

1	Introduction	1
1.1	Dry powder inhalers	2
1.1.1	Classical pulmonary powder formulations.....	4
1.1.2	Innovative pulmonary powder formulations	6
1.2	Deep lung delivery of biopharmaceuticals	8
1.2.1	Anatomy and physiology of the lung	9
1.2.2	Pulmonary resorption mechanisms for macromolecules	10
1.3	Formulation challenges: biopharmaceuticals in DPIs.....	12
1.3.1	Structure and instabilities of biopharmaceuticals	12
1.3.1.1	Physical instability of biopharmaceuticals	13
1.3.1.2	Chemical instability of biopharmaceuticals	15
1.3.1.3	Stabilization of liquid biopharmaceuticals	15
1.3.1.4	Stabilization of solid state biopharmaceuticals	16
1.3.2	Producing pulmonary applicable biopharmaceutical powders.....	18
1.3.2.1	Requirements on particle size for pulmonary application.....	18
1.3.2.2	Spray-drying.....	20
1.3.3	Flow properties of inhalable biopharmaceutical powders.....	28
1.3.3.1	Static and dynamic angle of repose.....	29
1.3.3.2	Vibrating spatula	33
1.3.3.3	Shear testing.....	37
1.3.3.4	Comparison of different methods for the determination of flow properties of pulmonary applicable powders.....	46
2	Objectives of this thesis	47
3	Materials and methods	49
3.1	Materials	49
3.1.1	Human serum albumin (HSA)	49
3.1.2	Type 1 immunoglobulin G (IgG1)	49
3.1.3	Dextran 1 (Dx1)	50
3.1.4	Lactosucrose	51
3.1.5	Coupling sugar.....	51
3.1.6	Compatible solutes.....	53
3.1.7	Excipients and reagents.....	54

3.2 Methods.....	55
3.2.1 Sugar analytics.....	55
3.2.1.1 Freeze-drying.....	55
3.2.1.2 Electrospray ionization mass spectroscopy (ESI-MS)	56
3.2.1.3 High performance liquid chromatography (HPLC).....	56
3.2.2 Producing protein DPIs	57
3.2.2.1 Preparation of spray-drying solutions	57
3.2.2.2 Spray-drying (sd).....	57
3.2.3 Material properties – physicochemical characterization.....	58
3.2.3.1 Differential scanning calorimetry (DSC)	58
3.2.3.2 X-ray powder diffractometry (XRD).....	58
3.2.3.3 Karl-Fischer titration	59
3.2.4 DPI particles – physicochemical characterization	59
3.2.4.1 Laserdiffractometry (LD)	59
3.2.4.2 Anderson Cascade Impactor (ACI)	59
3.2.4.3 Time of flight measurement (TOF)	60
3.2.4.4 HandiHaler [®]	61
3.2.4.5 Scanning electron microscopy (SEM).....	62
3.2.5 Flow properties (FP) of spray-dried powders.....	63
3.2.5.1 Powder flow analyzer (PFA)	63
3.2.5.2 Rotational shear tester (RST)	64
3.2.6 Protein stability	66
3.2.6.1 Spray-drying process stability screening.....	66
3.2.6.2 Vacuum-drying process stability screening.....	66
3.2.6.3 Equilibration process stability screening.....	66
3.2.6.4 Stress storage stability screening.....	66
3.2.6.5 Dry short-term storage stability screening	66
3.2.6.6 Equilibrated short-term storage stability screening	67
3.2.6.7 Dry long-term storage stability screening	67
3.2.6.8 Humid long-term storage stability screening.....	67
3.2.7 Protein analytics.....	68
3.2.7.1 Size exclusion high performance liquid chromatography	68
3.2.7.2 Turbidimetry.....	68

4 Results and Discussion	70
4.1 Quest for innovative sd excipients.....	70
4.1.1 New sugars	71
4.1.1.1 Dextran 1	71
4.1.1.2 Lactosucrose and Coupling Sugar.....	72
4.1.2 Compatible solutes.....	73
4.1.3 Additives	74
4.1.3.1 Amino acids	74
4.1.3.2 Tri-peptides	75
4.2 Sugar analytics.....	77
4.2.1 Electrospray ionization mass spectrometry (ESI-MS).....	77
4.2.2 Differential scanning calorimetry	79
4.2.3 HPLC-analysis of sugars	80
4.2.3.1 Ion-exchange HPLC.....	80
4.2.3.2 Amino bonded silica gel HPLC	82
4.2.4 Summary	87
4.3 Sd studies of new excipient formulations	88
4.3.1 Sd of non protein formulations	88
4.3.2 Sd of sugar/HSA formulations	89
4.3.3 Sd of sugar/citrulline formulations	91
4.3.4 Sd of sugar/Ile/HSA formulations	92
4.3.5 Sd of sugar/IgG1 formulations	94
4.3.6 Sd of various excipient to IgG1 ratios	96
4.3.7 Sd of sugar isoleucine IgG1 formulations.....	96
4.3.8 Sd of sugar/citrulline/IgG1 formulations	98
4.3.9 Sd of compatible solutes/IgG1 formulations.....	99
4.3.10 Sd of compatible solutes Ile IgG1 formulations	100
4.3.11 Summary	101
4.4 Protein stabilizing capabilities of new excipients.....	103
4.4.1 Process stability of new IgG1 formulations	103
4.4.2 Stress storage stability testing of new IgG1 formulations.....	105
4.4.3 Short-term stability testing of new IgG1 formulations	109
4.4.4 Summary	117

4.5 Development of Dextran 1 containing IgG1 DPIs	119
4.5.1 Aerodynamic performance (AP).....	119
4.5.1.1 Effect of total solid concentration and HE-cyclone on AP.....	119
4.5.1.2 Effect of Isoleucine on AP	121
4.5.1.3 Effect of Dextran 1 to Isoleucine Ratio on AP	122
4.5.1.4 Effect of Tri-Isoleucine on the AP	124
4.5.1.5 Effect of relative humidity on AP	126
4.5.2 Protein stability	128
4.5.2.1 Effect of TS on protein sd stability.....	128
4.5.2.2 Influence of formulation on sd and stress storage stability	129
4.5.2.3 Long term stability testing.....	130
4.5.3 Summary.....	133
4.6 Development of Lactosucrose containing IgG1 DPIs	135
4.6.1 Aerodynamic performance	135
4.6.1.1 Effect of HE-cyclone, AAF and TS on AP	135
4.6.1.2 Effect of Ile and Ile3 on AP of sd LS/IgG1 formulations	137
4.6.1.3 Effect of LS - Ile3 ratio on AP of LS/IgG1 powders.....	139
4.6.1.4 Effect of relative humidity on the AP of LS/Ile3/IgG1 powders	140
4.6.2 Protein stability	144
4.6.2.1 Effect of formulation and TS on protein sd stability	144
4.6.2.2 Influence of formulation on stress storage stability.....	146
4.6.2.3 Long term stability testing.....	147
4.6.3 Summary.....	154
4.7 Flow-properties of new protein-DPIs.....	157
4.7.1 Powder Flow Analyzer (PFA)	157
4.7.2 Peschl Rotation Shear Tester (RST)	158
4.7.2.1 FP quantification of sd IgG1 Formulations by shear testing.....	158
4.7.2.2 Influence of the Cyclone on FP	160
4.7.2.3 Influence of Ile and Ile3 content on FP	161
4.7.3 Summary.....	165
5 Final Summary	166