

Handbook of Polymers for Pharmaceutical Technologies

**Volume 3
Biodegradable Polymers**

Edited by

**Vijay Kumar Thakur and
Manju Kumari Thakur**



WILEY

Contents

Preface	xix
1 Bioactive Polysaccharides of Vegetable and Microbial Origins: An Overview	1
<i>Giuseppina Tommonaro, Annarita Poli, Paola Di Donato, Roberto Abbamondi Gennaro, Ilaria Finore and Barbara Nicolaus</i>	
1.1 Introduction	1
1.2 Anticarcinogenic Polysaccharides	3
1.2.1 Microbial Sources	3
1.2.2 Vegetable Sources	7
1.3 Anti-inflammatory/Immunostimulating Polysaccharides	8
1.3.1 Microbial Sources	9
1.3.2 Vegetable Sources	11
1.4 Antiviral Polysaccharides	13
1.4.1 Microbial Sources	13
1.4.2 Vegetable Sources	15
1.5 Antioxidant Polysaccharides	17
1.5.1 Microbial Sources	17
1.5.2 Vegetable Sources	19
1.6 Other Biotechnological Applications	21
1.7 Conclusions and Future Perspectives	23
Acknowledgments	23
Reference	24
2 Chitosan: An Emanating Polymeric Carrier for Drug Delivery	33
<i>Priti Girotra and Shailendra Kumar Singh</i>	
2.1 Introduction	33
2.2 Preparation of Chitosan	34
2.3 Physicochemical Properties of Chitosan	35
2.4 Biological Activities of Chitosan	36
2.4.1 Antimicrobial Activity	37
2.4.2 Hypolipidemic and Hypocholesterolemic Activity	37
2.4.3 Immunostimulatory Activity	37
2.4.4 Anti-Cancer Activity	38
2.4.5 Antioxidant Activity	38
2.4.6 Anti-Inflammatory Activity	38

2.4.7	Burn and Wound Healing Promoter	38
2.4.8	Antiulcer Potential	39
2.5	Pharmaceutical Applications of Chitosan	39
2.5.1	Mucoadhesive Drug Delivery Systems	44
2.5.1.1	Ophthalmic Drug Delivery	44
2.5.1.2	Buccal and Sublingual Drug Delivery	44
2.5.1.3	Nasal Drug Delivery	44
2.5.1.4	Gastro-Retentive Drug Delivery	45
2.5.1.5	Intravesicle Drug Delivery	45
2.5.1.6	Vaginal Drug Delivery	45
2.5.1.7	Rectal Drug Delivery	45
2.5.2	Targeted Drug Delivery	46
2.5.2.1	Brain Targeting	46
2.5.2.2	Colon Targeted Drug Delivery	46
2.5.3	Parenteral Drug Delivery	46
2.5.4	Transdermal Drug Delivery	46
2.5.5	Topical Drug Delivery	47
2.5.6	Proteins and Peptides Drug Delivery	47
2.5.7	Vaccine Delivery	47
2.5.8	Gene Delivery	48
2.5.9	Pharmaceutical Excipient in Tablets	48
2.5.10	Miscellaneous Applications	48
2.5.10.1	Wetting Agent	48
2.5.10.2	Coating Agent	49
2.5.10.3	Hair/Skin Care Cosmetics	49
2.5.10.4	Water Treatment	49
2.5.10.5	Food Industry Applications	49
2.5.10.6	Biomedical Applications	49
2.6	Functionalization of Chitosan	49
2.7	Conclusion and Future Perspectives	49
	Reference	51
3	Fungi as Sources of Polysaccharides for Pharmaceutical and Biomedical Applications	61
	<i>Filomena Freitas, Christophe Roca and Maria A. M. Reis</i>	
3.1	Introduction	61
3.2	The Fungal Cell	62
3.2.1	Cell Wall Structure	62
3.2.2	Capsular and Extracellular Polysaccharides	63
3.2.3	Polysaccharides Biosynthesis in Fungi	66
3.2.3.1	Carbohydrate Synthesis	66
3.2.3.2	Carbohydrate Polymerization	68
3.2.3.3	Polysaccharide Secretion	69
3.3	Polysaccharides Produced by Fungi	69
3.3.1	Glucans	69
3.3.2	Chitin, Chitosan and Their Complexes	73

