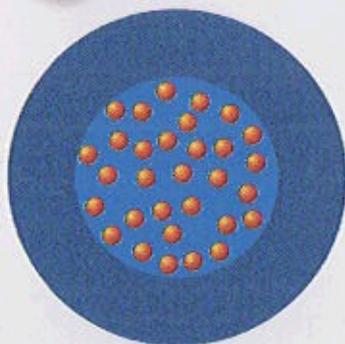
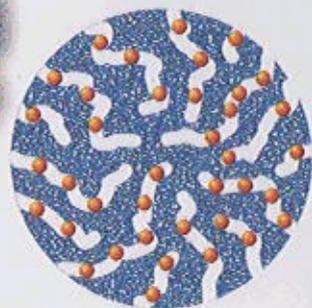
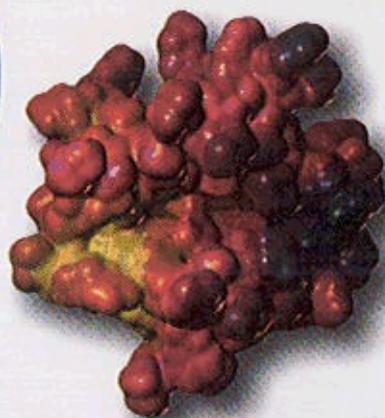
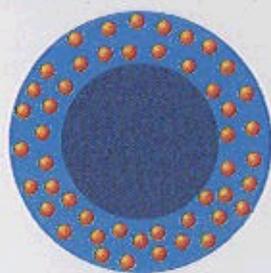
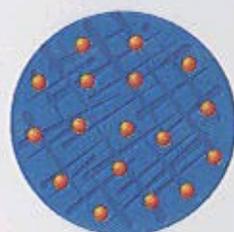
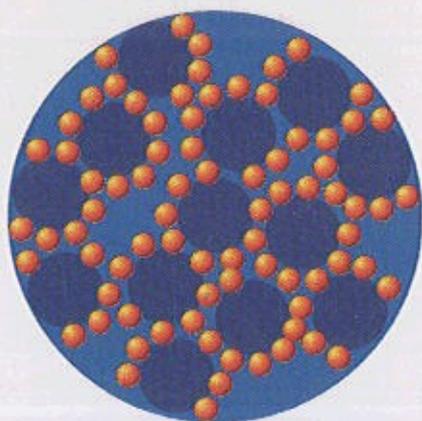


Linqiu Cao

WILEY-VCH

Carrier-bound Immobilized Enzymes

Principles, Applications and Design



Contents

	Foreword	V
1	Introduction: Immobilized Enzymes: Past, Present and Prospects	1
1.1	Introduction	1
1.2	The Past	4
1.2.1	The Early Days (1916–1940s)	5
1.2.2	The Underdeveloped Phase (1950s)	5
1.2.3	The Developing Phase (1960s)	7
1.2.4	The Developed Phase (1970s)	9
1.2.5	The Post-developed Phase (1980s)	14
1.2.6	Rational Design of Immobilized Enzymes (1990s–date)	16
1.3	Immobilized Enzymes: Implications from the Past	20
1.3.1	Methods of Immobilization	20
1.3.2	Diversity versus Versatility	21
1.3.3	Complimentary versus Alternative	23
1.3.4	Modification versus Immobilization	25
1.3.4.1	Enhanced Stability	25
1.3.4.2	Enhanced Activity	26
1.3.4.3	Improved Selectivity	29
1.4	Prospective and Future Development	34
1.4.1	The Room for Further Development	34
1.4.2	An Integration Approach	36
1.5	References	37
2	Adsorption-based Immobilization	53
2.1	Introduction	53
2.2	Classification of Adsorption	54
2.3	Principles Involved in Adsorptive Enzyme Immobilization	55
2.3.1	Monolayer Principle	56
2.3.2	Stabilization Principle	57
2.3.3	Enzyme Distribution	60
2.4	Requirement of the Carriers	61
2.4.1	Physical Requirements	61

