

# CONTENTS

	Page No.
<b>1. INTRODUCTION</b>	<b>1-23</b>
1.1 Amphiphilic Block Copolymers	1
1.2 Advantage of Amphiphilic Block Copolymers	2
1.3 Synthetic Approaches for Amphiphilic Block Copolymers	4
1.3.1 Living radical polymerization (LRP)	5
1.3.2 Ring Opening Polymerization (ROP)	6
1.3.3 Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization	8
1.3.4 Click Chemistry	11
1.4 Polymer Segments Employed for the Synthesis of Amphiphilic Block Copolymers	13
1.4.1 Poly(D,L-lactide) (PDLLA)	13
1.4.2 Poly(D,L-lactide- <i>co</i> -glycolide) (PLGA)	15
1.4.3 Poly( <i>N</i> -vinylpyrrolidone) (PNVP)	16
1.5 Literature review	18
1.5.1 PDLLA- <i>b</i> -PNVP block copolymers	19
1.5.2 PLGA based block copolymers	21
1.6 Aim of the Work	22
<b>Chapter 2: Synthesis, Characterization and Antitumor Activity of Methotrexate-loaded Poly(D,L-lactide)-<i>b</i>-Poly(<i>N</i>-vinylpyrrolidone) Amphiphilic Block Copolymers</b>	<b>24-76</b>
<b>2.1 Introduction</b>	<b>24</b>
<b>2.2 Experimental Section</b>	<b>27</b>
2.2.1 Materials	27
2.2.2 General Methods	28
2.2.2.1 <sup>1</sup> H NMR Spectroscopy and Gel Permeation Chromatography	28
2.2.2.2 Transmission Electron Microscopy (TEM) Study	29
2.2.2.3 Fluorescence Study	29

2.2.2.4 Light Scattering Study	30
2.2.2.5 Thermo Gravimetric Analysis (TGA)	30
2.2.2.6 Differential Scanning Calorimeter (DSC) Study	31
2.2.2.7 Wide Angle X-ray Diffraction (WXRd) Study	31
2.2.3 Typical Synthesis of Monohydroxy-terminated Poly(D,L-lactide) (PDLLA <sub>42</sub> -OH)	31
2.2.4 Typical Synthesis of PDLLA <sub>42</sub> -C(O)CH(CH <sub>3</sub> )Br (PDLLA <sub>42</sub> -Br)	32
2.2.5 Typical Synthesis of PDLLA <sub>42</sub> -C(O)CH(CH <sub>3</sub> )SC(S)OC <sub>2</sub> H <sub>5</sub> (PDLLA <sub>42</sub> -X)	33
2.2.6 Synthesis of the Block Copolymer PDLLA <sub>42</sub> - <i>b</i> -PNVP <sub>37</sub>	34
2.2.7 Synthesis of the Block Copolymer PDLLA <sub>42</sub> - <i>b</i> -PNVP <sub>63</sub>	34
2.2.8 Degradation of PDLLA Block in the Block Copolymers Obtained in the Kinetic Study	35
2.2.9 Drug Loading and Release Studies	35
2.2.10 Cell Lines and Cell Culture	36
2.2.11 Development of MTX-resistant DL and Raji cells	37
2.2.12 <i>In-vitro</i> Cell Viability Assay	37
2.2.13 <i>In-vitro</i> Cytotoxicity Assay	38
2.2.14 Tumor Cell Proliferation Assay	38
2.2.15 Detection of Apoptosis	39
2.2.16 Hemolysis assay	39
2.2.17 Statistical Analysis	40
<b>2.3 Results and Discussion</b>	<b>41</b>
2.3.1 Synthesis of PDLLA- <i>b</i> -PNVP Diblock Copolymers	41
2.3.2 Degradation of PDLLA Block in the Block Copolymers	50
2.3.3 Self-assembly of Amphiphilic PDLLA- <i>b</i> -PNVP Block Copolymers in Aqueous Solution	53
2.3.4 Thermal Study	59
2.3.5 XRD Study	62
2.3.6 MTX Loading and <i>In Vitro</i> Release Study	63
2.3.7 Effect of MTX-loaded PDLLA <sub>42</sub> - <i>b</i> -PNVP <sub>63</sub> on Tumor Cell Viability and Cytotoxicity	66