3.3.3	Mannan-Containing Polysaccharides	75
3.3.4	Other Polysaccharides	77
3.4	Production and Extraction of Polysaccharides from Fungi	77
3.4.1	Fungal Sources and Cultivation Conditions	77
3.4.2	Fractionation and Isolation of Cell Wall Polysaccharides	79
3.4.3	Extraction of Extracellular Polysaccharides	80
3.5	Fungal Polysaccharides in Biomedical and Pharmaceutical Applications	81
3.5.1	Bioactive Compounds	82
3.5.1.1	Immunomodulating Activity	82
3.5.1.2	Antimicrobial and Antiviral Properties	84
3.5.1.3	Anticancer Effect	84
3.5.1.4	Antioxidants	85
3.5.1.5	Other Biological Activities	86
3.5.2	Excipients	86
3.5.3	Drug Delivery Agents	87
3.6	Commercial Exploitation of Fungal Polysaccharides in Biomedical and Pharmaceutical Applications	89
3.7	Conclusion and Future Perspective	91
	Reference	91
4	Environmentally Responsive Chitosan-based Nanocarriers (CBNs)	105
	<i>Ankit Jain and Sanjay K. Jain</i>	
4.1	Introduction	105
4.2	Graft Copolymerized CBNs	107
4.3	pH-Sensitive CBNs	109
4.4	Thermosensitive CBNs	111
4.5	pH-Sensitive and Thermosensitive CBNs	112
4.6	pH- and Ionic-Sensitive CBNs	113
4.7	Photosensitive CBNs	114
4.8	Electrical-Sensitive CBNs	115
4.9	Magneto-Responsive CBNs	115
4.10	Chemo-Sensitive CBNs	115
4.11	Biodegradation of Chitosan and Its Derivatives	116
4.11.1	Enzymatic Degradation	116
4.11.2	Chemical Degradation	118
4.11.3	<i>In-Vitro</i> Biodegradation	119
4.11.4	<i>In-Vivo</i> Biodegradation of CBNs	119
4.12	Toxicity of CBNs	120
4.13	Conclusions and Future Perspectives	120
	References	120
5	Biomass Derived and Biomass Inspired Polymers in Pharmaceutical Applications	127
	<i>Elisavet D. Bartzoka, Claudia Crestini and Heiko Lange</i>	
5.1	Introduction	127
5.2	Biodegradable Polymers in Biomedical Applications – Relevant Aspects	129
5.3	Biodegradable Natural Polymers in Pharmaceutical Applications	133

5.3.1	Polyethers	133
5.3.1.1	Cellulose	133
5.3.1.2	Hemicelluloses and Other Pectic Substances	137
5.3.1.3	Cyclodextrin	145
5.3.1.4	Alginate	147
5.3.1.5	Carrageenan	149
5.3.1.6	Chitin and Chitosan	149
5.3.1.7	Hyaluronic Acid	151
5.3.1.8	Microbial Exopolysaccharides	152
5.3.1.9	Natural Gums	155
5.3.1.10	Poly(ethylene glycol)	157
5.3.2	Polyesters	157
5.3.2.1	Polyhydroxyalkanoates (PHA)	157
5.3.2.2	Hydroxyapatite	159
5.3.2.3	Lactide Polymers	160
5.3.2.4	Glycolides	161
5.3.2.5	Lactide-Glycolide Copolymers	161
5.3.2.6	Poly(orthoesters)	162
5.3.2.7	Poly(ϵ -caprolactone)	163
5.3.3	Polyamides	163
5.3.3.1	Collagen	163
5.3.3.2	Gelatin	165
5.3.3.3	Albumin	165
5.3.3.4	Fibrin	166
5.3.3.5	Synthetic Polyamides	167
5.3.4	Polyanhydrides	168
5.3.5	Polyurethanes	170
5.3.6	Polymers with Mixed Linkage Motifs	170
5.3.6.1	Polydioxanone	170
5.3.6.2	Polyfumarates	171
5.3.6.3	Lignin	172
5.4	Micro- and Nanocrystalline Natural Polymers and Fibrils – General Regulative Considerations	175
5.5	Concluding Remarks and Outlook	176
	Reference	177
6	Modification of Cyclodextrin for Improvement of Complexation and Formulation Properties	205
	<i>Tapan K. Dash and V. Badireenath Konkimalla</i>	
	Abbreviations	205
6.1	Introduction	206
6.2	Cyclodextrin and Its Degradation	206
6.3	Complexation by CDs and Release	207
6.4	Modifications and Scope with Respect to Pharmaceutical Application	208