2.4.1.1	Pore-size and Available Surface	61
2.4.1.2	Internal Structure	63
2.4.1.3	Density of Binding Functionality	63
2.4.1.4	Particle Size	64
2.4.2	Chemical Nature of the Carriers	65
2.4.2.1	Nature of Binding Functionality	65
2.4.2.2	The Role of the Spacer	66
2.4.2.3	The Nature of the Backbone	66
2.5	Factors Which Dictate Enzyme Catalytic Performance	67
2.5.1	Activity	67
2.5.1.1	Diffusion-controlled Activity	67
2.5.1.2	Conformation-controlled Activity	68
2.5.1.3	Substrate-controlled Activity	70
2.5.1.4	Loading-controlled Activity	70
2.5.1.5	Medium-dependent Activity	72
2.5.1.6	Microenvironment-dependent Activity	73
2.5.1.7	Carrier Nature-dependent Activity	74
2.5.1.8	Enzyme Nature-dependent Activity	75
2.5.1.9	Additive-dependent Activity	75
2.5.1.10	Hydrophilicity-dependent Activity	76
2.5.1.11	Orientation-determined Activity	78
2.5.1.12	Binding Nature-controlled Enzyme Activity	78
2.5.1.13	Binding Density-controlled Enzyme Activity	80
2.5.1.14	Reactor-dependent Activity	81
2.5.1.15	Pore-size-dependent Activity	82
2.5.1.16	Water-activity-dependent Activity	82
2.5.2	Stability	83
2.5.2.1	Conformation-controlled Stability	84
2.5.2.2	Confinement-controlled Stability	85
2.5.2.3	Enzyme Loading-dependent Stability	85
2.5.2.4	Diffusion-controlled Stability	86
2.5.2.5	Cross-linking-dependent Stability	86
2.5.2.6	Carrier Nature-controlled Stability	86
2.5.2.7	Aquaphilicity-controlled Stability	87
2.5.2.8	Medium-controlled Stability	88
2.5.2.9	Temperature-dependent Stability	88
2.5.2.10	Microenvironment-controlled Stability	89
2.5.2.11	Binding Nature-controlled Enzyme Stability	90
2.5.2.12	Binding Density-controlled Enzyme Stability	91
2.5.2.13	Additive-dependent Stability	91
2.5.2.14	Enzyme Orientation-dependent Stability	91
2.5.2.15	Enzyme-dependent Stability	91
2.5.3	Selectivity	92
2.5.3.1	Conformation-controlled Selectivity	93
2.5.3.2	Diffusion-controlled Selectivity	94

2.5.3.3	Binding Functionality-controlled Selectivity	94
2.5.3.4	Additive-controlled Selectivity	95
2.5.3.5	Orientation-controlled Selectivity	96
2.5.3.6	Medium-controlled Enantioselectivity	96
2.5.3.7	Water Activity-controlled Enantioselectivity	97
2.6	Preparation of Immobilized Enzymes by Adsorption	97
2.6.1	Conventional Adsorption	97
2.6.1.1	Non-specific Physical Adsorption	99
2.6.1.2	Ionic Adsorption	100
2.6.1.3	Hydrophobic Adsorption	108
2.6.1.4	Biospecific Adsorption	113
2.6.1.5	Affinity Adsorption	116
2.6.2	Unconventional Adsorption	121
2.6.2.1	Immobilization via Reversible Denaturation	123
2.6.2.2	Pseudo-covalent Immobilization	123
2.6.2.3	Mediated Adsorption	124
2.6.3	Adsorption-based Double Immobilization	128
2.6.3.1	Modification–Adsorption	128
2.6.3.2	Adsorption and Entrapment	131
2.6.3.3	Adsorption–Cross-linking	134
2.6.3.4	Adsorption–Covalent Attachment	142
2.7	References	145
3	Covalent Enzyme Immobilization	169
3.1	Introduction	169
3.2	Physical Nature of Carriers	171
3.2.1	The Surface of the Carriers	172
3.2.1.1	Internal and External Surface	173
3.2.1.2	Accessible Surface/Efficient Surface	173
3.2.1.3	A Theoretical Simulation	175
3.2.2	Density of Binding Sites	176
3.2.3	Pore Related Properties	177
3.2.3.1	The Porosity	177
3.2.3.2	Pore Size and Distribution	178
3.2.4	Particle Size	180
3.2.5	Shape of the Carriers	182
3.3	Chemical Nature of Carriers	183
3.3.1	Carrier-bound Active Groups (CAG)	185
3.3.2	Carrier-bound Inert Groups (CIG)	187
3.3.3	Spacer-Arm	188
3.4	Enzyme: Amino Acid Residues for Covalent Binding	190
3.4.1	Reactivity of Amino Acid Residues (AAR)	191
3.4.2	Position of Active Amino Acids	192
3.5	Factors Affecting Enzyme Performance	193
3.5.1	Activity Retention	194

