$ when h is very large or very small. At intermediate separations, the double-layer force gives rise to a potential energy barrier if the surface is sufficiently charged and if the electrolyte ions do not screen too much. When in a low electrolyte concentration, a substantial energy barrier exists in the total $V(h)$ and the colloidal system is typically stable.

Since, in the DLVO theory, colloidal stability is caused by a balance between double-layer repulsions and van der Waals attraction, the change of the nature and concentration of the electrolyte will greatly affect the stability of the colloidal system. Practically, three systems of colloidal stabilization can be used in aqueous medium: electrostatic

stabilization, steric stabilization and combined ionic and non-ionic stabilization. For our colloidal system, the stability of the colloid mostly depends on the steric stabilization. It is important for us to study this steric stabilization system when considering intravenous injection.

CFPT (Critical flocculation point) is related to the salt-added (concentration of the electrolyte) stability property of the micelle. The sterically stabilized system might flocculate when the solvency of the dispersion medium for the stabilizing moieties is greatly reduced. When the ionic strength of the medium is increased, PEG stabilized aqueous dispersions would flocculate because of the net breakdown of the hydrogen bonding between the polymeric ether oxygen or OH groups and the surrounding water molecules ^[212]. This behaviour has a direct relevance to intravenously-injected formulations. It would cause the physical entrapment by the capillary beds of the lungs if it agglomerates ^[213]. Stolnik ^[214] et al. studied the stability of PLGA uncoated and PLA-PEG coated nanoparticles. It was found that the nanoparticles coated with PLA-PEG were more stable than the uncoated one, as the flocculation was observed to occur at higher concentrations of the electrolyte. Riley et al ^[198] also studied the stability of micelle-like PLA-PEG nanoparticles with different PLA chain. They found that the CFPT decreased with increasing PLA chain length. In our research, it was also found that CFPT decreased greatly as the PLA chain length increased, which means with the increase of PLA block length, the micelle became more unstable. The star-shaped copolymer showed a little lower CFPT than the linear one, which means the aggregates are more unstable in the salt-added condition compared to their linear counterparts.

The main reason leading to the instability might be the segmental attraction when the solvency of the dispersion medium decreased. As the molecular weight of the PLA block increased, nanoparticles with larger hydrophobic surface areas were obtained. Compared with small PLA-PEG-PLA micellar-like nanoparticles with a relatively complete PEG surface coverage, dispersions prepared from higher PLA block copolymers experienced irreversible coagulation. So Riley ^[14] ascribed the decrease of the CFPT to the decrease in the PEG surface coverage and the presence of bare PLA “patches” on the particle surface. However, from our results, it is suggested that not only the PEG surface coverage but also the PEG chain length might cause the decrease of CFPT. The lower CFPT concentration of star-shaped derived aggregates might be caused by the steric architecture. The star-shaped PEO would have difficulty in covering all the surface of hydrophobic regions.

(a)

(b)

(c)

Figure 4-26 CFPT of (a) triblock, (b) diblock and (c) star-shaped copolymer

Another way to study the stability of the colloidal system is to test the cloud point with elevation of temperature. At certain concentration of electrolyte, with the increase of the temperature, the transmittance decreased dramatically. The cloud point was taken as the transmittance drop to 90% of original level and the measurements were performed at a $1^{\circ}\text{C}/\text{min}$. The cloud point of the micellar system can give us more information about the stability.

Figure 4-29 Transmittance as a function of temperature during a thermal process at 1°C/min for triblock copolymers colloidal system with 0.1M electrolyte (Na_2SO_4)

From the above results, we can note that first in the diblock copolymer, the one with short PEG chain is more unstable than other diblock copolymers. It should be attributed to the short PEG chains which offer less contribution to the steric barrier. For triblock copolymer, we found that the one with longer PEG chain showed better stability than the shorter one. For star-shaped copolymer, we found that the most stable one was the one with medium PLA chain length. This agrees well with the CFPT results we got in above context.

4.3.4. Micelle stability in PBS solution

In the clinical administration of the micellar like nanoparticle suspension, vessel occlusion due to the particle aggregation may occur. The steric stability of nanoparticles in the biological milieu is thus an important aspect to be considered. An improved safety profile of mPEG-PLA nanoparticles was observed in comparison with the PLA

nanoparticles, which was attributed to the presence of PEG on the particles surface to prevent the coagulation cascade.^[215] In this research, the stability of micellar like nanoparticles in *vitro* and in water was studied. By measuring the change of effective diameter of particle size, micelle-like nanoparticles are also investigated with dynamic light scattering. Compared to the change of effective diameter in water (almost constant during the observation period, data not shown), we can find the relatively quicker increase of particle size in PBS for some micellar nanoparticle systems. As we know, for the PBS solution, with the salt of di-sodium hydrogen phosphate and potassium dihydrogen phosphate present, the concentration of the salts is about 10mM. So it is not surprising that we found the agglomerate in the PBS solution for some micellar nanoparticle systems since there was no well covered steric barrier. However, when looking into details of those systems, we can still find some similarity. For example, for both diblock and triblock copolymers, the one with short PEG chain always leads into the quick agglomerates (e.g., PLA₉₇-PEG₄₅-PLA₉₇, PLA₅₄-mPEG₄₅). This is mostly caused by the uncompleted steric barrier for those short PEG copolymers and leads to the agglomeration of those micellar nanoparticles.

For star-shaped copolymer, we found a quick increase of effective diameter of micellar like nanoparticle PLA_{35*4}-sPEO_{56*4}, which has the shortest PLA chain length. In Sec 4.3.2.2, we hypothesized the conformation of the star-shaped micellar like nanoparticles (Figure 4-24). For PLA_{35*4}-sPEO_{56*4}, the short PLA chain length might cause instability of the particle and cause this quick increase of particle size.

Figure 4-30 Change of effective diameter of nanoparticles of (a) triblock , (b) diblock and (c)star-shaped copolymers in vitro (pH=7.0 PBS, Merck)

4.4 Surface property of polymer in forms of film and nanoparticles

Although many biodegradable polymers have been used in the aqueous environments in forms of colloidal drug carriers ^[216], there is still a lot to be researched, eg. the structure of polymer/colloid in contact with water or to what extent the surface rearrangement will be. A great challenge for this colloidal drug delivery system is how to extend the circulation time of the carrier system in the blood and escape the uptake of the reticulo-endothelial system (RES) ^[217]. As we know, the property of the long circulation time is greatly affected by the surface property of the drug carrier. A detailed investigation about the surface property of the drug delivery carrier was carried out with the help of XPS and dynamic contact angle testing. The surface charge of the colloidal system was also investigated by zeta potential measurements.

4.4.1. Surface composition of colloidal system and film investigated by XPS

XPS analysis can provide quantifiable data on biomaterial surface chemistry. XPS allows researchers to determine the elemental and average chemical composition of the material at its surface (5-10 nm depth) by measuring the binding energy of electrons associated with atoms. It can measure the enrichment of the surface of the particles with compositions, hence providing an insight into the mechanism of surface stabilization during micelle like nanoparticle preparation. Davis et al. studied the polystyrene colloids modified with polyethylene oxide on the surface. ^[218] With the help of XPS, they found in this model drug delivery system, the level of PEO was increased with the levels of macromonomer in the latex. For biodegradable polymer nanoparticle / microparticle, Gref. and his group first used XPS to study the surface composition of the nanospheres of PLGA-mPEG diblock copolymer. ^[77] They found the major amount of PEG is

concentrated with 5 nm of the outermost-layer of the nanospheres. Shakesheff ^[219] also researched the difference of the microparticles of PLGA-PEG and PLA-PEG and the parent copolymers in the absorption of a surfactant PVA (Poly (vinyl alcohol)). They found that relatively high surface contributions from PVA were evident for microparticles. However, Ruan et al. ^[156] found that the distribution of PEG segments was homogenous in the PLA-PEG-PLA microspheres, which was different from the diblock copolymer PLA-mPEG nanoparticles ^[220], in which the hydrophilic PEG migrated to the surface. From the above, it is evident that a systematic study of surface composition is lacking. Hence we undertook a detailed investigation of the surface composition of these systems, considering effects of different chain structure, LA or EG chain length and LA/EG ratio.

In our research, three different types of copolymer were investigated with different chain structure (di, tri and star-shaped), together with the research of the chain length effect of the copolymer and also the effect of the composition ratio.

4.4.1.1 Surface study of films and nanoparticles made from ABA triblock copolymers PLA-PEG-PLA

In our research, three triblock copolymers were prepared into film and nanoparticle forms. The PLA chain is between 43 to 90 units while the PEG chain is from 91 to 136 units respectively. The LA/EG ratio is from 0.63 to 1.98.

We found that surface composition of the triblocks were dependent on the overall composition (Figure 4-31 to Figure 4-33). Here, the important peak is peak B. If the surface contribution of this peak is different for the copolymers (in film or particle forms)

when compared to the theoretical value for the copolymer, surface segregation of PEO segments is confirmed.

When nanoparticles are considered, those with longer PLA chain lengths, such as PLA₉₀-PEG₉₁-PLA₉₀, have homogenous PEG distribution, whereas nanoparticles formed with shorter PLA chains, such as PLA₆₁-PEG₉₁-PLA₆₁, have higher PEG surface concentration than bulk copolymer. This implies surface segregation of PEG blocks, i.e. PEG attached to shorter PLA segments simply find it easier to migrate to the surface. In addition, for PLA₄₃-PEG₁₃₆-PLA₄₃, which has the longest PEG chain and also the highest PEG composition, a high PEG distribution on the surface was found in both the film and nanoparticles. This sort of surface segregation in film form, was also found by other researchers. K. B. Lewis et al. studied the surface of film made of diblock pHEMA-polystyrene and found the hydrophilic pHEMA –enrichment as a result of hydration ^[221]. Kiss also found a considerable accumulation of Pluronic® polymer (poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide)) (PEO rich) in the surface layer of the PLA/PLGA films, which were blended with Pluronic® ^[222]. The extension of the accumulation was dependent on the PLA/Pluronic® composition and also on the types of the Pluronic® polymer. So combined with their data, we might conclude that only if the composition of the hydrophilic part was high enough, would surface segregation of film be seen. That might be the reason for films, we only found the surface rearrangement on the film surface of the copolymer PLA₄₃-PEG₁₃₆-PLA₄₃.

These data clearly show that in order to obtain stable particles with potential “stealth” capability, particles with long hydrophilic PEG and short hydrophobic PLA segments are the best candidates, such as PLA₆₁-PEG₉₁-PLA₆₁. However, too short a PLA chain might

lead to less drug loading ability, so we need to find a balance. We are currently evaluating the interactions of these particles with macrophages *in vitro*.

