

Contents

Zusammenfassung	i
Summary	iii
Preface and Scopes of the work	v
Abbreviations	ix
1 Molecular Imprinting – State of Art	1
1.1 General Principle	1
1.1.1 Covalent and Non-Covalent Approach	2
1.1.2 Free Radical Copolymerisation	5
1.2 Synthesis and Conditions of MIP formation by Non-Covalent Imprinting	7
1.2.1 Template	7
1.2.2 Functional Monomers	8
1.2.3 Cross-Linkers	9
1.2.4 Solvents (Porogens)	10
1.2.5 Initiators	10
1.2.6 Polymer Stability	11
1.2.7 Thermodynamics and Modeling of the pre-polymerisation complex	12
1.2.8 Spectroscopic Studies of the pre-polymerisation complex	12
1.3 Polymer Formats	13
1.3.1 Monolithic MIPs	13
1.3.2 MIP Membranes	15
1.4 Polymer Characterisation used in this thesis	22
1.4.1 Elemental Micro-Analysis	22
1.4.2 Fourier-Transform Infra-Red Spectroscopy (FTIR)	22
1.4.3 Solid-State NMR	23
1.4.4 Nitrogen Sorption Porosimetry	23
1.4.5 Microscopy, e.g. SEM	23
1.4.6 X-ray Photoelectron Spectroscopy (XPS)	24
1.4.7 Characterisation of the Binding Sites and their Distribution	25
1.4.8 Equilibrium Partitioning Experiment	25

1.4.9	SPE-LC-MS	26
1.4.10	SPE-MALDI-TOF-MS	28
1.5	MIPs Targeting Peptides and Proteins	30
1.5.1	Obstacles in Large Molecular Weight Template Imprinting	30
1.5.2	Strategies for Large Molecular Weight Template Imprinting	31
1.6	MIPs for Solid Phase Extraction (SPE)	38
1.7	Conclusions	40
2	Phosphorylated Peptides	43
2.1	Proteomics and Phosphoproteomics: Importance of Phosphorylation and Tyrosine-Phosphorylation in Biochemistry	43
2.2	Difficulties for The Characterisation of Phospho-Peptides	46
2.3	Current Technology Strategies	46
2.3.1	Enrichment of Phosphoproteins	49
2.3.2	Chemical Derivatisation	49
2.3.3	Immobilised Metal Affinity Chromatography (IMAC)	50
2.3.4	Metal Oxide Affinity Chromatography (MOAC): TiO_2 , ZrO_2	51
3	Selective Capturing of Phosphotyrosine Containing Peptides by an Imprinted Polymer	53
3.1	Summary	53
3.2	Experimental Section	54
3.2.1	Polymer Preparation	54
3.2.2	Morphology, Physical and Chemical Polymer Characterisation	54
3.2.3	HPLC evaluation	55
3.2.4	Solid Phase Extraction (SPE)	55
3.2.5	LC-ESI-MS Analysis	56
3.2.6	MALDI-TOF-MS Analysis	57
3.3	Results and Discussion	58
3.3.1	Solution Complexation	58
3.3.2	Polymer Synthesis	58
3.3.3	Morphological, Physical and Chemical Polymer Characterisation	59
3.3.4	Chromatographic evaluation	61
3.3.5	Mobile Phase Optimisation	64
3.3.6	Frontal Analysis	69
3.3.7	Peptide Recognition by SPE	70
3.4	Chemicals	86
3.4.1	Chemicals for Synthesis	86
3.4.2	HPLC Solvents and Chemicals	86
3.5	Apparatus and Methods	86
3.5.1	Morphological Analysis	86
3.5.2	Elemental Analysis	87
3.5.3	Phosphorous Analysis	87

3.5.4	FT-IR spectroscopy	87
3.5.5	Nitrogen sorption	87
3.5.6	Measurement of swelling	87
3.5.7	HPLC	87
3.5.8	ESI-MS	87
3.5.9	Matrix assisted laser desorption ionisation (MALDI) mass spectrometry	88
4	The Vitamin B₂ Riboflavin	89
4.1	Riboflavin: Importance in Biochemistry	89
4.1.1	Riboflavin Photolysis	91
4.1.2	Riboflavin Complexes and Cell Toxicity	91
4.2	Characterisation of Riboflavin	93
4.3	Role of Riboflavin in Beer Flavor Instability	95
4.3.1	Oxygen Radicals in Living Systems and in Food	95
4.3.2	Mechanism for Formation of the Lightstruck Flavor in Beer	95
4.3.3	Current Technologies for Maintenance Flavor Stability in Beer	97
4.3.4	Quantitative Determination of Riboflavin Levels in Beer	98
4.4	Molecular Imprinting Approach for Detection of Riboflavin	98
5	Molecularly Imprinted Membranes for Selective Removal of Riboflavin	101
5.1	Summary	101
5.2	Experimental Section	101
5.2.1	Membrane Preparation	101
5.2.2	Morphological Analysis	102
5.2.3	Equilibrium Partitioning Experiments in H ₂ O/EtOH 95/5 v/v for the membranes functionalised for the recognition of Riboflavin	102
5.2.4	Dynamic Partitioning experiments for the membranes functionalised for the recognition of Riboflavin	104
5.3	Results and Discussion	107
5.3.1	Morphological Analysis	107
5.3.2	Equilibrium Partitioning Experiments in H ₂ O/EtOH 95/5 v/v for the membranes functionalised for the recognition of Riboflavin	111
5.3.3	Dynamic Partitioning Experiments for the membranes functionalised for the recognition of Riboflavin	116
5.3.4	Conclusions	119
5.4	Chemicals	120
5.4.1	Chemicals for Synthesis	120
5.4.2	HPLC Solvents and Chemicals	120
5.5	Apparatus and Methods	120
5.5.1	HPLC	120
5.5.2	UV- Reader	120
5.5.3	FT-IR spectroscopy	120

5.5.4	SEM analysis	120
5.5.5	Machine for Membrane preparation	121
6	Conclusions	123
	Acknowledgements	125
	Bibliography	129
	Curriculum Vitae	137
	Lebenslauf	139