DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES

DER FAKULTÄT FÜR CHEMIE UND PHARMAZIE

DER LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

DEVELOPMENT OF MICROPARTICLES FOR SUSTAINED RELEASE OF PROTEINS BASED ON LIPID AND SILICA MATERIALS

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aus

Hubei, China

2017

Erklärung

Dieser Dissertation wurde im Sinne von § 7 der Promotionsordnung vom 28. November 2011 von Herrn Prof. Dr. Wolfgang Frieß betreut.

EIDESSTATTLICHE VERSICHERUNG

Diese Dissertation wurde eigenständig und ohne unerlaubte Hilfe erarbeitet.

München, den ……..

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Bifeng Wang

Dissertation eingereicht am: 21.02.2017

1. Gutachter: Prof. Dr. Wolfgang Frieß

2. Gutachter: Prof. Dr. Gerhard Winter

Mündliche Prüfung am: 17.03.2017

子曰：學而不思則罔，思而不學則殆。

《论语・为政》

The Master said, "Learning without thought is labor lost; thought without learning is perilous."

(The Analects. Weizheng)

ACKNOWLEDGEMENTS

The present work was performed under the supervision of Prof. Dr. Wolfgang Frieß at the Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-Universität (LMU), München.

First of all, I would like to express my deepest gratitude to my supervisor, Prof. Dr. Wolfgang Frieß. It was my great fortune to have him as my doctor father. Throughout the years, he has always been supportive and encouraging both in research and in life. I am deeply grateful to him for his kindness and generous support through every stage of my foreign Ph.D life in Germany, as well as for offering interesting projects, excellent supervision, brilliant ideas, fantastic working conditions and the patience and time towards me during my writing time. His invaluable advices and constant encouragement during the PhD years would be deeply engraved in my mind.

I also would like to thank Prof. Dr. Gerhard Winter, not only for acting as the second examiner of the thesis, but also for providing excellent working conditions and Thursday's seminars as well as many interesting team activities every years at the chair of Pharmaceutical Technology and Biopharmaceutics. Many thanks also go to Prof. Dr. Olivia Merkel for providing a harmonious atmosphere as one big family.

Particularly I want to express the gratitude to China Scholarship Council for providing me the scholarship to study in Germany, and also the great help from the

international office of LMU for the orientation course, language and culture programs helping me to get used to the new life in Munich.

Thanks are also extended to Prof. Dr. Franz Bracher, Prof. Dr. Angelika Vollmar, Prof. Dr. Stefan Zahler, and Prof. Dr. Christian Wahl-Schott, members for my thesis advisory committee.

Further many thanks go to all the members in AK Frieß, AK Winter and AK Merkel. I would never forget the moments we shared during skiing, hiking trip, boating, Oktoberfest visit, conference visit, coffee breaks, and other social events. In particular, I want to acknowledge Shohel Horiuchi, Ellen Köpf, Kerstin Hoffmann, Corinna Dürr for being my lab mates as well as for the happy time and scientific discussions we had. I did not feel lonely at the beginning of my study with Shohel, my first Japanese friend. Ellen, Kerstin and Corinna were always there for helping me about my life issue. Especially in the bike accident, Corinna and Jacqueline helped me dealing with the emails and contacting with insurance company. Mrs. Imke Leitner was so nice to assist me a lot in the practical experiments and instruments over the past years. I want to thank Kerstin who also worked on spray drying project for many valuable discussions. And I also want to thank Dr. Madeleine Witting for introducing me to the nano spray dryer, Christoph Marschall for sharing with me many interesting Chinese experiences, Christoph Korpus and Yordanka Yordanova for helping me on the computer problems, Dr. Verena Saller for subleasing her nice apartment to me, Kay Strüver and Dr. Stefanie Funke for organizing many interesting

social events. And many thanks also go to all of you for the nice presents when my baby was born. I would also like to thank Mrs. Gabi Hartl, Mrs. Sabine Kohler and Mrs. Ayla Tekbudak for all the support. Thank you very much for always helping me with regards to work-related but also life issues. Also I would like to acknowledge Mr. Minke for SEM measurements.