Peak	Pure Copolymer			Nanoparticles	
	Binding energy (eV)	Theoretical %	Film %	Binding energy (eV)	Experimental %
A	284.995	22.68	22.22	285.009	21.16
C	287.327	22.68	22.22	287.119	23.01
D	289.241	22.69	22.98	289.258	23.44
B	286.150	31.95	32.57	286.050	32.39

Figure 4-31 C 1s regions of XPS data of PLA₉₀-PEG₉₁-PLA₉₀ (a) film and (b) nanoparticles

Pure Copolymer				Nanoparticles	
Peak	Binding energy (eV)	Theoretical %	Film %	Binding energy (eV)	Experimental %
A	285.000	19.09	19.11	285.000	17.88
C	287.370	19.09	19.11	287.470	17.72
D	289.243	19.08	19.12	289.656	16.08
B	286.254	42.74	42.66	286.344	48.32

Figure 4-32 C 1s regions of XPS data of PLA₆₁-PEG₉₁-PLA₆₁ (a) films and (b) nanoparticles

Peak	Pure Copolymer			Nanoparticles	
	Binding energy (eV)	Theoretical %	Film %	Binding energy (eV)	Experimental %
A	285.002	12.86	9.68	285.007	6.59
C	287.321	12.86	8.62	287.667	6.56
D	289.573	12.86	8.95	289.530	5.28
B	286.592	41.42	72.75	286.697	81.57

Figure 4-33 C 1s regions of XPS data of PLA₄₃-PEG₁₃₆-PLA₄₃ (a) films and (b) nanoparticles

4.4.1.2 Surface study of films and nanoparticles made from AB diblock copolymers PLA-mPEG

Two diblock copolymers were prepared into film and nanoparticles forms for XPS testing. The PLA chain was between 202 to 341 units while the PEG chain is fixed at 114 units respectively. The LA/EG ratio was from 1.86 to 3.0.

In the diblock copolymers, independent of PLA chain length (both PLA₃₄₁-mPEG₁₁₄ and short PLA chain length PLA₂₀₂-mPEG₁₁₄), there is surface migration of the PEG for the nanoparticles (Figure 4-34 and Figure 4-35). This is different from what we have found in the triblock copolymer, where the segregation is seen only with short PLA chain length/high PEG composition. This difference could be ascribed to the chain structure difference between diblock and triblock copolymers. For the triblock copolymer, the PEG chain is anchored to the two PLA chains, the migration might be difficult if the PLA chain is too long (more extensive PLA inter-chain interactions). However, for diblock copolymer, it is easier for PEG chain migration to the surface. To date, in most reference related to surface characterization of nanoparticles from diblock copolymer, increase of PEG content in the surface layer was found.^[157, 223] So this observation also corroborates the assumption that the chain structure will affect the surface migration ability.

Pure Copolymer				Nanoparticles	
Peak	Binding energy (eV)	Theoretical %	Film %	Binding energy (eV)	Experimental %
A	285.000	21.4	22.6	285.009	17.0
C	287.327	21.4	19.1	287.119	16.6
D	289.241	21.4	23.7	289.258	17.6
B	286.150	35.8	34.6	286.050	48.8

Figure 4-34 C 1s regions of XPS data of PLA₂₀₂-mPEG₁₁₄

(a) films and (b) nanoparticles

(a)

(b)

Peak	Pure copolymer			Nanoparticles	
	Binding energy (eV)	Theoretical %	Film %	Binding energy (eV)	Experimental %
A	285.000	25.0	23.4	285.000	22.6
C	287.248	25.0	23.5	287.393	19.0
D	289.221	25.0	28.5	289.512	24.1
B	286.083	25.0	24.6	285.801	34.4

Figure 4-35 C 1s regions of XPS data of PLA₃₄₁-mPEG₁₁₄
(a) films and (b) nanoparticles

4.4.1.3 Surface study of films and nanoparticles made from (AB)₄ star-shaped copolymers (PLA-sPEO)₄

Two star-shaped block copolymers were prepared into film and nanoparticle forms for XPS test. The PLA chain was between 74 to 203 units per arm while the PEG chain was fixed at 56 units per arm. The LA/EG ratio is from 1.29 to 3.63

To date, the surface migration of star-shaped copolymer PLA-sPEO has never been documented in film or in nanoparticle form. So it is interesting to compare results of the branched star-shaped copolymer to the linear counterpart. From Figure 4-36 and Figure 4-37, for both star-shaped copolymers, we found only slight migration of the PEG on the surface independent of the PLA chain length and form type. This might be caused by the large space group of 4-arm PEO, the steric hindrance and localized viscosity of which might lead to the difficulty of PEO enrichment on the surface.

However, another factor needs to be considered. As the nanoparticles we tested were prepared after freeze drying, agglomeration might have happened during this process if the hydrophilic components protecting colloidal system were insufficient.

Peak	Pure copolymer			Nanoparticles	
	Binding energy (eV)	Theoretical %	Film %	Binding energy (eV)	Experimental %
A	285.000	26.1	26.3	285.000	21.7
C	287.050	26.1	26.2	287.050	29.4
D	289.200	26.2	26.2	289.200	26.2
B	286.029	21.6	21.3	286.111	22.7

Figure 4-36 C 1s regions of XPS data of PLA₂₀₃*4-sPEO₅₆*4

(a) films and (b) nanoparticles

Peak	Pure copolymer			Nanoparticles	
	Binding energy (eV)	Theoretical %	Film %	Binding energy (eV)	Experimental %
A	285.000	19.0	20.2	285.000	18.6
C	287.174	19.0	20.7	287.223	20.2
D	289.256	19.1	18.7	289.298	18.2
B	286.134	42.9	40.3	286.220	43.0

Figure 4-37 C 1s regions of XPS data of PLA_{74*4}-sPEO_{56*4}

(b) films and (b) nanoparticles

4.4.2. Copolymer surface characterization with dynamic contact angle

Modification with hydrophilic polymer is the most common way to improve surface properties of devices used for biomedical applications. One of the most sensitive methods that provide information about the outermost polymer surface of a few angstroms is the contact angle measurement. The contact angle is defined as in Figure 4-38.

Figure 4-38 Definition of contact angle

Figure 4-39 Definition of advancing contact angle and receding contact angle and hysteresis between them

The dynamic contact angle was measured to estimate the dynamics of the uppermost surface. When the volume of the droplet was increased, the contact angle at first increased but the three-phase line remained fixed (pinned) in place until it suddenly began moving. The contact angle during the motion was defined as the advancing contact angle. The receding contact angle was defined correspondingly as the contact angle during the receding motion, when the volume of the droplet decreased (Figure 4-38). This measurement procedure turned out to be the most reliable and reproducible method for characterizing the polymer wetting behavior.

The variation in the advancing (θ_{adv}) and receding (θ_{rec}) contact angles and the hysteresis with the PLA segments are shown in Figure 4-40 to Figure 4-42. Johnson and Dettre have shown that θ_{adv} was associated predominantly with high free energy surfaces while θ_{rec} was associated with predominantly high free energy surfaces^[224]. When one makes a receding measurement for a surface covered with water droplets under a water-contracting process, the high energy and low-angle phase tends to keep the water drop from being pulled away. As a result, in such a measuring system, a marked difference between θ_{adv} and θ_{rec} can be seen. From these graphs related above, we can see that with the increase of LA/EG ratio, the advancing angle increases accordingly for all three types (tri, di and star-shape) copolymers. Moreover, we can also find the increase of receding angle at the same time. This can be explained that, with the increase of the hydrophobic block in the copolymer, the polymer will be more similar to the hydrophobic homopolymer (e. g. PLA). So it is not unexpected that we found the increase in advancing angle. Then, we can find for all of samples, with the increase of the LA/EG ratio, the reduced hysteresis value H decrease. The surface rearrangement and interfacial

orientation of PLA/PEG at the water interface occur due to the high surface tension of water, which provides a strong driving force for polymer strands to orient so as to minimize the interfacial tension. On hydration, the receding contact line experiences a more hydrophilic surface than the advancing counterpart. Accordingly the maximum H value corresponding to the highest EG content of the copolymer can be determined

However, for the hysteresis vs LA/EG ratio, the relationship is more complicated. So it is worthwhile discussing the reason that causes the hysteresis, in order to explain the results.

Figure 4-40 (a) Advancing and receding angles of triblock copolymers (b) The effect of copolymer composition ratio on the Hysteresis and reduced hysteresis

(a)

(b)

Figure 4-41(a) Advancing and receding angles of diblock copolymers (b) The effect of copolymer composition ratio on the Hysteresis and reduced hysteresis

(a)

(b)

Figure 4-42 (a) Advancing and receding angles of star-shaped copolymers (b) The effect of copolymer composition ratio on the Hysteresis and reduced hysteresis

Contact angle hysteresis has long been a subject of numerous investigations, but the mechanisms are still controversial because so many factors of surface properties are involved in the hysteresis. The possible cause of contact angle hysteresis mentioned in literature includes two classes of hysteresis: Thermodynamic and kinetic [225] Thermodynamic hysteresis was thought to be caused by the surface roughness and surface chemical heterogeneity.

Surface roughness:

Surface roughness can be characterized by a factor R , called the rugosity of the substrate

$$R = \text{Real surface area } A_{SL} / \text{apparent surface area } A'_{SL} \quad (R \geq 1)$$

For a small movement of the contact line of the drop at equilibrium ($dF=0$)

$$\cos\theta_c = R \cos\theta_c (\text{smooth})$$

As for the reference, the effect of the surface roughness might be neglected, for its effect on the contact angle hysteresis if it is smaller than $0.1 \mu\text{m}$ [225]. With the AFM result, the surface roughness of block copolymer film was about 0.1 to $0.2 \mu\text{m}$. Therefore the effect of surface roughness on hysteresis might be neglected.

Chemical heterogeneity

If the substrate consists of patches of chemically different species which are microscopic and uniformly distributed over a macroscopic area of substrate, then a liquid drop sitting on the substrate experiences different surface tensions from place to place on the substrate. Newman studied surfaces with different geometric arrangements of energetically different areas, searching the effect of surface heterogeneity on average contact angles. [226] He found, for surfaces which are not homogeneous there will exist domains on the surface which present barriers to the motion of the contact line. For the

case of chemical heterogeneity these domains represent areas with different contact angles than the surrounding surface. For example when wetting with water, hydrophobic domains will pin the motion of the contact line as the liquid advances thus increasing the contact angles. When the water recedes the hydrophilic domains will hold back the draining motion of the contact line thus decreasing the contact angle. From this analysis, it can be seen that, when testing with water, advancing angles will be sensitive to the hydrophobic domains and receding angles will characterize the hydrophilic domains on the surface.