- 3.5.1.1 Pore-size-dependent Activity 194
- 3.5.1.2 CAG-controlled Activity 196
- 3.5.1.3 CIG-controlled Retention of Activity 200
- 3.5.1.4 Spacer-controlled Activity 205
- 3.5.1.5 Enzyme Orientation-controlled Activity 210
- 3.5.1.6 Binding Density-controlled Activity 210
- 3.5.1.7 Diffusion-controlled Enzyme Activity 211
- 3.5.1.8 Reactive Amino Acid Residues (RAAR)-controlled Activity 212
- 3.5.1.9 Loading-dependent Activity 213
- 3.5.1.10 Other Factors Controlling Activity 213
- 3.5.2 Stability of Immobilized Enzymes 214
 - 3.5.2.1 Multipoint Attachment/binding density 215
 - 3.5.2.2 CAG-controlled Stability 217
 - 3.5.2.3 CIG-controlled Enzyme Stability 221
 - 3.5.2.4 Spacer-dependent Stability 224
 - 3.5.2.5 Molecular Confinement-controlled Stability 225
 - 3.5.2.6 Microenvironment-controlled Stability 227
- 3.5.3 Selectivity of Immobilized Enzymes 228
 - 3.5.3.1 CAG-controlled Selectivity 228
 - 3.5.3.2 CIG-controlled Selectivity 230
 - 3.5.3.3 Spacer-controlled Enzyme Selectivity 232
 - 3.5.3.4 Diffusion-controlled Selectivity 233
 - 3.5.3.5 Aquaphilicity-controlled Selectivity 233
 - 3.5.3.6 Conformation-controlled Enantioselectivity 233
 - 3.5.3.7 Selectivity and Particle Size 234
- 3.6 Preparation of Active Carriers 235
 - 3.6.1 Synthetic Active Carriers 237
 - 3.6.1.1 Polymers Bearing Acyl Azide 237
 - 3.6.1.2 Polymers Bearing Anhydrides 238
 - 3.6.1.3 Polymers Bearing Halogen Atoms 240
 - 3.6.1.4 Oxirane Functional Polymers 241
 - 3.6.1.5 Isocyanate/Thioisocyanate Functional Polymers 244
 - 3.6.1.6 Polycarbonate 245
 - 3.6.1.7 Activated Carbonyl Polymers 247
 - 3.6.1.8 Polyphenolic Polymers 248
 - 3.6.1.9 Polymeric Carriers Bearing Aldehyde Groups 249
 - 3.6.1.10 Polymers Bearing Activated Ester 251
 - 3.6.1.11 Polymers Bearing Active Azalactone 252
 - 3.6.2 Inactive Pre-carriers 253
 - 3.6.2.1 Hydroxyl Functionality 253
 - 3.6.2.2 Polyacrylamide 254
 - 3.6.2.3 Insoluble Polyacrylic Acid or Derivatives 255
 - 3.6.2.4 Polymers Bearing Nitrile Groups 257
 - 3.6.2.5 Semi-synthetic Polysaccharides 258
 - 3.6.2.6 Synthetic Polypeptide 259