6.4.1	Modification to Improve Complexation Efficacy	209
6.4.1.1	Chemical Modification	209
6.4.1.2	Ionic Modification or Salt Formation	210
6.4.1.3	Environmental Modification	211
6.4.2	Modification for Improvement of Formulation Properties	213
6.4.2.1	Modulation of Release and Formulations	213
6.4.2.2	Targeting Features	216
6.4.2.3	Fluorescence and Its Attenuation	217
6.5	Concluding Remarks	218
	Acknowledgements	218
	Reference	218
7	Cellulose-, Ethylene Oxide- and Acrylic-Based Polymers in Assembled Module Technology (Dome Matrix')	225
	<i>Camillo Benetti, Paolo Colombo and Tin Wui Wong</i>	
7.1	Dome Matrix® Technology	225
7.1.1	Advantages as Drug Carrier	227
7.1.2	Preparation Methods	227
7.2	Polymers for Controlled Drug Release	228
7.3	Cellulose Derivatives	230
7.4	Acrylic Acid Polymers	232
7.5	Polymethacrylates	234
7.6	Polyethylene Oxide	236
7.7	Conclusions	237
	Acknowledgments	237
	Reference	237
8	Structured Biodegradable Polymers for Drug Delivery	243
	<i>Nishi Mody, Udita Agrawal, Rajeev Sharma and S. P. Vyas</i>	
8.1	Introduction	243
8.1.1	Advantages of Biodegradable Polymers	244
8.1.2	Disadvantages of Biodegradable Polymers	244
8.1.3	Factors Governing Biodegradation of Polymers	244
8.1.3.1	Effect of Polymer Structure	247
8.1.3.2	Effect of Polymer Morphology	247
8.1.3.3	Effect of Molecular Weight	248
8.1.3.4	Effect of Physical Properties	248
8.2	Classification	249
8.2.1	Polymer Classification	249
8.2.1.1	On the Basis of Origin	249
8.2.1.2	On the Basis of Polymerization	249
8.2.1.3	On the Basis of Degradation	249
8.2.1.4	On the Basis of Interaction of Polymer with Water	249
8.2.1.5	On the Basis of Type of Degradation	250
8.2.1.6	Smart Polymers	250
8.2.2	Characterization of Polymers	253

8.3	Degradation Processes in Biodegradable Polymers	254
8.3.1	Mechanism of Biodegradation	254
8.3.2	Hydrolytically Degradable Polymers as Biomaterials	255
8.3.2.1	Polyanhydrides	256
8.3.2.2	Poly(Alkyl Cyanoacrylate)s	257
8.3.2.3	Polyphosphoesters	257
8.3.3	Enzymatically Degradable Polymers	257
8.3.3.1	Proteins and Poly(Amino Acid)s	258
8.3.3.2	Natural Poly(Amino Acid)s	258
8.3.3.3	Synthetic Poly(Amino Acid)s	259
8.3.3.4	Albumin	259
8.3.3.5	Polysaccharides	259
8.4	Responsive Stimuli-Sensitive Polymers	260
8.4.1	pH-Sensitive Polymers	262
8.4.1.1	Applications	263
8.4.2	Temperature Responsive Polymers	269
8.4.3	Polymers with Dual Stimuli Responsiveness	270
8.4.4	Phase-Sensitive Smart Polymers	270
8.4.5	Light-Sensitive Smart Polymers	271
8.5	Conclusion and Future Prospects	271
	References	271
9	Current State of the Potential Use of Chitosan as Pharmaceutical Excipient	275
	<i>A. Raquel Madureira, Bruno Sarmento and Manuela Pintado</i>	
9.1	The World of Pharmaceutical Excipients	275
9.2	Chitosan	276
9.3	Activities Found for Chitosan	277
9.3.1	Antimicrobial Activity	278
9.3.2	Antioxidant Activity	279
9.3.3	Anti-Inflammatory Activity	279
9.3.4	Haemostatic Activity	279
9.3.5	Antitumor Activity	280
9.3.6	Hypocholesterolemic Activity	280
9.4	Properties of Chitosan	280
9.4.1	Viscosity	280
9.4.2	Biocompatibility	281
9.4.3	Biodegradation	281
9.5	Applications as a Pharmaceutical Excipient	282
9.5.1	Tablets	282
9.5.2	Chitosan Microspheres/Nanoparticles	284
9.5.3	Drug Delivery	284
9.5.4	Tissue Engineering Agent	288
9.6	Conclusion	289
	References	290