Kinetic hysteresis

Kinetic hysteresis is usually the result of time- or rate-dependent interactions of the liquid with the solid surface, resulting in swelling, liquid penetration into the surface region, and segmental or conformational reorientation on the surface.^[226]

From the above discussion, we might assume that contact angle hysteresis in the present study may be caused by these factors: (1) short time-scale molecular reorganization with the surface environment changes or (2) surface chemical heterogeneities due to different property of the two components in the block copolymer.

Large hydrophilic PEG segments in the block copolymer facilitated the wettability and reduced the contact angle, so we find the largest hysteresis at lower LA/EG ratio for diblock and star-shaped copolymers. The triblock behaves differently. There is little change of the hysteresis. This might indicate for triblocks, the effect of the surface roughness might not be negligible. Compared to linear counterparts, the star-shaped copolymer shows to be more hydrophobic (higher advancing contact angle) with

comparable PLA length. (PLA_{35*4}-SPEO_{56*4}, PLA₁₅₂-mPEG₁₁₄ and PLA₆₈-PEG₁₁₄-PLA₆₈)

4.4.3. Surface charge of the particle

Now we take into consideration of the surface charge of colloidal system. The surface charge, more specifically the surface charge density, plays an important role not only in the colloidal stability but also in influencing protein adsorption and thus the particulate uptake by the MPS. Zeta potential is the potential located at the shear plane of the diffuse ionic layer at the particles surface. It is often used to characterize the stability of colloids and can be taken as an indirect measure of the surface charge.

Table 4-11 Zeta potential of micellar like nanoparticles (in mV)

	Triblock copolymer					Diblock copolymer			Star-shaped	
	PLA ₆₁ - PEG ₉₁ - PLA ₆₁	PLA ₉₀ - PEG ₉₁ - PLA ₉₀	PLA ₄₁ - PEG ₄₅ - PLA ₄₁	PLA ₉₇ - PEG ₄₅ - PLA ₉₇		PLA ₅₀ - mPEG ₁₁₄	PLA ₃₄₁ - mPEG ₁₁₄	PLA ₁₅₉ - mPEG ₄₅	PLA _{74*4} - sPEG _{56*4}	PLA _{203*4} - sPEG _{56*4}
ξ	-54	-2.1	-12.0	-23.7	-33.7	-0.6	-27.8	-29.0	-8.7	-16.8
S	1.35	0.85	0.79	1.08	3.76	0.82	0.93	1.58	0.54	0.66
D										

It is well known that for the blank PLA or PLGA nanoparticles, as the existence of end carboxyl group will lead to the negative surface charge for the colloid system. With this negative charge, the C3 complements in the blood might more easily deposit on the colloid surface as the colloid system is intravenous injected. It will result in the uptake of these particles by the MPS organs. However, the block copolymers of PLA/PEG show

only a small value of negative charge and are close to neutral. So this property is ideal for the colloid system used as the carrier of drug for intravenous injection. In Table 4-11, usually the polymer with short PLA chain but long PEG chain will have near neutral value due to the sufficient surface PEG density. ^[227, 228]

However, not all reported work agreed about the influence of surface charge on particulate uptake. Other surface properties, such as hydrophobicity might also affect the opsonization process. ^[229] Park et al. suggested that while some negatively charged liposomes can increase liver and spleen uptake, others can actually also avoid uptake leading to prolonged circulation time. ^[230] Davis et al. reported no effect on the biodistribution of emulsions on reversal of the charge ^[231] and L. Illum et al also showed that there was no direct correlation between uptake and charge. ^[232]

Due to all these uncertainties, the macrophage uptake research is currently carrying in our group to find the correlation between the uptake amount and the particle properties.

Summary of the characterization findings:

- (a) Relatively high-MW polymers were synthesized; Mw's determined by GPC and light-scattering agreed to each other well
- (b) Thermal measurements show good phase separation between PLA and PEG blocks, provided each block is sufficiently long.
- (c) DOC of the PLA segment range from 19-53% for triblocks; 35-57% for diblocks and 9-40% for star-branched polymers. The DOC and T_m of the PLA block are the highest when PLA length is long.
- (d) CMCs were relatively low, indicating that these polymers form micelles easily in aqueous medium.

- (e) Sizes of micelles were determined by light-scattering in the region of 50-200 nm and they were affected by the concentration and the organic solvent during preparing procedure.
- (f) The aggregate number (i.e, number of molecules per micelle) was relatively high for triblock copolymers; micelles from these polymers also had rod-like aggregates as seen by AFM. This indicates the development of a unique morphology for triblocks, as stated in the context, whereas micelles from di- and four-armed blocks were spherical.
- (g) Micelle stability, which is related to the injectability of the formulation, was correlated to CFPT; in general, the CFPT decreases (stability decreases) with increasing PLA segment length. Most micelles were stable *in vitro* except for those copolymers with short PEG chain which will lead into the quick agglomerating
- (h) Nanoparticles formed by block copolymer with short PLA chain but longer PEG chain had nearly neutral value due to the sufficient surface PEG density. In contrast, those with shorter PEG chain or longer PLA chain had large absolute value of negative zeta potential.
- (i) The diblock copolymer PLA₁₅₂-mPEG₁₁₄ and star-shaped copolymer PLA_{74*4}-sPEO_{56*4} were suitable for the drug delivery carrier according the stability and surface charge testing
- (j) Surface segregation of PEG units occurs in film and nanoparticle forms, provided (i) the PLA segment is short, and (ii) the length of the PEG segment is above a minimum limit for triblock copolymer: for the diblock copolymers, independent with PLA chain length, there is surface segregation of PEG unit exiting

4.5 Hydrolytic degradation of copolymer in form of film and nanoparticle

One of the most important criteria for biodegradable polymers to be used as drug delivery carriers is their degradation profile. A review of the literature shows that the long-term (up to 90 weeks) degradation characteristics have been studied extensively [233, 202]. However, very few studies have been done on the shorter-term profile (1-3 weeks), which is more important for drug release prediction of common chemotherapeutics. Furthermore, there is no reported systematic study comparing the three types of copolymers. Therefore in our work, the polymer film degradation and the drug release profile of four triblock copolymers, four diblock copolymers and three star-shaped copolymers in a buffer, for up to 3 weeks were studied. (See Table 4-12) For a few selected copolymers (PLA₉₀-PEG₉₁-PLA₉₀, PLA₁₅₂-mPEG₁₁₄ and PLA_{74*4}-sPEO_{56*4}), the degradation study was done on both film and particulate form.

Table 4-12 Polymers for degradation study

triblock	PLA ₄₁ -PEG ₄₅ - PLA ₄₁	PLA ₉₀ -PEG ₉₁ - PLA ₉₀	PLA ₉₀ -PEG ₉₁ - PLA ₉₀ *	PLA ₆₂ -PEG ₁₈₂ - PLA ₆₂
diblock	PLA ₅₅ -mPEG ₄₅	PLA ₅₀ - mPEG ₁₁₄	PLA ₁₅₂ - mPEG ₁₁₄ *	PLA ₃₄₁ -mPEG ₁₁₄
starshpe	PLA _{74*4} -sPEO _{56*4}	PLA _{74*4} - sPEO _{56*4} *	PLA _{203*4} - sPEO _{56*4}	

* For film form and nanoparticle form study

4.5.1. Degradation of triblock copolymers

Four synthesized triblock copolymers have been chosen to study the degradation profiles with different PLA chain length of PLA and PEG. The MW data for the four polymers in film form are compared in Figure 4-43, while Figure 4-44 shows how the

MWD changes with degradation of representative polymers. First of all, it was noticed that for all copolymers there was an obvious decrease in MW after three weeks. Most copolymers had a significant drop in MW initially but the degradation rate decreased over time.

Figure 4-43 Molecular weight changes as a function of time of PLA/PEG block copolymer triblock copolymers

For triblock copolymer, it was found that both PLA chain and PEG chain affected the polymer degradation rate. For samples PLA₉₇-PEG₄₅-PLA₉₇ and PLA₉₀-PEG₉₁-PLA₉₀, doubling the PEG chain length enhanced the degradation rate (The molecular weight still remained at 80% of initial value for PLA₉₇-PEG₄₅-PLA₉₇ while it decreased to 55% for PLA₉₀-PEG₉₁-PLA₉₀ after three weeks *in vitro* degradation). Similarly, the reduction in PLA chain length also enhanced degradation (PLA₉₇-PEG₄₅-PLA₉₇ and PLA₄₁-PEG₄₅-PLA₄₁). The crystallinity of PLA chain, the water uptake rate of the copolymer and also the degradation product are believed to influence the degradation profile. So longer PLA chain means higher crystallinity and could reduce the polymer hydrolysis rate. On the other hand, longer PEG chain increases water uptake and hence enhance the polymer degradation rate. Another thing that needs to be considered is the degradation products.

As for short PLA chain copolymer, the chain cleavage led to segments of PEG connected to very short PLA chain. Due to their aqueous solubility, these components were dissolved in the buffer solution, which made the faster molecular weight loss in the initial stage compared to the longer PLA chain copolymers.

(a) PLA₉₀-PEG₉₁-PLA₉₀

(b) PLA₉₇-PEG₄₅-PLA₉₇

From the MWD results, two distinct patterns of degradation can be seen (Figure 4-44). All copolymers initially exhibited a monomodal molar mass distribution. However, two of the copolymers with longer PEG chain $\text{PLA}_{62}\text{-PEG}_{182}\text{-PLA}_{62}$ (Figure 4-44(c)) and $\text{PLA}_{90}\text{-PEG}_{91}\text{-PLA}_{90}$ (Figure 4-44 (a)) showed bimodal profiles while copolymers with shorter PEG chains ($\text{PLA}_{97}\text{-PEG}_{45}\text{-PLA}_{97}$, Figure 4-44 (b) and $\text{PLA}_{41}\text{-PEG}_{45}\text{-PLA}_{41}$, Figure 4-44(d)) show no obvious bimodal profile after 20 days of degradation. To further understand the cause leading to this bimodal phenomenon, the two peaks were analysed separately. The nonlinear curve fitting method was applied to separate the two peaks and a two-peak-Gauss model was chosen to fit the weight fraction curve of the curve. It was found that the ratio of the new peak to the “older” peak increased with the time of degradation (Figure 4-45). It was also found that the molecular weight of the two peaks decreased at the same time.

