

Table of Contents

1	Abstract	1
2	Thesis Overview	5
3	Introduction.....	6
3.1	Preface.....	6
3.2	Glycosylation.....	6
3.2.1	General Aspects of Glycosylation.....	6
3.2.2	IgG Structure and Glycosylation.....	9
3.2.3	Effects of Process Conditions on Glycosylation.....	12
3.3	Recombinant Proteins	12
3.3.1	PankoMab-GEX™	12
3.3.2	Cetuximab.....	13
3.3.3	Trastuzumab	13
3.3.4	Factor VII	13
3.4	GlycoExpress Expression Platform	14
3.5	Upstream Process Development.....	15
3.6	Mammalian Cell Culture & Metabolism.....	16
3.6.1	Batch.....	16
3.6.2	Fed-batch.....	17
3.6.3	Chemostat & Perfusion	18
3.6.4	Mammalian Central Metabolism	21
3.6.5	Cell Culture Media for Biopharmaceutical Production.....	24
3.6.6	Bioprocess Scale Down	26
4	Material & Methods.....	28
4.1	Cell Culture	28
4.1.1	Cell Culture Media.....	28
4.1.2	Preculture.....	28
4.1.3	Transfection	29
4.1.4	Single Cell Cloning	29
4.1.5	Bioreactor Cultivations	29
4.1.5.1	1L Perfusion Bioreactor.....	30
4.1.5.2	1L DASGIP Fed-Batch Bioreactor	30
4.1.5.3	AMBR Microbioreactor System	30
4.1.5.4	Feeding Strategies for Fed-Batch Cultivations.....	31
4.2	Analytical techniques.....	31
4.2.1	Cell Concentration.....	31
4.2.2	Metabolites.....	31
4.2.3	Osmolality Determination	31
4.2.4	ELISA.....	32

4.2.4.1	Titer ELISA for IgG Quantification	32
4.2.4.2	Titer ELISA for FVII Quantification.....	32
4.2.4.3	Antigen ELISA.....	32
4.2.5	Octet QK ^e	33
4.2.6	SPR Determination	33
4.2.6.1	SPR in perfusion process.....	33
4.2.6.2	SPR Assay.....	33
4.2.7	SDS PAGE.....	34
4.2.8	Western Blot	34
4.2.9	FACS	34
4.2.10	Isoelectric Focusing (IEF)	35
4.2.11	Weak Cation-Exchange Chromatography.....	35
4.2.12	Size Exclusion Chromatography	35
4.2.13	FcγRIIIa Alpha Screen.....	35
4.2.14	ADCC assay	36
4.2.15	Fab/Fc Separation	36
4.2.16	Glycan Analysis	36
4.2.17	Peptide Map Fingerprinting (deamidation, lysine-clipping, oxidation).....	37
4.3	Software.....	37
5	Results and Discussion	38
5.1	Feeding Strategies for low Glucose Fed-batch and Perfusion Cultivation.....	38
5.1.1	Feeding Strategies for low Glucose Fed-batch.....	38
5.1.2	Development of pH-stat Feeding Strategy for Fed-batch Cultivation	40
5.1.3	Transfer and Scale up of pH-stat fed-batch from Micro Bioreactor to 1L Scale.....	47
5.1.4	Implementation of pH-stat Feeding Strategy to an ATF Perfusion System	49
5.1.5	Product Quality of pH-stat Fed-batch and Perfusion.....	53
5.2	Design and Development of Scale-down System for Perfusion Bioreactors	54
5.2.1	Establishment of Sedimentation as Cell Retention in a Microbioreactor	54
5.2.2	Comparison of ambr Perfusion to Benchtop-scale Perfusion.....	60
5.2.3	Comparison of ambr Perfusion to Chemostat and Batch Cultivation.....	63
5.2.4	Application to CHO Cells.....	65
5.2.5	DoE Studies using ambr Perfusion.....	67
5.2.5.1	DoE Study for USP Parameters	67
5.2.5.2	DoE Study for Product Quality Attributes.....	70
5.3	Cell culture Media Optimization for a Perfusion Process	75
5.3.1	Supplementation with chemical Chaperone 5.....	75
5.3.2	Supplementation with GlycoMix	84
5.3.3	Combination of chemical Chaperone 5 and GlycoMix	87
5.4	Cell Line Development applying Overexpression of human SOD1	99

5.4.1	Generation of SOD1 overexpressing Cell Line	99
5.4.2	Evaluation of Transfection Efficiency with GFP	102
5.4.3	Transfection of SOD1 overexpressing Clones with PankoMab-GEX™	106
5.4.4	Transfection of SOD1 overexpressing Clones with FVII	109
6	Appendix	112
6.1	List of Abbreviations	112
6.2	List of Cultivations	113
6.3	List of Figures.....	117
6.4	List of Tables.....	120
6.5	Bibliography	121