2.3.8 Cellular Growth Inhibition by MTX-loaded PDLLA <sub>42</sub> - <i>b</i> -PNVP <sub>63</sub> Micelles	68
2.3.9 Induction of Tumor Cell Apoptosis by MTX-loaded PDLLA <sub>42</sub> - <i>b</i> -PNVP <sub>63</sub> Micelles	71
2.3.10 RBC Integrity and Size Distribution	72
2.3.11 MTX-loaded PDLLA <sub>42</sub> - <i>b</i> -PNVP <sub>63</sub> Micelles Tolerant to Normal Cell Viability and Cytotoxicity	73
<b>2.4 Conclusions</b>	<b>75</b>
<hr/>	
<b>Chapter 3: Synthesis, Characterization and Antitumor Activity of Methotrexate-loaded Four-arm Star Poly(D,L-lactide)-<i>b</i>-Poly(<i>N</i>-vinylpyrrolidone) Amphiphilic Block Copolymer</b>	<b>77-107</b>
<hr/>	
<b>3.1 Introduction</b>	<b>77</b>
<b>3.2 Experimental section</b>	<b>80</b>
3.2.1 Materials	80
3.2.2 General methods	80
3.2.3 Synthesis of four-arm star poly(D, L-lactide)[S-(PDLLA <sub>15</sub> -OH) <sub>4</sub> ]	80
3.2.4 Synthesis of four-arm star [PDLLA-COCH (CH <sub>3</sub> ) Br] <sub>4</sub>	81
3.2.5 Synthesis of four-arm star [PDLLA-C(O)CH(CH <sub>3</sub> )SC(S)OC <sub>2</sub> H <sub>5</sub> ] <sub>4</sub>	82
3.2.6. <i>In Vivo</i> Therapeutic Efficacy	83
3.2.7 Ethics statement	84
<b>3.3 Results and Discussion</b>	<b>82</b>
3.3.1 Synthesis of Four-arm Star Block Copolymers S-(PDLLA- <i>b</i> -PNVP) <sub>4</sub>	85
3.3.2 Self-assembly of Amphiphilic S-(PDLLA- <i>b</i> -PNVP) <sub>4</sub> Block Copolymers in Aqueous Solution	91
3.3.3 MTX Loading and In Vitro Release Study	92
3.3.4 Effect of MTX-loaded Star-(PDLLA <sub>15</sub> - <i>b</i> -PNVP <sub>10</sub> ) <sub>4</sub> on Tumor Cell Viability and Cytotoxicity	95
3.3.5 Cellular Growth Inhibition by MTX loaded star-(PDLLA <sub>15</sub> - <i>b</i> -PNVP <sub>10</sub> )	97
<hr/>	