3.6.2.7	Polymers Bearing Amino Groups	260
3.6.3	Interconversion of Inert Carriers	260
3.6.3.1	Polymers Containing Hydroxyl Groups	261
3.6.3.2	Activation of Separate Hydroxyl Groups	268
3.6.3.3	Polymers Containing Carboxylic or Ester Groups	272
3.6.3.4	Polymers Containing Amino Groups	277
3.6.3.5	Polymers Containing Amide Groups	283
3.6.3.6	Polymers Containing Nitrile Groups	286
3.6.3.7	Polymers Bearing Isonitrile Functional Groups	287
3.6.4	Interconversion of Active Functionality	288
3.6.4.1	Converting Epoxy Groups	289
3.6.4.2	Converting Anhydride to New Functionality	290
3.6.4.3	Aldehyde	292
3.7	References	293
4	Enzyme Entrapment	317
4.1	Introduction	317
4.2	Definition of Entrapment	319
4.3	Requirement of the Carriers	321
4.3.1	Physical Requirements	321
4.3.1.1	Pore Size	321
4.3.1.2	Porosity	322
4.3.1.3	Geometry	322
4.3.1.4	Particle Size	323
4.3.2	Chemical Requirements	323
4.3.2.1	Nature of the Active Functionality	323
4.3.2.2	Aquaphilicity of the Carriers	324
4.4	Effect of Entrapment	324
4.4.1	Activity of the Entrapped Enzyme	324
4.4.1.1	Loading-dependent Activity	325
4.4.1.2	Matrix-dependent Activity	325
4.4.1.3	Diffusion-controlled Enzyme Activity	326
4.4.1.4	Conformation-controlled Enzyme Activity	327
4.4.1.5	Additives-controlled Enzyme Activity	328
4.4.2	Stability	328
4.4.2.1	Confinement-determined Stability	329
4.4.2.2	Matrix-nature-dependent Stability	329
4.4.2.3	Enzyme-dependent Stability	330
4.4.2.4	Enzyme Structure-dependent Stability	331
4.4.3	Selectivity	331
4.4.3.1	Microenvironment-dependent Selectivity	331
4.4.3.2	Conformation-dependent Selectivity	332
4.4.3.3	Carrier Nature-dependent Selectivity	333
4.5	Preparation of Various Entrapped Enzymes	333
4.5.1	Conventional Entrapment Process	334

4.5.1.1	Formation of Entrapment Matrix by Chemical Cross-linking	334
4.5.1.2	Physical Entrapment	342
4.5.1.3	Covalent Entrapment	351
4.5.1.4	Sol–Gel Process	356
4.5.2	Non-conventional Entrapment	362
4.5.2.1	Post-loading Entrapment (PLE)	362
4.5.2.2	Entrapment-based Double-immobilization Technique	364
4.5.2.3	Modification and Entrapment	368
4.5.2.4	Supported Entrapment	371
4.6	References	379