Figure 4-45 Analysis of the triblock copolymer $\text{PLA}_{91}\text{-PEG}_{90}\text{-PLA}_{91}$ components of the two peaks of the whole copolymer molecular weight got from the Gauss model (a) Separation of the two peaks using two peak Gauss model (b) molecular weight change of the two peaks got after the simulation

Li et al.^[28] reported bimodality during degradation, in all their triblocks copolymers with different ratio of LA to EG (from about 1 to 5). However, in their work, the second peak appeared only after about 11 weeks in 3 of the polymers, and after 25 weeks for the polymer with a LA/EG ratio of 5. Moreover, the PEG block length was kept constant at 41 in their studies. The mechanism proposed by Li et al. is that the bimodality is a consequence of the different rates of hydrolysis in the crystalline (or crystallizable) phase of PLA blocks. In our study, bimodality was observed within 20 days, for all triblocks with PEG lengths above 90 DP. This might be due to faster water ingress for those copolymers with longer PEG chains, due both to the higher hydrophilicity as well as the reduced crystallinity. Clearly, from Table 4-3, the triblock polymers with higher PEG content, had much lower degrees of crystallinity for the PLA block. This also translates to greater differentiation between attacks on amorphous and crystalline regions for the same polymers, perhaps at a very early stage in the degradation process. In addition, from the result of the simulation, it can be roughly assumed that the most components in the low molecular weight peak are short chain PLA segments while for the high molecular weight are PLA chains connected with PEG. Thus, the early observation of bimodality for the high PEG content triblocks, is due to the reduced crystallinity of the PLA block in these polymers, with the amorphous regions being attacked first, thus creating a bimodal distribution. For polymers with lower PEG lengths (contents), water ingress is slower, and the DOC of the PLA segments are higher, leading to less differentiation between the amorphous and crystalline portions of the film.

Considerable difference of morphological changes of the films can be found after 11 days degradation between PLA₄₁-PEG₄₅-PLA₄₁, PLA₉₇-PEG₄₅-PLA₉₇ and PLA₉₀-PEG₉₁-PLA₉₀ in Figure 4-46. For the polymer with the short PLA chain, the film looked like the mesh after 11 days (Fig 4-46b), while only small holes could be found for the one with longest PLA chain (PLA₉₇-PEG₄₅-PLA₉₇) (Fig4-46a). It was also consistent with slower water ingress for the latter copolymer. For the one with longer PLA chain and also with longer PEG chain (PLA₉₀-PEG₉₁-PLA₉₀)(Fig 4-46c), we could find the increase of surface roughness greater than the one with longer PLA chain and shorter PEG chain (Fig 4-46a) yet not so obvious mesh like as the one with shorter PLA chain(Fig 4-46b),

(a) PLA₉₇-PEG₄₅-PLA₉₇ (b) PLA₄₁-PEG₄₅-PLA₄₁ (c) PLA₉₀-PEG₉₁-PLA₉₀

Figure 4-46 Change of film morphology of PLA-PEG-PLA triblock copolymer after 11 days degradation investigated by FESEM

NMR provides a means to examine the composition changes of the degrading copolymer. It was found from the ¹H-NMR spectra that all the copolymers showed a very slight decrease in LA/EG ratio after 20 days (Fig 4-47). This certainly indicates very tiny loss of either EG- or LA-containing groups up to 20 days (Table 4-13), which further substantiates our earlier explanation for bimodality.

Table 4-13 LA/EG ratios of the copolymers at different degradation time

Degradation time (day)	0 day	20 day
PLA ₄₁ -PEG ₄₅ -PLA ₄₁	1.8	1.8
PLA ₉₇ -PEG ₄₅ -PLA ₉₇	4.3	4.1
PLA ₉₀ -PEG ₉₁ -PLA ₉₀	2.0	1.7
PLA ₆₂ -PEG ₁₈₂ -PLA ₆₂	0.7	0.6

Figure 4-47 ^1H -NMR characterization of select sample PLA₉₇-PEG₄₅-PLA₉₇ before and after 20 days in *vitro* degradation (data for other triblock copolymer not shown)

Figure 4-48 FTIR of select sample PLA₉₇-PEG₄₅-PLA₉₇ before and after 20 days *in vitro* degradation (data for other triblock copolymer not shown)

Figure 4-48 is the FTIR spectra for PLA₉₇-PEG₄₅-PLA₉₇ before and after *in vitro* degradation. From the graph, it can be seen that the C=O group of LA segment at 1760cm⁻¹ remained nearly unchanged while the peak intensity at 755cm⁻¹ associated with PLA crystals decreased after degradation. The decrease of crystallinity of PLA was also found by the thermal analysis.

Thermal property changes were also investigated by MDSC. For all the copolymers, the melting peak of PLA block decreased with the degradation time. Figure 4-49 shows that for triblock copolymers with a relatively long PLA chain, T_m decreased slowly in the first 20 days, whereas for the copolymer with relatively short PLA chain, T_m decreased faster initially. The decrease in T_m attests to attack on the crystalline regions.

Figure 4-49 Melting and crystalline temperature change with the degradation time of selected triblock copolymers

Finally, we compared the degradation profiles of the same polymer in the form of particle and film, using PLA₉₀-PEG₉₁-PLA₉₀ and PLA₉₇-PEG₄₅-PLA₉₇ as an examples (Figure 4-50A and Figure 4-50B). In film form, PLA₉₀-PEG₉₁-PLA₉₀ exhibited bimodality after about 7 days, but this phenomenon was much less pronounced when seen in nanoparticle form, although MW reduction rates are comparable. This can only be attributed to the fact that thickness plays a role in enhancing the heterogeneity of MW reduction. In other words, in the thicker film, the degradation in the interior of the film is substantial, and the low-MW species are retained in the interior, whereas at the surface, the low-MW “tail”, when generated, leaches out. Moreover, when the film form does not exhibit any bimodality, neither does the nanoparticle form, as seen with PLA₉₇-PEG₄₅-PLA₉₇.

Figure 4-50A Comparison of degradation data for nanoparticles and film of PLA₉₀-PEG₉₁-PLA₉₀ (a) GPC chromatograms of nanoparticles (b) Molecular weight loss

Fig 4-50B Comparison of degradation data for nanoparticle and film of PLA₉₇-PEG₄₅-PLA₉₇ (a) GPC chromatograms of nanoparticles (b) Molecular weight loss

We have also shown, by XPS (see above context), that there is little surface segregation of PEG segments for particles or for films of PLA₉₀-PEG₉₁-PLA₉₀, thus

reinforcing that the more heterogeneous nature of the degradation for films is purely a consequence of the thickness of the film.

4.5.2. Diblock copolymer

Four diblock copolymers with different PLA and PEG chain length were used in our study. PLA chain units ranged from 50 to 341 while PEG chain units were fixed at either 114 or 45. The results showed that longer PLA chain (PLA₃₄₁-mPEG₁₁₄) had a greater initial molecular weight loss compared to the shorter PLA chain (PLA₁₅₂-mPEG₁₁₄). The former copolymer lost about 58% of its original molecular weight (M_n) left after 20 days but for the latter copolymer this value was only 38%. . At the same time, it was also found that PLA₅₅-mPEG₄₅ had slower initial degradation rate compared to the PLA₅₀-mPEG₁₁₄, mostly due to the its short chain length of PEG block.

Figure 4-51 Molecular weight changes as a function of time of PLA/PEG diblock copolymer

(a) PLA₅₅-mPEG₄₅ (b) PLA₅₀-mPEG₁₁₄ (c) PLA₁₅₂-mPEG₁₁₄ (d) PLA₃₄₁-mPEG₁₁₄

Figure 4-52 GPC chromatograms of series of PLA-mPEG diblock copolymers with degradation

From the MWD results, as with the triblock copolymer discussed above, we found two distinct patterns of degradation. (Figure 4-52) All copolymers initially exhibited a monomodal molar mass distribution. Except for the one with short PEG chain (PLA₅₅-mPEG₄₅), all the other three showed a bimodal (PLA₅₀-mPEG₁₁₄ and PLA₁₅₂-mPEG₁₁₄) or broad distribution profile (PLA₃₄₁-mPEG₁₁₄). Again, where bimodality is observed, the trend is that the lower the crystallinity, the earlier the onset of bimodality (as seen with the triblocks).

Figure 4-53 Change of film morphology of PLA-mPEG diblock copolymer after 11 days degradation investigated by FESEM

Figure 4-53 shows the changes in morphology change for the diblock copolymer film during *in vitro* degradation. It was found that there were many cracks on the film of PLA₅₀-mPEG₁₁₄ compared to PLA₅₅-mPEG₄₅ film. The formation of cracks should be

mostly due to the hydrophilic PEG chain length as they have almost same PLA chain length. The PLA₅₀-mPEG₁₁₄ has longer PEG chain which leads to the quick uptake of water; this creates channels for water ingress, and the subsequent efflux of degraded polymer.

Thermal property changes of diblock copolymers during degradation were also investigated by MDSC. It was found that the melting peaks of PLA block decreased with the time (Figure 4-54), which was very similar to triblock copolymers. The decrease in melting temperature (T_m) was greater for copolymers having shorter PLA chain (PLA₅₄-mPEG₄₅). The decrease in T_m indicates changes in the crystalline regions. The results suggest that hydrolytic degradation affects the crystalline region of PLA chain and hence leads to the decrease in the melting temperature.

We also compared the degradation profiles of polymer in the form of nanoparticles and film using PLA₁₅₂-mPEG₁₁₄ as example. In film form (Figure 4-52 C), it exhibited bimodality after about 9 days, but this phenomena was less obvious in the nanoparticle form (Figure 4-55(a)) even though their molecular weight reduction rates are comparable. (Figure 4-55(b)) This result is similar to what we obtained from the triblock copolymers earlier.

Figure 4-54 Melting and crystalline temperature change with the degradation time of selected diblock copolymers

Figure 4-55 Comparison of degradation data for nanoparticle and film of PLA₁₅₂-mPEG₁₁₄ (a) GPC chromatograms of nanoparticle (b) Molecular weight loss

4.5.3. Star-shaped block copolymer

Figure 4-56 shows that the longer PLA chain length copolymer had lesser rate of decrease in molecular weight (M_n) and a similar trend was also observed for triblock copolymers. After 20 days, the percentage of M_n remained at 60.2% (PLA_{203*4}-sPEO_{56*4}), 54.4% (PLA_{74*4}-sPEO_{56*4}) and 43.4% (PLA_{35*4}-sPEO_{56*4}) respectively.