Finally and most importantly I would like to express my profound gratitude to my family: to my parents for raising me and for giving me the possibility to study, to Duoyiqi for being my son and for being so sweet and healthy while I was working, I am really proud of you, to my brother Shengbo and my sister Lei for encouraging me all the time, and most of all to Mancy for being always with me and supporting me every single moment of my life.

TABLE OF CONTENT

CHAPTER 1

General introduction

1. Introduction

With the rapid development of recombinant DNA technology a broad variety of protein drugs has become available for therapy of a wide range of conditions, including various cancer types, heart attack, stroke, cystic fibrosis, Gaucher's disease, diabetes, or anaemia, haemophilia [1, 2]. Compared to small-molecule drugs that still account for the majority of the pharmaceutical market share, proteins are more specific, which may result in less side effects and lower toxicity [3, 4]. The 2013 Pharmaceutical Research and Manufacturers of America (PhRMA) report on "Biologic Medicines in Development" claimed that over 900 kinds of protein and peptide-based medicines are in development, aiming at treating more than 100 diseases, of which 353 candidates target cancer and related conditions, 187 infectious diseases, 69 autoimmune diseases and 59 cardiovascular diseases [5]. It has been estimated that the global therapeutic proteins market worth 113.4 billion dollars in 2016 and will increase up to 141.5 billion by 2017 [6].

Proteins are large amino acid based macromolecules characterized by a unique three-dimensional structure corresponding to their biologically active state [7]. The native structure of a protein molecule is the result of a fine balance among various interactions including covalent linkages, hydrophobic interactions, electrostatic

interactions, hydrogen bonding and van der Waals forces [7]. This specific structure is rather sensitive and formulation of proteins with optimal efficacy and safety needs special attention to external factors such as pH, temperature, and surface interaction, as well as contaminants and impurities of excipients affecting chemical and physical stability [4].

2. Parenteral administration of protein drug

Although non-parenteral routes benefit from convenience and patient acceptability, they exhibit low bioavailability of proteins and peptides [8]. Permeation enhancers, enzyme inhibitors and special formulation vehicles have been tested but still currently, no clinically useful oral formulations have been established [9].

Therefore, subcutaneous and intravenous injections are the most common form of protein administration [4]. Many therapeutic proteins have a short *in vivo* half-life and treatment of chronic diseases necessitates frequent injections [7]. An attractive way to overcome this problem would be a dosage form that delivers the proteins over a longer period rendering plasma concentrations within the therapeutic window for an extended time. Such sustained release formulation would provide numerous and distinct advantages, both therapeutic and financial, including protecting protein from clearance, improving ability of delivering the protein locally to a particular site or body compartment, and increasing patient comfort, convenience, and compliance [10]. In fact, parenteral sustained release systems are relatively mature for delivering small molecular drugs. The difficulty of developing similar delivery systems for proteins mainly results from protein instability during manufacturing but

additionally the protein must remain stable after administration at body temperature in these systems for weeks or months [4]. These challenges have necessitated new materials and methods to achieve parenteral depot formulation for protein drugs.

3. Materials for parenteral protein drug delivery

Materials used for parenteral protein drug delivery must be at least non-toxic and biocompatible and a broad number of natural and synthetic polymers, lipids and silica materials have been applied [11-17].