5 Enzyme Encapsulation 397

5.1	Introduction	397
5.1.1	General Considerations	398
5.1.2	An Historical Overview	398
5.1.3	Pros and Cons of Micro-encapsulation	399
5.2	Classification of Encapsulation	399
5.2.1	Conventional Encapsulation	399
5.2.1.1	Encapsulation by an Interfacial Process	400
5.2.1.2	Encapsulation by Phase Inversion	401
5.2.2	Non-conventional Encapsulation	401
5.2.2.1	Encapsulation in Liquid Membrane	401
5.2.2.2	Encapsulation–Reticulation	401
5.2.3	Double Immobilization Based on Encapsulation	402
5.2.3.1	Encapsulation–Cross-linking	402
5.2.3.2	Encapsulation and Coating	402
5.2.3.3	Entrapment and Coating	403
5.2.3.4	Immobilization and Encapsulation	403
5.2.4	Post-loading Encapsulation	404
5.2.4.1	Encapsulation in Non-swellable Microcapsules	405
5.2.4.2	Encapsulation in Soft Microcapsules	405
5.3	Effect of Encapsulation	406
5.3.1	Activity of the Encapsulated Enzymes	406
5.3.2	Stability of the Encapsulated Enzymes	407
5.3.3	Enantioselectivity	407
5.4	Processes for Preparation of Encapsulated Enzymes	407
5.4.1	Interfacial Processes	407
5.4.1.1	Interfacial Cross-linking/Polymerization	408
5.4.1.2	Interfacial Physical Gelation	411
5.4.2	Surfactant-related Hollow Microsphere	412
5.4.2.1	Microemulsion-based Encapsulated Enzymes	412
5.4.2.2	Polymeric Micelles/Liposomes	416
5.4.2.3	Liposome capsules	416
5.4.2.4	Colloidal Liquid Aphrons (CLA)	421
5.4.3	Phase Inversion	423

- 5.4.3.1 Coacervation 423
- 5.4.3.2 The Double-emulsion Method 423
- 5.4.3.3 Modified Double-emulsion Methods 426
- 5.4.3.4 Other Methods 429
- 5.4.4 Pre-designed Capsules for Post-loading Encapsulation 429
 - 5.4.4.1 Introduction 429
 - 5.4.4.2 Theoretical Considerations 430
- 5.4.5 Non-conventional Encapsulation Processes 430
 - 5.4.5.1 Encapsulation/Coating 430
 - 5.4.5.2 Encapsulation/Cross-linking 432
 - 5.4.5.3 Immobilization and Encapsulation 434
 - 5.4.5.4 Immobilization and Encapsulation 434
- 5.5 References 438

- 6 Unconventional Enzyme Immobilization 449**
 - 6.1 Introduction 449
 - 6.2 Coating-based Enzyme Immobilization 450
 - 6.2.1 Monolayer Enzymes 451
 - 6.2.2 Phase-inversion Coating 451
 - 6.2.3 Multiple Enzyme Coating by Physical Adsorption 452
 - 6.2.4 Mediated Formation of Multiple Enzyme Layers 452
 - 6.2.5 Affinity-ligand-mediated Formation of Enzyme Coatings 455
 - 6.2.6 Coating of Soluble Enzyme–Polymer Complexes 456
 - 6.2.7 Enzymatically Gelified Multienzyme Layer 456
 - 6.2.8 Sol–Gel Coating and Covalent Attachment 457
 - 6.2.9 Electrochemical Deposition 457
 - 6.2.10 Enzyme Coating by Use of Small Pore-size Carriers 458
 - 6.3 Site-specific Immobilization 458
 - 6.3.1 Site-specific Immobilization via Biospecific Ligand–Enzyme Interaction 463
 - 6.3.2 Introduction of Chemical Tags 464
 - 6.3.2.1 Oxidation of the Sugar Moiety of Enzymes 466
 - 6.3.2.2 Introduction of a Cofactor into the Enzyme 466
 - 6.3.2.3 Orientation and Covalent Binding 466
 - 6.3.3 Immobilized Ligand (Substrate Analogue) Enzyme-binding 468
 - 6.3.3.1 Immobilized Substrate or Substrate Analogues 470
 - 6.3.3.2 Immobilized Non-substrate Ligand 470
 - 6.3.4 Genetically Engineered Tags 473
 - 6.3.4.1 Non-covalent Oriented Enzyme Immobilization 473
 - 6.3.4.2 Covalent Orientation in Enzyme Immobilization 477
 - 6.4 Immobilization in Organic Solvents 477
 - 6.4.1 Covalent Attachment in Organic Solvents 478
 - 6.4.2 Entrapment of Enzyme in Organic Solvent 479
 - 6.4.3 Immobilization of Organic-soluble Enzyme Derivatives 480
 - 6.4.4 Adsorption of Enzyme on to the Carrier in Organic Solvents 480