Figure 4-56 Molecular weight changes as a function of time of PLA-sPEO star-shaped block copolymer

All three copolymers initially showed a unimodal distribution (Figure 4-57) before *in vitro* degradation. However, two of the copolymers, which have shorter PLA chain length, developed an obvious bimodal distribution as degradation proceeded, while the one with longest PLA chain length developed a very broad MWD profile. These results suggest that bimodality occurs when PLA and PEO chain lengths are comparable (PLA_{74*4}-sPEO_{56*4} and PLA_{35*4}-sPEO_{56*4}). Possible reasons are more rapid water ingress and heterogeneity of hydrolysis of the copolymer films. Copolymer with the longest PLA

chain showed a broad molecular distribution. (PLA_{203*4}-sPEO_{56*4}) This result is very similar to those of the diblock copolymer PLA₃₄₁-mPEG₁₁₄ (Figure 4-52(d)).

(a) PLA_{35*4}-sPEO_{56*4} (b) PLA_{74*4}-sPEO_{56*4} (c) PLA_{203x4}-sPEO_{56x4}

Figure 4-57 GPC chromatograms of series of PLA-sPEO star-shaped block copolymers with degradation

The bimodal curve of PLA_{74*4}-sPEO_{56*4} was also fitted with the two-peak Gauss model. From Figure 4-58, we can find that the content of the low molecular weight peak also increased as degradation proceeded.

Figure 4-58 Analysis of the components of the two peaks of the whole copolymer molecular weight got from the Gauss model PLA_{74*4}-sPEO_{56*4} (a) Separation of the two peaks using two peak Gauss model (b) molecular weight change of the two peaks obtained after the simulation

The star-shaped copolymer showed a relatively more rapid initial loss of PEG content, as seen in ¹H NMR (Figure 4-59). This might be related to the greater solubilization of PEO-rich chains in the initial stage, followed by the usual PLA-based scission.

Figure 4-59 Composition of star-block copolymers during *in vitro* degradation, obtained from ^1H -NMR

In the only other reported work on degradation of star-shaped copolymers of PLA, Li et al.^[26] found that star-shaped copolymers of comparable MW, degraded slower than the diblock counterpart. However, for our star-shaped copolymer, we found a sudden decrease of PEO content after 13 days. During the same period, we can find a rapid increase of the ratio of the low molecular weight peak in the total molecular weight. (Figure 4-46 (b)) The decrease of the PEO content is most likely due to degradation at either on or near to the bonding site of LA-EO. The degraded products, PEO or PEO with short PLA chain, would dissolve in buffer easily and diffuse out from the film. This explains the decrease of the PEO content after 13 days, as shown in NMR.

Figure 4-60 FTIR of select sample PLA_{74*4}-sPEO_{56*4} before and after 20 days *in vitro* degradation

In Figure 4-60, the peak intensity at 755cm^{-1} associated with PLA crystals decreased after degradation for the star-branched polymer. This indicates attack on the crystalline domains around day 20, similar to what was observed for triblocks.

Degradation of copolymer will also affect its thermal properties. In Figure 4-61 (a), both star-shaped copolymers showed a gradual decrease in the T_m of the PLA block. For the longer PLA chain copolymer, the crystalline temperature T_c decreased with time. However, for the copolymer with shorter PLA chain, there was an increase of T_c for the first few days (Figure 4-61 (b)). This is similar to what we have found in the diblock copolymer (Figure 4-54).

Figure 4-61 Melting and crystalline temperature change with the degradation time of selected star-shaped copolymers

Figure 4-62 Comparison of degradation data for nanoparticle and film of star-shaped copolymer PLA₇₄*4-SPEO₅₆*4 (a) GPC chromatograms of nanoparticles (b) Molecular weight loss

When we compared the film and micelle forms of the same star-shaped polymer, we find that overall degradation rates and patterns of MWD are similar, but accelerated in the micellar case. Also, the bimodality present in film degradation was much less pronounced in the case of micelles. Both of these differences can be attributed to the relative differences in surface areas.

4.5.4. Comparison of (a) linear and star-shaped copolymer (b) film and nanoparticle form

We also compared the degradation property of star-shaped copolymer and linear copolymer with approximately same LA/EG ratio. From Figure 4-63, there was not much difference for linear and star-shaped copolymer in film forms. However, when we compared micelle form of star-shaped and linear copolymer, the MWD of the former copolymer decreased much more rapidly than the linear diblock copolymer. This might be caused by the higher PEO content (more water uptake).

We found another difference between the result of linear block copolymer and star-shaped block copolymer. For triblock copolymer, the LA/EG ratio remained stable and only provided a small drop after three weeks of in vitro degradation. For the star-shaped copolymer, a decrease of EO content was found. To explain this difference, the lability of hydrolytic degradation on the bond site of these two type copolymers might be taken into consideration. From our result, for star-shaped copolymer, the degradation happened mostly at either on or near to the bonding site of LA-EO.

As for the comparison of the degradation rate in the form of film form and nanoparticle form, many factors might to be considered. First, for nanoparticles, the small particle size leads to a large increase in surface area.^[234] The higher surface area might be associated with accelerating the buffer contact/penetration into particles. However,

another effect should also be taken into consideration. Since the PLA degradation might be autocatalyzed by acidic monomers and oligomers, as degradation products, the high surface area might also lead to the fast diffusion of these degradation products, which decrease the release rate. So it is not surprising as some nanoparticles degrade faster than film while others did not (compensation of competing factors).

Figure 4-63 Comparison of degradation profile of diblock and star-shaped copolymer with approximate LA/EG ratio

4.6 In vitro anti-cancer drug release from micelle- like nanoparticles

Among all the hydrophilic polymers, PEG has the advantage of being non-toxic and it is approved (in various dosage forms) by the Food and Drug Administration (FDA) for internal use in humans. PEG significantly increases the nanoparticle blood circulation time. Besides hydrophilicity, chain flexibility is another major feature necessary for the coating polymers to provide long-circulating particles. Due to the transient, flexible and rapidly changing structure of PEG, the immune system would have difficulties in modeling an antibody around it.^[88] The protective layer of PEG is considered as a “cloud” of possible chain conformations, with a density high enough to prevent the interactions of opsonins with the surface of the particles. If a polymer chain possess both hydrophilicity and flexibility properties (to enable a high number of possible chain conformations), it can serve as an effective coating protector for particles against opsonization. All these properties make these block copolymers good candidates for long-circulating drug delivery carriers.

Three types of PEG block copolymers (di, tri and star-shaped) were synthesized and incorporated with the anti-cancer drug. Two kinds of anti-cancer drugs were entrapped and their drug release profiles were studied. One is 5-FU (5-Fluorouracil (5-fluoro-2, 4-pyrimidinedione)), which is slightly soluble in water, and the other one is Paclitaxel, which has very low solubility in water. Both of them are widely used for the anti-cancer treatment. So it is very useful to study the entrapment efficiency and their drug release profiles from different types block copolymers.

4.6.1. Drug Release Data: 5-FU

As shown in Table 4-14., particle sizes of micelle- like nanoparticles increased slightly after the incorporation of drug, 5-FU. Table 4-15 and Table 4-16 show that the drug entrapment efficiency was greatly affected by the initial polymer/drug concentration ratio of triblock copolymer, but little effect observed for diblock and star-shaped copolymer.

Table 4-14 Hydrodynamic diameter, polydispersity of PLA-PEG-PLA nanoparticles w/o drug

	Particle size (nm) mean $\pm$ SD (without drug)	Particle size (nm) mean $\pm$ SD (with drug)	Polydispersity mean $\pm$ SD (without drug)	Polydispersity mean $\pm$ SD (with drug)
PLA ₆₁ -PEG ₉₁ - PLA ₆₁	63.8 $\pm$ 3.0	75.0 $\pm$ 6.1	0.31 $\pm$ 0.03	0.17 $\pm$ 0.04
PLA ₆₈ -PEG ₉₁ - PLA ₆₈	138.0 $\pm$ 0.5	179.1 $\pm$ 2.0	0.22 $\pm$ 0.01	0.22 $\pm$ 0.01
PLA ₉₀ -PEG ₉₁ - PLA ₉₀	210.7 $\pm$ 0.5	219.4 $\pm$ 1.7	0.25 $\pm$ 0.01	0.24 $\pm$ 0.01

Table 4-15 Effect of different PLA block chain length and concentration on 5-FU drug entrapment (triblock)

	Different copolymer ¹			Different polymer/drug ratio ²		
	PLA ₆₁ - PEG ₉₁ - PLA ₆₁	PLA ₆₈ - PEG ₉₁ - PLA ₆₈	PLA ₉₀ - PEG ₉₁ - PLA ₉₀	Polymer /drug =10:1	Polymer /drug =10:2	Polymer /drug =10:5
Theoretical drug loading (%)	9.1	9.1	9.1	9.1	16.7	33.4
Drug entrapment (%)	14.2	15.5	12.6	13.1	19.7	38.8

¹ the polymer/drug ratio maintained at 10:1

² PLA₆₈-PEG₉₁-PLA₆₈ was used as the polymer

Table 4-16 Effect of concentration on 5-FU drug entrapment

	PLA ₁₅₂ -mPEG ₁₁₄			PLA _{35*4} -sPEO _{56*4}		
	Polymer /drug =10:1	Polymer /drug =10:2	Polymer /drug =10:5	Polymer /drug =10:1	Polymer /drug =10:2	Polymer /drug =10:5
Theoretical drug loading (%)	9.1	16.7	33.4	9.1	16.7	33.4
Drug entrapment (%)	23.8	23.9	24.8	19.4	20.7	19.8

4.6.1.1 Effect of PLA chain length

The chemical structure of 5-Fluorouracil (5-fluoro-2, 4-pyrimidinedione) is shown in Figure 4-64

Figure 4-64 Chemical structure of 5-FU

5-FU dissolves sparingly in water, about 12.5mg/ml. The drug was continuously released *in vitro* over 300 hrs. The rate of release was high and this concurs with the findings of other researchers^[235]. In Figure 4-65, three triblock copolymers were chosen as carriers for 5-FU delivery. The unit number of PEG chain was fixed while the PLA chain was from 122 to 180. The results obtained indicate that the different PLA chain length has not much effect on the release profiles.

Figure 4-65 Release profile of 5-FU from nanoparticles of triblock copolymer PLA-PEG-PLA with different PLA blocks at same drug loading

4.6.1.2 Effect of drug loading on profiles of different copolymers

Figure 4-66 Release profile of 5-FU from nanoparticles of block copolymer (a) triblock PELA₆₈-PEG₉₁-PLA₆₈ (b) diblock PLA₁₅₂-mPEG₁₁₄ and (c) star-shaped PLA_{35*4}-sPEO_{56*4} with different polymer/drug concentration

The effect of drug loading on the drug release profile is shown in Figure 4-66. It was noticed that drug loading concentration did not affect the drug release profiles much. The initial stage of drug release profile was very similar for all types of copolymers and the release was very rapid. Most of the drug was released within 2 to 3 days and this could be due to the rapid burst release effect. It was then followed by a much slower release rate caused by diffusion. We believe that this profile is due to the hydrophilic nature of the drug which does not like to reside in the micelle hydrophobic core. Bhadra et al. used dendrimers to complex with the 5-FU, and they found that the PEGylated formulation showed release over 144hr when doing the *in vitro* study, while non PEGylated formulation completed release all the drug within one day. This suggests that drug need to be well encapsulated inside the core in order to achieve a sustained drug release profile [167].