10	Modification of Gums: Synthesis Techniques and Pharmaceutical Benefits	299
	<i>Vikas Rana, Sunil Kamboj, Radhika Sharma and Kuldeep Singh</i>	
10.1	Introduction	299
10.2	Synthesis of Modified Gums	302
10.2.1	Gum Modification Using Chemical Reaction	302
10.2.1.1	Carboxymethylation	302
10.2.1.2	Carbamoylethylation	304
10.2.1.3	Sulfation	305
10.2.1.4	Phosphorylation	306
10.2.1.5	Thiolation	306
10.2.1.6	Gums Grafted with Acrylic Acid or Its Derivatives	308
10.2.2	Modification of Gums via Crosslinking Technique	313
10.2.2.1	Crosslinking with Glutaraldehyde Group	313
10.2.2.2	Phosphate Crosslinking of Natural Gums	315
10.2.2.3	Crosslinking with Ions	316
10.2.2.4	Crosslinking with Epichlorohydrin	317
10.2.2.5	Mechanism of Crosslinking: Modification of Gums	318
10.2.2.6	Crosslinker Interaction Chemistry	318
10.3	Characterization	320
10.3.1	Spectral Attributes	320
10.3.1.1	X-ray Powder Diffraction (XpRD)	320
10.3.1.2	Nuclear Magnetic Resonance (NMR)	321
10.3.1.3	Fourier Transform Infrared Spectroscopy (FTIR)	323
10.3.2	Thermal Attributes	325
10.3.3	Scanning Electron Microscopy (SEM)	327
10.3.4	Rheology	329
10.3.5	Average Molecular Weight	331
10.4	Pharmaceutical Applications of Modified Gums	332
10.4.1	Tablet Formulations	332
10.4.2	Mucoadhesion-Based Delivery System	347
10.4.3	Colon-Specific Drug Delivery System	348
10.4.4	Nanoparticles	351
10.4.5	Microspheres	352
10.4.6	Hydrogels	353
10.5	Conclusion and Future Prospective	354
	Reference	355
11	Biomaterials for Functional Applications in the Oral Cavity via Contemporary Multidimensional Science	365
	<i>V. Tamara Perchyonok, Vanessa Reher, Nicolaas Basson and Sias Grobler</i>	
11.1	Introduction	365
11.2	Free Radical Formation, Antioxidants and Relevance in Health	366
11.2.1	Generation of Free Radicals in Living Systems	366
11.2.2	Antioxidants	367
11.2.3	Oxidative Stress	368
11.2.4	Advantages of Free Radicals in the Cell	368