3.3.6 Induction of Tumor Cell Apoptosis by MTX-loaded Star-(PDLLA <sub>15</sub> - <i>b</i> -PNVP <sub>10</sub> ) <sub>4</sub> Micelles	98
3.3.7 RBC Integrity and Size Distribution	100
3.3.8 MTX-loaded Star-(PDLLA <sub>15</sub> - <i>b</i> -PNVP <sub>10</sub> ) <sub>4</sub> Tolerant to Normal Cells	101
3.3.9 Comparative <i>In Vivo</i> Antitumor Activity of MTX-loaded linear PDLLA <sub>42</sub> - <i>b</i> -PNVP <sub>63</sub> and star-(PDLLA <sub>15</sub> - <i>b</i> -PNVP <sub>10</sub> ) <sub>4</sub> Micelles	103
<b>3.4 Conclusions</b>	<b>106</b>
<hr/>	
<b>Chapter 4: Synthesis and Characterization of DOX-loaded Amphiphilic Poly(<i>N</i>-vinyl pyrrolidone) (PNVP)-<i>b</i>-PDLLA-<i>b</i>-PNVP Triblock Copolymers and Its <i>In Vitro</i> Drug Release Study</b>	<b>108-122</b>
<hr/>	
<b>4.1 Introduction</b>	<b>108</b>
<b>4.2 Experimental section</b>	<b>109</b>
4.2.1 Materials	109
4.2.2 General methods	109
4.2.3 Synthesis of dihydroxyl-terminated poly(D,L-lactide) (HO-PDLLA <sub>48</sub> -OH)	109
4.2.4 Synthesis of dibromo-terminated PDLLA [Br(CH <sub>3</sub> )CHCO-PDLLA <sub>48</sub> -COCH (CH <sub>3</sub> ) Br] (Br-PDLLA <sub>48</sub> -Br)	110
4.2.5 Synthesis of Dixanthate-terminated PDLLA (X-PDLLA <sub>48</sub> -X)	111
4.2.6 Typical Synthesis of the ABA type Double Hydrophilic Amphiphilic Tri Block Copolymer PNVP <sub>23</sub> - <i>b</i> -PDLLA <sub>48</sub> - <i>b</i> -PNVP <sub>23</sub>	112
4.2.7 Drug Loading and Release Study	112
<b>4.3 Results and Discussion</b>	<b>113</b>
4.3.1 Synthesis of PNVP- <i>b</i> -PDLLA- <i>b</i> -PNVP Amphiphilic Triblock Copolymers	113
4.3.2 Self-assembly of Amphiphilic PNVP- <i>b</i> -PDLLA- <i>b</i> -PNVP Triblock Copolymers in Water	118
4.3.3 DOX Loading and <i>In Vitro</i> Release	120
<b>4.4 Conclusions</b>	<b>122</b>
<hr/>	

<b>Chapter 5: Synthesis and Study of the Self-assembly of Novel Poly(D,L-Lactide-co-Glycolide)-<i>b</i>-Poly(<i>N</i>-Vinylpyrrolidone)(PLGA-<i>b</i>-PNVP) Amphiphilic Diblock Copolymers: Its Antitumo</b>	<b>123-142</b>
<b>5.1 Introduction</b>	<b>123</b>
<b>5.2 Experimental Section</b>	<b>125</b>
5.2.3 Synthesis of Alkyne-terminated Copolymer of D, L-lactide and Glycolide using Propargyl Alcohol	125
5.2.4 Typical Synthesis of Azide-terminated Poly(NVP) Macro-RAFT agent	126
5.2.5 Synthesis of PLGA <sub>46</sub> - <i>b</i> -PNVP <sub>68</sub> Amphiphilic Block Copolymer by Alkyne-Azide Click Reaction	127
5.2.6 Synthesis of PLGA <sub>46</sub> - <i>b</i> -PNVP <sub>89</sub> Amphiphilic Block Copolymer by Alkyne-Azide Click Reaction	127
<b>5.3 Results and Discussion</b>	<b>128</b>
5.3.1 Synthesis of Alkyne-terminated PLGA	128
5.3.2 Synthesis of Azide-terminated Poly (NVP) Macro-RAFT Agent	131
5.3.3 Synthesis of PLGA- <i>b</i> -PNVP Block Copolymers by Click Reaction	132
5.3.4 Self-assembly of Amphiphilic PLGA- <i>b</i> -PNVP Block Copolymers in Water	133
5.3.5 Drug Loading and Release	136
5.3.6 Cellular Viability and Toxicity of DOX-loaded PLGA <sub>46</sub> - <i>b</i> -PNVP <sub>89</sub> Micelles	138
5.3.7 Induction of Tumor Cell Apoptosis by DOX-loaded PLGA <sub>46</sub> - <i>b</i> -PNVP <sub>89</sub> Micelles	140
<b>5.4 Conclusions</b>	<b>142</b>
<b>Summary and Future Work</b>	<b>143-146</b>
<b>REFERENCES</b>	<b>147-164</b>
<b>PUBLICATIONS</b>	
<b>CARRICULAM VITE</b>	