

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA
INDUSTRIAL DE BARCELONA

UNIVERSIDAD POLITÉCNICA DE CATALUÑA

**“Synthesis, characterization and biomedical
applications of microbial polymalic
and polyglutamic acids derivatives.”**

Presentado por: José Antonio Portilla Arias

Trabajo realizado bajo la dirección de los Drs.
Sebastián Muñoz Guerra y Montserrat García Álvarez

Barcelona, Febrero 2008

Índice

1. Introducción	1
2. Polímeros biotecnológicos funcionalizados para aplicaciones biomédicas I.	
El ácido poli(β,L-málico)	5
2.1. Introducción	6
2.2. Estructura y propiedades	7
2.2.1. Estructura molecular	7
2.2.2. Propiedades físico-químicas.	7
2.2.3. Degradación hidrolítica y biodegradabilidad	8
2.3. Síntesis y biosíntesis	9
2.3.1. Síntesis química	9
2.3.2. Biosíntesis	10
2.4. Derivados del PMLA	12
2.4.1. Modificación del polímero de biosíntesis	12
2.4.2. Poli (β -malato)s de síntesis química	13
2.4.3. Complejos iónicos del PMLA	15
2.5. Aplicaciones biomédicas	16
2.6. Referencias	20
3. Polímeros biotecnológicos funcionalizados para aplicaciones biomédicas II.	
El ácido poli(γ-glutámico)	21
3.1. Introducción	22
3.2. Estructura y propiedades	22
3.2.1. Estructura molecular	22
3.2.2. Propiedades físico-químicas	23
3.2.3. Degradación hidrolítica y biodegradabilidad	24
3.3. Síntesis y biosíntesis	25
3.3.1. Síntesis química	25
3.3.1.1. Policondensación de ésteres activos	25
3.3.1.2. Policondensación por apertura de ciclos.	27
3.3.2. Biosíntesis	27

3.4. Derivados del PGGA	30
3.4.1. Modificación del polímero de biosíntesis	31
3.4.2. Poli(γ -glutamato)s de síntesis química	32
3.4.3. Complejos iónicos del PGGA	32
3.5. Aplicaciones biomédicas.	33
3.6. Referencias	36
4. Synthesis, Degradability and Drug Releasing Properties of Methyl Esters of Fungal Poly(β,L-malic acid)	39
4.1. Introduction	40
4.2. Experimental	41
4.2.1. Materials	41
4.2.2. Esterification	41
4.2.3. Hydrolytic and enzymatic degradation	42
4.2.4. Preparation of microspheres, hydrodegradation and erythromycin release	42
4.2.5. Measurements	43
4.3. Results and discussion	44
4.3.1. Partially methylated poly(β ,L-malic acid)s. Synthesis and characterization	44
4.3.2. Hydrolytic degradation	50
4.3.3. Microspheres of PMLA-Me: Preparation, hydrolytic degradation and erythromycin release	54
4.4. Conclusions	60
4.5. References	62
5. Nanostructured Complexes of Poly(β,L-malate) and Cationic Surfactants: Synthesis, Characterization and Structural Aspects	63
5.1. Introduction	64
5.2. Experimental	65
5.2.1. Materials	65
5.2.2. Conditions for Growth and PMLA Production.	66
5.2.3. Isolation and Purification of PMLA from Culture	66
5.2.4. Synthesis of Complexes	67
5.2.5. Measurements	67
5.3. Results and discussion	68
5.3.1. Synthesis and Characterization	68
5.3.2. Solid-State Structure	72
5.3.3. Liquid Crystal Behavior	82

5.4. Conclusions	84
5.5. References	85
6. Thermal Decomposition of Fungal Poly(β,L-malic acid) and Poly(β,L-malate)s	86
6.1. Introduction	87
6.2. Experimental	88
6.2.1. Materials	88
6.3. Results and discussion	90
6.3.1. Thermal Degradation of PMLA	90
6.3.2. Thermal Degradation of PAALM-1	96
6.3.3. Thermal Degradation of nATMA·PMLA Complexes	100
6.4. Conclusions	106
6.5. References	107
7. Thermal decomposition of microbial poly(γ-glutamic acid) and poly(γ-glutamate)s	108
7.1. Introduction	109
7.2. Experimental	110
7.2.1. Materials	111
7.3. Results and discussion	112
7.3.1. Thermal degradation of poly(γ -glutamic acid) and poly(α -methyl γ -glutamate)	112
7.3.2. Thermal degradation of n-alkyltrimethylammonium poly(γ -glutamate)s	118
7.4. Conclusions	122
7.5. References	124
8. Biodegradable nanoparticles of partially methylated fungal poly(β,L-malic acid) as a novel protein delivery carrier	125
8.1. Introduction	126
8.2. Experimental	127
8.2.1. Materials	127
8.2.2. Esterification	128
8.2.3. Nanoparticles: preparation and degradation	128
8.2.4. Protein loading	129
8.2.5. In vitro release studies and protein activity measurements	130
8.2.6. Measurements	130

8.3. Results and discussion	130
8.3.1. Preparation and characterization of coPMLA-(Me ₇₅ H ₂₅) nanoparticles	130
8.3.2. Hydrolytic degradation	131
8.3.3. Entrapment of protein into the nanoparticles	135
8.3.4. In vitro protein releasing from loaded coPMLA-(Me ₇₅ H ₂₅) nanoparticles	139
8.3.5. Protein activity loss	140
8.4. Conclusions	142
8.5. References	143

9. Nanoparticles made of microbial poly(γ-glutamate)s for encapsulation and delivery of drugs and proteins	144
9.1. Introduction	145
9.2. Experimental	146
9.2.1. Materials	146
9.2.2. Esterification of PGGA	147
9.2.3. Nanoparticles preparation and hydrolytic degradation	147
9.2.4 Erythromycin loading, encapsulation efficiency and release measurements	148
9.2.5. α -Chymotrypsin: loading, in vitro release studies and assays of activity	148
9.2.6. Measurements	149
9.3. Results and discussion	149
9.3.1. Synthesis of polyglutamates	149
9.3.2. Preparation, characterization and hydrolytic degradation	
of coPAAG-(R _x H _y) nanoparticles	151
9.3.3. Erythromycin encapsulation and release	153
9.3.4. Evaluation as protein carriers: α -Chymotrypsin encapsulation and release	155
9.4. Conclusions	158
9.5. References	160

10. Ionic complexes of biosynthetic polymalic and polyglutamic acids as prospective drug delivery systems	161
10.1. Introduction	162
10.2. Experimental	164
10.2.1. Materials	164
10.2.2. Preparation of Discs and Hydrolytic Degradation	165
10.2.3. Measurements	166

10.3. Results and discussion	167
10.3.1. Hydrolytic Degradation of Complexes	167
10.3.2. Effect of pH and Temperature	168
10.3.3. Degradation Mechanisms	170
10.3.4. Complexes as Drug-Delivery Systems	176
10.4. Conclusions	180
10.5. References	181
 11. Conclusions	182
 Apéndice A	185
Producción y purificación del ácido poli(β ,L-málico)	
A.1. Fermentación semilla	185
A.2. Fermentación en reactor de 20 L	185
A.3. Proceso de purificación del PMLA	187
A.4. Ensayo cuantitativos del ácido poli(β ,L-málico)	189
 Agradecimientos	191
Curriculum Vitae	192
Lista de publicaciones	192
Patentes	193
Comunicaciones a congresos y reuniones científicas	194