11.3	Oral Diseases: Oxidative Stress and the Role of Antioxidant Defenses in the Oral Cavity	369
11.3.1	Oral Mucosa and Design of Buccal Drug-Delivery Systems	370
11.4	Biomaterials and Intelligent Design of Functional Biomaterials	371
11.4.1	Hydrogels as Carrier Molecules	371
11.4.2	Chitosan Hydrogels as a Vehicle for Optimal Oral Drug-Delivery Systems	371
11.5	<i>In-Vitro</i> Developments of Free Radical Defense Mechanisms and Drug-Delivery Systems	372
11.6	Practical <i>In-Vitro</i> Applications of Chitosan-Based Functional Biomaterial Prototypes in Dentistry	375
11.6.1	Preventive Dentistry and Chitosan	375
11.6.2	Restorative Dentistry and Chitosan	377
11.6.2.1	Mechanistic Problems in Bonding to Dentin: Can it be overcome?	378
11.6.2.2	Development of Dentin Adhesives	379
11.6.2.3	Chitosan as a Bioadhesive Material	380
11.6.2.4	Chitosan and Derivatives as Bioactive Materials	381
11.6.2.5	Cytotoxic Effect of Chitosan Hydrogels	382
11.6.2.6	Antioxidant Containing Chitosan Hydrogels on Dentine Bond Strength: <i>In-Vitro</i> Approach	383
11.6.3	Antibiotics in Dentistry	390
11.6.3.1	A Brief Introduction	390
11.6.3.2	The Target: Microorganism	390
11.6.3.3	Functional Antibiotic/Antioxidant-Containing Chitosan Hydrogels	391
11.6.3.4	Insights into Performance of Antibiotic-Chitosan Gels as Effective Free Radical Defense Functional Material	396
11.6.4	Analgesics/Anti-Inflammatories in Dentistry	397
11.6.4.1	Pain and Antioxidants Control	397
11.6.4.2	Pain and Aspirin, Naproxen and Ibuprofen Containing Hydrogels: <i>In-Vitro</i> Studies	398
11.7	Conclusion	398
	References	399
12	Role of Polymers in Ternary Drug Cyclodextrin Complexes	413
	<i>Renu Chadha, Madhu Bala, Parnika, Kunal Chadha and Maninder Karan</i>	
12.1	Introduction	413
12.2	Cyclodextrins (Cycloamyloses, Cyclomaltoses, Schardinger Dextrins)	414
12.2.1	Properties and Characteristics	414
12.2.2	Cyclodextrin Inclusion Complexes	415
12.3	Role of Biodegradable/Water-Soluble Polymers in Efficacy of Inclusion Complexes	416
12.3.1	Merits of Water-Soluble Polymers	417
12.3.2	Polymer-Cyclodextrin Interactions	418

12.3.3	CD-Containing Polymers	419
12.3.4	Characterization of Ternary Complexes Containing Drug, Polymer and CD	420
12.3.4.1	Solid-State Characterization of Complexes	420
12.3.4.2	Characterization of Inclusion Complexation in Solution State	422
12.4	Solubility, Dissolution and Bioavailability Enhancement: Case Studies	423
12.5	Conclusion	433
	References	433
13	Collagen-Based Materials for Pharmaceutical Applications	439
	<i>Daniela Pamfil, Manuela Tatiana Nistor and Cornelia Vasile</i>	
13.1	Introduction	439
13.2	Collagen Structure and Its Properties	440
13.2.1	Structure	440
13.2.2	Collagen Properties	440
13.3	Preparation Methods of Collagen-Based Biomaterials	443
13.3.1	Blends Based on Collagen	444
13.3.2	Chemical Analogous Modifications in Collagen Molecule	445
13.3.3	Crosslinked Collagen-Based Structures	446
13.3.3.1	Chemical Crosslinking of Collagen	446
13.3.3.2	Collagen-Based Hydrogels	449
13.4	Pharmaceutical Applications of Collagen-Based Products	450
13.4.1	Available Forms of Collagen-Based Materials in the Pharmaceutical Area	450
13.4.1.1	Collagen Sponges for Wound Dressing	450
13.4.1.2	Collagen-Based Hydrogels	452
13.4.1.3	Nanoparticles/Nanospheres/Microspheres	452
13.4.1.4	Liposome	452
13.4.1.5	Collagen Membranes	452
13.4.2	Collagen Wound Dressings	452
13.4.2.1	Modifications in Collagen Structure for Wound Dressings Applications	454
13.4.2.2	Drug Delivery Applied for Wound Dressings	454
13.4.3	Collagen-Based Materials for Drug Delivery	455
13.5	Concluding Remarks and Future Perspectives	462
	Acknowledgments	468
	References	468
14	Natural Polysaccharides as Pharmaceutical Excipients	483
	<i>Nazire Deniz Yılmaz, Gülbanu Koyundereli Çılgı and Kenan Yılmaz</i>	
14.1	Introduction	483
14.2	Natural Polysaccharides	485
14.2.1	Plant Polysaccharides	485
14.2.1.1	Cellulose	485
14.2.1.2	Hemicellulose	489
14.2.1.3	Pectins	490
14.2.1.4	Starches	492