4.6.1.3 Effect of different chain type copolymers on drug release profile

In Figure 4-67, we compared the drug (5-FU) release profile of triblock, diblock and star-shaped copolymers. All of them showed a rapid burst release within 2 days. This demonstrates that all of the profiles showed very little control over the release, a testing to relatively inefficient “core” trapping for this hydrophilic drug.

However, among all these copolymers, star-shaped copolymer had the slowest release. It might be due to its unique molecular structure.

Figure 4-67 Release profile of 5-FU from nanoparticles of triblock PLA₆₈-PEG₉₁-PLA₆₈, diblock PLA₁₅₂-mPEG₁₁₄ and star-shaped block copolymer PLA_{35*4}-sPEO_{56*4} with polymer : drug=10:2

Thus for a relatively hydrophilic drug such as 5-FU, the three types of block copolymers offer the same degree of control over its release, without sufficient distinction among them. Most of the release profiles appear to be dominated by a “burst” effect in the first 24 hours, indicating the uncompleted encapsulation of this drug in the core of the micelles.

We also studied the surface of nanoparticles encapsulating the 5-FU, the N 1s peak was found. It indicated that the entrapment of hydrophilic drug as 5-FU in the nanoparticles was insufficient (Figure 4-68). This is also one of the reasons for the burst effect found in our experiments.

Figure 4-68 XPS of surface investigation of nanoparticles loaded with 5-FU: N 1s region of star-shaped copolymer PLA_{35*4}-PEO_{56*4}

4.6.2. Drug Release Data: Paclitaxel

Paclitaxel is a microtubule-stabilizing agent which promotes polymerization of tubulin causing cell death by disrupting the dynamics necessary for cell division. Paclitaxel, formulated in a 50:50 mixture of Cremophor[®] EL (polyoxyethylated castor oil): absolute ethanol as Taxol[™], is the world's most successful chemotherapeutic drug. In fiscal year 2001, Bristol-Myers Squibb posted nearly \$1.2 billion in sales from Taxol[™]. It has neoplastic activity especially against primary epithelial ovarian carcinoma, breast, colon and non-small cell lung cancers.

The main limitation of paclitaxel for clinical application is its difficulty in administration. Paclitaxel is highly hydrophobic so an adjuvant consisting of Cremophor[®] EL and absolute alcohol has to be used in current clinical administration of paclitaxel,

which causes side-effect. Due to the limitation of paclitaxel in its clinical administration, its dosage and infusion period have thus been restricted. Best clinical effects have not been achieved.

The use of biodegradable polymeric micro/nanoparticles for controlled delivery of anticancer agents has advantages in enhancing therapeutic efficacy and reducing systemic side-effects. ^[236] In addition, nanoparticles enable intravenous injection as well as intramuscular and subcutaneous administration by minimizing possible irritant reactions. ^[237, 238] Feng et al. have investigated the feasibility to apply the nanoparticles of biodegradable polymers PLA and PLGA as an alternative administration system for paclitaxel. ^[155]

paclitaxel

Figure 4-69 molecular structure of paclitaxel

It is well known that surface modification of micelle-like nanoparticles with PEG prolongs its circulation time in the human body. In addition, the size of PEG modified particulate system will also determine its biological fate. It has been suggested that the mechanism of removal of the particles above approximately 200 nm by the spleen is a

result of mechanical filtration, followed by eventual phagocytosis in red pulp macrophages. Due to the EPR (enhanced permeation and retention effect), particles smaller than 200 nm can deliver the drug to the tumor cell as passive targeting. Jain and collaborators studied the permeability of the vasculature in tumors in more detail and indeed found areas with a high permeability compared to vasculature in normal tissue.^[239] On the other hand, it has been demonstrated that long circulating effect has a lower size limit. For example, irrespective of their composition, liposomes with diameters below 70nm demonstrated an increased accumulation in the liver.^[240] The effect of the surface modification with PEG on prolonged circulation and the site of deposition of a particulate carrier appear to be limited to a relatively narrow size range of 70-200 nm.

Das et al. encapsulated the Paclitaxel inside the PEG coated PLA microspheres. They found the initial burst release of 25-30% followed by a constant slow release of drugs from PEG coated PLA microspheres.^[241] Feng et al. studied the encapsulation of Paclitaxel in microparticles from triblock copolymer PLA-PEG-PLA and nanoparticles from PLA-mPEG^[155, 156]. In the following context, our results will be compared to their results.

In our study, paclitaxel was entrapped into the micellar nanoparticles made of triblock, diblock and star-shaped copolymer. The particle sizes of the micelle-like nanoparticles were tested by the dynamic light scattering (DLS). Table 4-17 shows the effective diameter of micelle-like nanoparticle loaded with/without paclitaxel. It can be noted that the size of all particles fell within the range of 70 to 200nm even after loaded with drug, which means they are ideal for long circulation drug carrier. In addition, incorporation of drug seems to have little effect on the particle size, except for one of the

triblock copolymer which showed a 10% increase in size. Furthermore, the amount of drug loaded did not affect the particle size significantly.

Table 4-17 Particle size of drug loaded and blank micelle-like nanoparticle

	Blank (nm) particle size $\pm$ SD	100μg (nm) particle size $\pm$ SD	300 μg (nm) particle size $\pm$ SD	500 μg (nm) particle size $\pm$ SD
PLA ₉₀ - PEG ₉₁ -PLA ₉₀	133 $\pm$ 1.0	145 $\pm$ 0.8	145 $\pm$ 0.9	147 $\pm$ 0.7
PLA ₉₇ - PEG ₄₅ -PLA ₉₇	130 $\pm$ 0.7	127 $\pm$ 0.8	133 $\pm$ 1.0	128 $\pm$ 1.0
PLA ₁₅₉ - mPEG ₄₅	83 $\pm$ 0.7	72 $\pm$ 5.4	68 $\pm$ 4.2	78 $\pm$ 4.8
PLA ₁₅₂ - mPEG ₁₁₄	83 $\pm$ 0.2	86 $\pm$ 0.1	73 $\pm$ 0.1	73 $\pm$ 0.3
PLA _{74*4} - sPEO _{56*4}	161 $\pm$ 2.1	158 $\pm$ 0.6	164 $\pm$ 1.5	157 $\pm$ 1.0
PLA _{203*4} - sPEO _{56*4}	113 $\pm$ 1.0	126 $\pm$ 0.5	104 $\pm$ 0.8	119 $\pm$ 0.7

Figure 4-70 TEM graph of paclitaxel loaded micelle-like nanoparticles
(PLA_{203x4}-SPEO_{56x4}, negative stained with PTA)

Figure 4-70 is the graph for the morphology of the micelle-like nanoparticles prepared from the star-shaped copolymer PLA₂₀₃*4-SPEO₅₆*4.

$$\text{Theoretical Drug Content (\%)} = \frac{\text{Mass of drug used in formulation}}{\text{Mass of copolymer and drug in formulation}} \times 100 \quad \text{Equation 4-17}$$

$$\text{Drug Entrapment Efficiency (\%)} = \frac{\text{Mass of drug in particles}}{\text{Mass of drug used in formulation}} \times 100 \quad \text{Equation 4-18}$$

$$\text{Drug Content (\%)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles recovered}} \times 100 \quad \text{Equation 4-19}$$

$$\text{Nanoparticle recovery (\%)} = \frac{\text{Mass of nanoparticles recovered} \times 100}{\text{Mass of polymeric material, drug and any formulation excipient used in formulation}} \quad \text{Equation 4-20}$$

(a)

Figure 4-71 XPS of surface investigation of nanoparticles loaded with Paclitaxel: N 1s regions of star-shaped copolymer PLA_{203*4}-sPEO_{56*4} and PLA_{74*4}-sPEO_{56*4}

XPS was used to study the presence of paclitaxel on the surface of micelle like nanoparticles. From Figure 4-71, it was noted that there was no N 1s signal (nitrogen element) on the surface of the micelle-like nanoparticles. If there was any paclitaxel on the surface of the nanoparticles, there should be visible N 1s signal. **So this suggests that the drug is totally encapsulated in the core of nanoparticles.**

Table 4-18 presents the results of entrapment efficiency of triblock, diblock and star-shaped copolymers with different polymer drug feed ratios. The theoretical drug content, drug entrapment efficiency, drug content and nanoparticle recovery were calculated with above equations (Eq. 4-17 to Eq. 4-20). Recovery of nanoparticle was low as the centrifugation speed might not be high enough to spin down all the particles. From the

table, we noted that with the increase of the theoretical drug loading, the drug content and entrapment efficiency also increased.

An important property of micelles that has particular significance in pharmacy is their ability to increase the solubility of hydrophobic drug in water. The solubilization in micelles can be defined as the spontaneous dissolving of a substance by reversible interaction with the micelles of a surfactant in water to form a thermodynamically stable isotropic solution with reduced thermodynamic activity of the solubilized material. ^[242]

As for the polymeric micelles systems, they present even better solubilization capacity when compared to surfactant micelles due to the higher number of micelles and /or larger cores of the formers^[243]. For paclitaxel, Kim et al also found the increase of solubility in the PLA/PEG copolymeric micelles^[244].

In the previous section, we have demonstrated that the release of a relatively more hydrophilic drug 5-FU from particles could not be controlled. However, for a very hydrophobic drug, such as paclitaxel, it seemed that diffusion controlled release is more dominant than the rapid burst effect release profile of 5-FU over this time period. First, the drug release rate was much slower, and complete release of drug was accomplished only after 12 days compared to the fast release of the 5-FU. Second, at lower theoretical drug loading (1%-3%), the drug entrapment efficiency still remained comparable to 5-FU with high theoretical drug loading (9%-33%), as seen in Table 4-15 and Table 4-18

Also, with the increase of the theoretical drug loading, the drug content and drug entrapment efficiency increased accordingly, concurring with other results. ^[245,246]

Nanoparticle recoveries were independent of the increase of theoretical drug loading. T. Govender found an increasing theoretical drug (procaine hydrochloride) loading that

could lead to a decrease in the PLGA nanoparticle recovery ^[247]. For the PLA/PEG block copolymer systems, steric rather than electrostatic stabilization is mainly responsible for preventing particle agglomeration ^[198]. Later they also found the independence of nanoparticle recovery to the theoretical drug loading in the study of PLA-mPEG nanoparticles ^[246], which is in agreement with our results.