- 6.5 Imprinting–Immobilization 481
 - 6.5.1 Imprinting–Multipoint Attachment 481
 - 6.5.2 Imprinting–Cross-linking 482
 - 6.5.3 Entrapment–Imprinting 484
 - 6.5.4 Crystallization and Cross-linking 484
 - 6.5.5 Aggregation and Cross-linking 485
 - 6.5.6 Intra-molecular Cross-linking – Imprinting 485
 - 6.5.7 Post-immobilization Imprinting 486
 - 6.5.8 Lyophilization Imprinting 486
- 6.6 Stabilization–Immobilization 487
 - 6.6.1 Stabilization by Ligand Binding 488
 - 6.6.2 Stabilization by Addition of Stabilizer as the Excipient of the Conformation 490
 - 6.6.3 Stabilization by Pre-immobilization Modification 490
 - 6.6.3.1 Stabilization by Pre-immobilization Modification with Soluble Polymer 490
 - 6.6.3.2 Stabilization by Pre-immobilization Chemical Modification 492
 - 6.6.3.3 Chemical Cross-linking/Covalent Immobilization 493
- 6.7 Modification-based Enzyme Immobilization 493
 - 6.7.1 Immobilization then Modification 494
 - 6.7.2 Modification then Polymerization 494
 - 6.7.3 Pre-immobilization Improvement Techniques (PIT) 496
 - 6.7.3.1 Introduction of Extra Charge 497
 - 6.7.3.2 Alteration of Enzyme Hydrophobicity 498
 - 6.7.3.3 Formation of Polymer–Enzyme Conjugate 498
 - 6.7.3.4 Introduction of Active Functionality for Covalent Binding 503
 - 6.7.3.5 Introduction of Mediators 505
 - 6.7.3.6 Interconversion of Amino Acid Residues (AAR) 505
 - 6.7.3.7 Cross-linking/Immobilization 506
- 6.8 Post-Immobilization Techniques 506
 - 6.8.1 Introduction 506
 - 6.8.2 Classification of Post-treatments 507
 - 6.8.3 Physical Methods 507
 - 6.8.3.1 Increasing the pH 509
 - 6.8.3.2 Solvent Washing 510
 - 6.8.3.3 Lyophilization/Drying/Addition of Additives 510
 - 6.8.3.4 pH Imprinting 511
 - 6.8.3.5 Physical Entrapment 512
 - 6.8.3.6 Thermal Activation 512
 - 6.8.3.7 Activation by Denaturants 513
 - 6.8.3.8 Post-immobilization by Physical Coating 513
 - 6.8.3.9 Rehydration/water Activity Adjustment 514
 - 6.8.3.10 Sonication 515
 - 6.8.3.11 Acid or Alkaline Treatment 515
 - 6.8.4 Chemical Methods 515

6.8.4.1	Consecutive Cross-linking of the Immobilized Enzymes	516
6.8.4.2	Consecutive Chemical Modification of Immobilized Enzymes	518
6.8.4.3	Conversion	518
6.8.4.4	Consecutive Modification of the Carrier	519
6.8.4.5	Chemical Coating	521
6.8.5	Outlook	522
6.9	Reversibly Soluble Immobilized Enzymes	522
6.9.1	pH-responsive Smart Polymer	522
6.9.2	Temperature-sensitive Smart Polymers	526
6.9.3	Solvent-sensitive Enzyme–Polymer Conjugates	526
6.9.4	Reversibly Soluble Immobilized Enzyme Based on Ionic Strength-sensitive Polymers	528
6.9.5	Reversibly Soluble Immobilized Enzyme Based on Light-sensitive Polymers	528
6.10	References	531
	Subject Index	551