14.2.1.5	Inulin	494
14.2.1.6	Gums and Mucilages	494
14.2.2	Seaweed Polysaccharides	502
14.2.2.1	Alginates	502
14.2.2.2	Carrageenans	503
14.2.2.3	Gum Agar	504
14.2.3	Microbial Polysaccharides	505
14.2.3.1	Xanthan Gum	505
14.2.3.2	Gellan Gum	507
14.2.3.3	Pullulan	508
14.2.4	Animal Polysaccharides	508
14.2.4.1	Chitin	508
14.2.4.2	Chitosan	509
14.3	Conclusion	510
	Reference	510
15	Structure, Chemistry and Pharmaceutical Applications of Biodegradable Polymers	517
	<i>Mazhar Ul-Islam, Shaukat Khan, Muhammad Wajid Ullah and Joong Kon Park</i>	
15.1	Introduction	517
15.2	History of Polymers	518
15.3	Concept of Biodegradability	522
15.4	Biodegradable Polymers and Their Classification	522
15.4.1	Agropolymers	523
15.4.1.1	Starch-Based Polymers	523
15.4.1.2	Protein-Based Biodegradable Polymers	525
15.4.2	Biodegradable Polymers from Natural or Microbial Sources (Polyesters)	525
15.4.2.1	Microbial Polyesters	525
15.4.2.2	Bacterial Cellulose	525
15.4.2.3	Polyhydroxyalcanoates (PHAs)	526
15.4.2.4	Polylactic Acid (PLA)	526
15.4.3	Synthetic Biodegradable Polymers	528
15.5	Biocompatibility of Biodegradable Polymers	528
15.6	Biodegradable Polymers in Pharmaceutical Applications	530
15.6.1	Biodegradable Polymers in Drug Delivery	530
15.6.2	Limitations of Conventional Drug-Delivery Systems	530
15.6.3	Advantages of Using Biodegradable Polymers in Drug Delivery	531
15.6.4	Factors Affecting the Degradation of Polymers	531
15.6.5	Mechanisms of Action of Drug Release from Biodegradable Polymers	532
15.7	Development of Various Biodegradable Polymer Systems for Drug Delivery	532
15.7.1	Microparticles	533
15.7.2	Emulsions	534

15.7.3	Liposomes	534
15.7.4	Micelles	534
15.7.5	Injectibles	534
15.7.6	Elastomers	535
15.7.7	Hydrogels	535
15.8	Future Prospects	535
	Acknowledgment	536
	Reference	536
16	Preparation and Properties of Biopolymers: A Critical Review	541
	<i>Selvaraj Mohana Roopan, T. V. Surendra and G. Madhumitha</i>	
16.1	Introduction	541
16.2	Nature of Biopolymers	543
16.2.1	Life Cycle Assessment of Biopolymers	543
16.2.2	Life Cycle Assessment as a Method for Quantifying Environmental Impacts	543
16.3	Common Biopolymers	544
16.4	Biopolymers in Drug Development	545
16.4.1	Cellulose and Its Derivatives	546
16.4.2	Starch	546
16.4.3	Hemicellulose	547
16.4.4	Pectin	547
16.5	Biobased Polymers Production	548
16.5.1	Polylactic Acid	548
16.5.2	Polyhydroxy Alkanoates	549
16.5.3	Polybutylene Succinate	550
16.5.4	Biopolyethylene	550
16.6	Properties of Biopolymers	551
16.6.1	Physical Properties	551
16.6.2	Mechanical Properties	552
16.6.3	Specific Mechanical Properties	552
16.6.4	Fiber Mechanical Properties	553
16.6	Conclusion and Remarks	553
	Acknowledgement	553
	Reference	553
17	Engineering Biodegradable Polymers to Control Their Degradation and Optimize Their Use as Delivery and Theranostic Systems	557
	<i>Ilaria Armentano, Loredana Latterini, Nicoletta Rescignano, Luigi Tarpani, Elena Fortunati and Josè Maria Kenny</i>	
17.1	Introduction	557
17.2	Nanotechnology	559
17.3	Nanostructured Biodegradable Polymers	560
17.3.1	Biopolymeric Nanoparticles	560

17.3.2	BioPolymeric Hybrid Nanoparticles	561
17.3.2.1	Magnetic Nanoshell	562
17.3.2.2	Metal Nanoshell	563
17.3.3	Bionanocomposites	563
17.4	Design Strategies for Fluorescent Biodegradable Polymeric Systems	566
17.4.1	Fluorescence Spectroscopy	566
17.4.2	Generation and Detection of Fluorescence Signals on Nanostructured Polymers	567
17.4.3	Fluorescence Imaging Methods	568
17.5	Conclusions and Perspectives	570
	Reference	570
	Index	577