Table 4-18 Drug entrapment efficiency of nanoparticles prepared with triblock, diblock and star-shaped copolymer with different polymer drug feed ratio

	Paclitaxel in feed (μg)	Nanoparticle recovery (%)	Theoretical drug loading (%)	Drug content (%)	Entrapment Efficiency (%)
PLA _{74x4} - SPEO _{56x4}	100.00	44.55	1.00	0.13	12.72
	300.00	75.73	3.00	0.79	26.99
PLA _{203*4} - sPEO _{56*4}	100.00	46.44	1.00	0.29	29.22
	300.00	47.09	3.00	0.81	26.89
PLA ₉₇ - PEG ₄₅ - PLA ₉₇	100.00	49.01	1.00	0.30	29.85
	300.00	36.41	3.00	1.42	47.43
PLA ₉₀ - PEG ₉₁ - PLA ₉₀	100.00	41.82	1.00	0.13	13.55
	300.00	69.71	3.00	0.76	25.87
PLA ₁₅₂ - mPEG ₁₁₄	100.00	42.43	1.00	0.13	9.38
	300.00	26.57	3.00	0.76	11.90
PLA ₃₄₁ - mPEG ₁₁₄	100.00	24.06	1.00	0.13	13.36
	300.00	23.30	3.00	0.63	21.79

4.6.2.1 Drug release profiles of paclitaxel from triblock copolymers

Two triblock copolymers were chosen in this study, PLA₉₀-PEG₉₁-PLA₉₀ and PLA₉₇-PEG₄₅-PLA₉₇. The PLA chain length was quite comparable but both have different PEG chain lengths.

Figure 4-72 : Release of Paclitaxel from (a) PLA₉₀-PEG₉₁-PLA₉₀ and (b) PLA₉₇-PEG₄₅-PLA₉₇ micelle-like nanoparticles

In comparison with reported data on similar systems,^[20, 156] we found that the release rate from our triblock copolymer was faster compared to the nanoparticle release reported by Ruan^[159]. Reasons for the discrepancy may be due to the lower particle size in our work (150 nm) compared to Ruan's (20 microns); the size difference may also explain the relatively higher encapsulation efficiency reported in Ruan's work. We found an inverse dependence of release rate on drug loading, i. e., lower loadings appeared to have faster rate of release. This is somewhat in agreement with Ruan's result.

Comparing these two release profiles, one prominent difference can be seen as the cumulative release of PLA₉₇-PEG₄₅-PLA₉₇ was much lower. We have studied the stability of the micelle-like nanoparticles (Chapter 4.3.3). For triblock copolymer PLA₉₇-PEG₄₅-PLA₉₇, the hydrophilic PEG chain was too short to protect the nanoparticle well. So it was more unstable than PLA₉₀-PEG₉₁-PLA₉₀ (See Figure 4-26 (a)). So it might be much easier to form agglomerates of this colloidal system for PLA₉₇-PEG₄₅-PLA₉₇. The

drug inside these agglomerates may not be able to diffuse out as fast as from isolated micelles. This might be the main reason that we only found about 40% of paclitaxel released out of PLA₉₇-PEG₄₅-PLA₉₇ micelle after two weeks.

The inverse dependence of release rate on drug loading is puzzling. At this moment, we can only postulate that at higher drug loadings, more of the drug is better encapsulated inside the core, hence diffuses out slower. This warrants further study, and the use of more sophisticated techniques to quantify the extent core-shell distribution of drug.

4.6.2.2 Drug release profiles of paclitaxel from diblock copolymers

Two diblock copolymers, PLA₁₅₂-mPEG₁₁₄ and PLA₃₄₁-mPEG₁₁₄ were chosen in this study. PEG chain length was fixed at 114 but PLA chain length varied from 152 to 341.

Figure 4-73 Release of Paclitaxel from (a) PLA₁₅₂-mPEG₁₁₄ (b) PLA₃₄₁-mPEG₁₁₄ micelle-like nanoparticles

For PLA₁₅₂-mPEG₁₁₄, the initial release rates for different drug loading were quite similar (Figure 4-73); beyond about 100 hours, we see a divergence in the extent of release. The high-loaded micelles appear to release more drug than the low of high-loaded micelles. This is contrary to what is expected from purely diffusion-controlled release. Again the reason for the anomaly lies either in different drug encapsulation levels or differing effects of degradation on the release profile. Given the particular morphology of micelles formed from diblocks (which are bilayers), it is also possible that better entrapment of the hydrophobic drug occurs in the core of the micelle, resulting in some retention of drug ^[248]; the effect is likely to be exaggerated at lower drug loadings.

For PLA₃₄₁-mPEG₁₁₄, the overall behaviour is similar.

Effect of different PLA length

In Figure 4-74, we compare the drug release profile from two diblock copolymers with different PLA chain length at higher drug loading. It can be noticed that little effect of PLA chain length on the drug release profile. Comparing our result with Feng's result for paclitaxel release from diblock PLA/PEG nanoparticles, it is noticed that his result is similar to our result of PLA₁₅₂-mPEG₁₁₄ while their copolymer has much longer PLA chain (Mn=65,000, mPEG 5k). ^[160]

Figure 4-74 Comparison of diblock copolymer with different PLA chain length for higher drug loading

4.6.2.3 Drug release profiles of paclitaxel from star-shaped block copolymers

Star-shaped copolymer PLA_{74x4}-sPEO_{56x4} and PLA_{203x4}-sPEO_{56x4} were chosen in this study. As shown in Figure 4-75, paclitaxel was released much slower compared to 5-FU due to its high hydrophobicity. In contrast with 5-FU, the release profile of paclitaxel was greatly affected by the initial polymer drug ratio. However, further study would be needed to verify it.

The effect of different drug loading on the release profile is shown in Figure 4-75. It was noted that high-drug-loading released most drug for both star-shaped copolymers. This release profile is also consistent with the diblock copolymers.

Figure 4-75 Release of Paclitaxel from nanoparticles (a) PLA₂₀₃*4-sPEO₅₆*4 (b) PLA₇₄*4-sPEO₅₆*4

Figure 4-76 Paclitaxel release from nanoparticle PLA_{74x4}-sPEO_{56x4} at high drug loading

The effect of different PLA chain length on the release profile is shown in Figure 4-76. No significant burst release was observed in both of micelles, and there was 20% of paclitaxel released during the first day. The release of drug during the first two days was similarly independent of the PLA chain length. However, a dependence on PLA chain length of release profile was observed where slower release was obtained from micelle-

like like nanoparticles comprising longer PLA chain length. Lin et al. obtained the similar results in their study of indomethacin released from the PLA/PEG triblock copolymer micelle ^[249]. From Figure 4-26 (c) and Figure 4-28, we can conclude that PLA_{74*4}-sPEO_{56*4} has better colloidal stability than PLA_{203*4}-sPEO_{56*4}. The explanation is similar to that given for tri- and di-blocks. Here better entrapment inside the longer PLA chains is also possible.

There has been no publication about the paclitaxel delivery using star-shaped copolymer micelle-like like nanoparticles. A. Breitenbach used star-shaped copolymer to prepare microparticles for protein delivery ^[250]. Almost zero-order kinetics was found.

4.6.2.4 Comparison of all these three types of copolymers

Finally, we compared the release profile of diblock, triblock and star-shaped copolymer at different drug loading. At high drug loading, the triblock appeared less efficiency for drug release, although rates were similar. At low drug loading, the star-branched polymer had high efficiency for drug release.

Figure 4-77 Paclitaxel release from different type of copolymer micelle-like nanoparticles
(a) high drug loading and (b) low drug loading

4.6.3. Modelling the drug release

The release mechanism and kinetics are usually the most important issues considered by researchers. Mathematical modeling has been employed to simulate the release kinetics of different delivery systems which provides a scientific knowledge base concerning the mass transport mechanism.

In 1963, Higuchi described the release of drugs from an insoluble matrix as square root of the time-dependent process on the basis of Fickian diffusion.^[177]

Higuchi model,

$$Q_t = K_H t^{1/2} \quad \text{Equation 4-21}$$

Where K_H is the Higuchi release constant and Q_t is the amount of drug at time t .

Lapidus and Lordi^[251] showed that, based on the Higuchi diffusional law, there is a linear dependence between release rate and drug dose, W_0 (soluble drugs) or $W^{1/2}$ (poorly soluble drugs), i.e., the ratio of diffusion rate constant at different drug loading k_{h1}/k_{h2} should equal to $\sqrt{C_{d2}/C_{d1}}$. (C_d is the drug loading concentration)

Since paclitaxel is very difficult to dissolve in water, so we took it as poorly soluble drug for the following simulation of the release profile with drug release data.

In our research, paclitaxel release profiles from triblock, diblock and star-shaped micelle-like nanoparticles were simulated with the Higuchi model. However, only the diblock copolymer PLA₁₅₂-mPEG₁₁₄ seemed to fit the Higuchi model well in the first few days.

Figure 4-78 Higuchi model for the drug release study of the PLA₁₅₂-mPEG₁₁₄

Table 4-19 Correlation of K vs Sqrt (c)

	Drug amount (SQRT)	K value	R ²
C1	3.063	0.019	0.995
C2	5.976	0.102	0.997
C3	10.102	0.207	0.999

As discussed above, if the Higuchi equation were applicable, the increased drug loading would have increased the diffusion rate constant, k , by a factor of $\sqrt{C_{d2}/C_{d1}}$. Therefore from the results as shown in Figure 4-78 and Table 4-19, we conclude that for diblock copolymer with medium PLA chain length, the diffusion Higuchi model can be used to fit the release profile well. However, this is the only case we found that fits the Higuchi model.

Figure 4-79 Higuchi model for the drug release study of the PLA₉₀-PEG₉₁-PLA₉₀

Figure 4-80 Higuchi model for the drug release study of the PLA₂₀₃*4-sPEO₅₆*4

Table 4-20 Correlation of K vs Sqrt (c) for PLA₉₀-PEG₉₁-PLA₉₀

	Drug amount (SQRT)	K value	R ²
C1	1.966	0.009	0.988
C2	4.672	0.043	0.989
C3	7.236	0.125	0.994

Table 4-21 Correlation of K vs Sqrt (c) for PLA_{203*4}-sPEO_{56*4}

	Drug amount (SQRT)	K value	R ²
C1	5.405	0.0175	0.993
C2	8.982	0.0687	0.996
C3	13.039	0.0963	0.999

From Figure 4-79, Figure 4-80 and Table 4-20, Table 4-21, we can see that for triblock copolymer PLA₉₀-PEG₉₁-PLA₉₀ and star-shaped copolymer PLA_{203*4}-sPEO_{56*4}, the concentration dependence Higuchi model was not well fitted. It suggests that the degradation of those copolymers might also influence the drug release rate.

Annex

(DSC thermograms for copolymer degradation)

PLA₄₁-PEG₄₅-PLA₄₁

PLA₇₄*4-sPEO₅₆*4

PLA₂₀₃*4-sPEO₅₆*4

PLA152-mPEG114

PLA₃₄₁-mPEG₁₁₄

Chapter 5

5. Conclusions

A series of triblock, diblock and star-shaped PLA/PEG block copolymers have been synthesized by ring opening polymerization with stannous octoate as catalyst. Products with narrow polydispersity were obtained.

The polymers were characterized for their molecular properties, as well as their surface compositions. The second part of the study involved micellization, degradation and drug release, with a view to assessing their performance as “stealth” anti-cancer drug carriers.

5.1 Copolymer characterization

5.1.1. Molecular structure

FTIR was used to confirm covalent linkages between the PLA and PEG segments.

The molecular structures were further verified by the ^1H -NMR and ^{13}C -NMR. The number average molecular weight can also be calculated with the ^1H -NMR. For star-shaped copolymer, NMR showed that all the hydroxyl end groups of 4-arm PEO are esterified by L-lactide.

5.1.2. Molecular weight

The number average and weight average molecular weight were also determined by the GPC and SLS. The data we obtained from light scattering agree with the GPC data.

5.1.3. Thermal property

Thermal measurements show good phase separation between PLA and PEG blocks, provided each block is sufficiently long. The reduced interactions between the molecules

of star-shaped copolymers due to their steric architecture lowered the degree of crystallinity, as well as the T_m , compared to linear PLA/PEG block copolymer.

5.2 Formation and characterization of micelle- like nanoparticles

5.2.1. Particle size study

Sizes of micelles determined using light-scattering were in the region of 50-200 nm; sizes were affected by the concentration of the polymer and by the nature of the organic solvent used to prepare the micelles.

5.2.2. Aggregation property

The aggregation number (i.e, number of molecules per micelle) was relatively high for triblock copolymers; micelles from these polymers also had rod-like aggregates as seen by AFM. This indicates development of a unique rod-like aggregate morphology for triblocks, whereas micelles from di- and four-armed blocks were spherical.

5.2.3. Stability of micelle in dilute condition and salt-added condition

The CMC is very low, which is favorable for drug delivery carrier applications. It was found that, with the increase of PLA block length, the micelle became more unstable. The star-shaped copolymer showed greater instability than the linear one, which is because of the inability of a star-shaped PEG to cover all the surface of hydrophobic regions.

From the various techniques used, we found that:

- (a) Shorter PEG chain lengths cause greater instability of the micelles.
- (b) For star-shaped polymer, the most stable polymer is the one with the medium PLA chain length.

Most micelles are stable *in vitro*. However, in salt-containing media, linear copolymers with short PEG chain always lead to quick agglomerates. It is mostly caused by the incomplete steric barrier for short PEG copolymers.

5.3 Surface property of polymer in forms of film and nanoparticles

5.3.1. Surface composition of colloidal system and film investigated by XPS

Surface segregation of PEG units occurs in film and nanoparticle forms, provided

- (i) the PLA segment is short, and (ii) the length of the PEG segment is above a minimum limit for triblock copolymer: for the diblock copolymers, independent with PLA chain length, there is little surface segregation of PEG unit exiting

5.3.2. Surface charge of nanoparticle

Nanoparticles formed by block copolymer with short PLA chain but longer PEG chain had nearly neutral value due to the sufficient surface PEG density. In contrast, those with short PEG chain or longer PLA chain had large absolute value of negative zeta potential. Neutral values are preferable as these discourage attachment by plasma proteins.

5.4 Hydrolytic degradation of copolymer in form of film and nanoparticle

In our work, the polymer film and nanoparticle degradation of all the three types of copolymers was studied. Most copolymers had a significant drop in MW initially but the degradation rate decreased over time.

For triblock copolymer, longer PLA chain also resulted in higher crystallinity and this reduces the polymer hydrolysis rate. The bimodality was observed within 20 days, for all triblocks with PEG lengths above 90 DP. This might be due to faster water ingress for those copolymers with longer PEG chains, both to the higher hydrophilicity as well as the

reduced crystallinity. The bimodality phenomenon was much less pronounced in nanoparticle form.

For diblock copolymers, Bimodality is also observed. The lower the crystallinity, the earlier the onset of bimodality (as seen with the triblocks). Bimodality indicates heterogeneity of the degradation process.

The star-shaped copolymer showed a relatively more rapid initial loss of PEG content. This might be related to the greater solubilization of PEO-rich chains in the initial stage, followed by the usual PLA-based scission.

In summary, all the block copolymers show relatively faster degradation rates than PLGA due to the incorporation of hydrophilic PEG chain into the molecule chain. For triblock copolymer, the LA/EG ratio remained stable and only provided a small drop after three weeks of in vitro degradation. For the star-shaped copolymer, a decrease of EO content was found. From our result, for star-shaped copolymer, the degradation happened mostly at either on or near to the bonding site of LA-EO.

5.5 In *vitro* anti-cancer drug release from micelle-like nanoparticles

5.5.1. 5-FU

Particle sizes of micelle-like nanoparticles increased slightly after the incorporation of drug. The drug entrapment efficiency was greatly affected by the initial polymer/drug concentration ratio while the PLA chain length had little effect on it. However, different PLA chain length had little effect on the release profiles. The drug loading concentration did not affect the drug release profiles much. For a relatively hydrophilic drug such as 5-FU, the three types of block copolymers offer the same degree of control over its release, without sufficient distinction among them. Most of the release appears to be dominated

by a “burst” effect in the first 24 hours, testifying to the lack of encapsulation of this drug in the core of the micelles.

5.5.2. Paclitaxel

The particle sizes of the micelle-like nanoparticles were in the range of 70 to 200nm even after loaded with drug, which means they are ideal for long circulating drug carrier applications. From the XPS result, the drug was totally encapsulated in the core of nanoparticles. It was noted that with the increase of the theoretical drug loading, the drug content and entrapment efficiency also increased.

For triblocks with different PEG lengths (but same PLA length), it was found that the short-chain PEG copolymer releases less drug, possibly due to agglomeration (and hence greater diffusion path lengths). An inverse dependence of release rate on drug loading was found, i. e., lower loadings appear to have faster rate of release.

For diblock copolymers with same PEG chain length but different PLA chain lengths, it was found that the longer PLA chain slows down drug release, once again due to better encapsulation and greater diffusion path length in the core.

Two star-shaped copolymers with different PLA chain length were also investigated. The release of drug during the first two days was independent of the PLA chain length. Over a longer time period, slower release is obtained from particles that have greater PLA length, similar to the observations above.

Finally we compared the release profile of diblock, triblock and star-shaped copolymer at different drug loading. Compared to the linear block copolymer, the star-shaped one released faster at first stage and later became slower at the high drug loading.

In low drug loading, we found faster and more complete release from star-shaped copolymer compared to the linear ones.

5.5.3. Drug release modelling

Paclitaxel release profiles from triblock, diblock and star-shaped micelle-like nanoparticles were simulated with the Higuchi model. However, only the diblock copolymer PLA₁₅₂-mPEG₁₁₄ seemed to fit the Higuchi model well in the first few days. Together with the degradation result, it indicates that not only the diffusion, but also degradation need to be considered as the factors affecting the drug release profile.

5.5.4. Optimization

From our results, we conclude that these copolymers are unsuitable as carriers for hydrophilic drugs. For hydrophobic drugs, to be a good candidate for anti-cancer drug carrier, there are two important considerations: the “stealth” characteristics, and the ability to encapsulate/control release of drug. For these block copolymers, requirements for one appear to be in conflict with the other. Stealth characteristics are enhanced with short PLA chains and long PEG blocks; drug release/encapsulation is enhanced with longer PLA blocks. In view of these conflicting requirements, combined with the stability and surface property result, the best compromise candidates for stealth hydrophobic drug carriers are:

- (a) the diblock copolymer PLA₁₅₂-mPEG₁₁₄ and
- (b) the star-shaped copolymer PLA_{74*4}-sPEO_{56*4}

Chapter 6

6. Suggestions for future work

6.1 The synthesis of 3-arm or 8-arm star-shaped copolymer

For a detailed investigation of the branched block copolymer, three arm or eight arm star-shaped copolymers might be synthesized and compared to the four-arm star-shaped copolymer. From this, the effect of the molecular structure on the drug delivery can be more systematically researched.

6.2 Polymer structure and micelle-like aggregates studies

The crystallization of the copolymers can be further studied by observing the formation of the spherulites. The nucleation mechanism and the relative growth rates will be studied.

The influence of polymers in confined spaces will also be studied. The entanglements observed especially obvious in triblock copolymer nano aggregates. The origin of these entanglements would be suitable to explore.

The difference between the micelles and micelle-like nanoparticles also need to be explored

6.3 Degradation studies

A more detailed study of the degradation mechanisms in all types of block copolymers will be necessary. By evaluating the crystallinity, MW, weight loss and other parameters during degradation, the origin of bimodality or multimodality will be explained more thoroughly

6.4 Drug release studies

A more systematic study of the effect of drug loading on release is warranted. For example, it is not clear whether the incorporation of more drugs may disrupt the regular

Chapter Six

Suggestions for future work

morphology of the “core” of the micelle; hence the release kinetics may depend in a non-linear fashion with drug loading. We have seen some evidence of this (as discussed on page 173-174). Both micelle size and stability may be affected by high drug loadings; these need to be evaluated. The partition coefficient of the drug will be quantitatively measured by plotting the drug concentration in the polymer with respect to the equilibrium drug concentration in the external solution. Also the drug release experiment might carry on in a flowing medium rather than a static one. The effect of gases dissolved in the system should also be studied. More anti-cancer drugs can be evaluated for a systematically research.

6.5 The biodistribution of nanoparticles

The biodistribution of drug loaded nanoparticles is an important piece of data which indicate the long-circulating ability of nanoparticles. The increase of the distribution of nanoparticles to plasma is caused by the decrease of the biodistribution to liver and spleen. All of these should be verified with the biodistribution experiments in vivo. With suitable animal models, copolymeric micelles of differing surface hydrophilicities and PEG conformation should be injected intravascularly, and the blood lifetimes, as well distribution in the likely organs (liver, spleen and target tumours) should be quantified.

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