3.1. Natural polymers

Among the natural polymers, polysaccharides and proteins are common carrier materials used in the parenteral depots of protein drugs [17, 18]. The polysaccharides have abundant resources from algal origin (e.g. alginate), plant origin (e.g. pectin, guar gum), microbial origin (e.g. dextran, xanthan gum), and animal origin (hyaluronic acid, chitosan, chondroitin) as well as low cost in their processing [19]. Furthermore polysaccharides can be easily modified chemically and biochemically due to the presence of various groups on the polymer chain which can be derivatized, leading a broad variety of polysaccharide derivatives which are used for sustained release application [19]. Protein e.g. gelatin, collagen, albumin or fibrin are another promising class of materials [20]. They are degraded by the normal protein turnover pathways and are in general biocompatible and biodegradable which makes them attractive for biopharmaceutical drug delivery [20]. Among all the natural polymers, alginate, hyaluronic acid, chitosan and gelatin are the most popular carrier materials for protein drug delivery.

Alginate, an anionic polysaccharide extracted from various species of algae, consists of D-mannuronic and L-guluronic acid units. The ratio and distribution of D-mannuronic and L-guluronic acids determines the functionality of alginate as carrier material [11]. Aqueous alginate solution dropped into a calcium bath gel by rapid crosslinking between the alginate guluronic acid units and the cation [11]. Due to the simplicity, non-toxicity, biocompatibility, low cost and mild formulation conditions, alginate has been fabricated into large beads, microbeads, block gels, fibers, and used for *in situ* gelling systems for the entrapment and/or delivery of a variety of proteins [21-23]. Factors such as alginate concentration, cation concentration, hardening time, viscosity of alginate solution have been investigated [22]. Despite the broad use of ionically cross-linked alginate hydrogels, the systems usually lead to poor control over the swelling behavior and the mechanical properties of gels. Additionally, due to the loss of cations under physiological conditions, ionically cross-linked alginate hydrogels exhibit limited long-term stability and release [24].

Hyaluronic acid (HA), a linear polysaccharide of a wide molecular weight range (10^3-10^7 Da), consists of alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine with (1→4) inter glycosidic linkage and is distributed throughout the extracellular matrix, connective tissues, and organs of all higher animals [25, 26]. Due to its strong hydration, viscoelasticity and high biocompatibility, it has received great attention for protein drug delivery system development [23, 27]. Natural HA is highly hydrated and rapidly degraded, which constraints its application in prolonged release formulations. An efficient method to offset its deficiencies is to

chemically modify HA [28]. The pore size of HA hydrogels can be controlled by changing the crosslinking density for the encapsulation of protein drugs within the HA hydrogel networks [23, 29]. However, protein drugs were released rapidly within a week due to the difficulties in the preparation of highly crosslinked HA hydrogel network and preserving injectability in many cases [29].

Chitosan is another polysaccharide consisting of varying amounts of (1-4)-glycosidic bonds linking glucosamine and N-acetyl-D-glucosamine [30]. It is made by alkaline treatment of chitin from shells of shrimp and other crustaceans [31]. With its different functional groups it allows versatile chemical modification [30]. Compared to many other natural polymers, chitosan carries positive charge under physiological conditions which makes it a preferred candidate for drug encapsulation and controlled release of negatively charged compounds [32]. A broad variety of chitosan based protein drug delivery systems in the forms of gels and particles have been developed and studied [31, 33]. As a result of the combined effects of hydrogen bonds and hydrophobic interaction, chitosan tends to form aggregates making it difficult to dissolve in the neutral media, which poses a substantial limitation [32].

Gelatin is obtained by hydrolysis of collagen, which is a fibrous biomaterial typically derived from skin, tendon and in connective tissues of animals [34]. The high number of amino and carboxylic groups enable an easy crosslinking of gelatin with a variety of crosslinkers. The isoelectric point of gelatin can be modified during the fabrication process to yield either gelatin which is either negatively or positively charged at physiological pH. This allows substantial electrostatic interactions between a charged biomolecule. Various forms of gelatin carrier matrices can be

fabricated for controlled release studies [13]. In spite of these advantages, gelatin is still limited due to its high degree of swelling in aqueous environments leading to fast drug release in the body. Swelling and degradation can be reduced by chemical cross linking e.g. by glutaraldehyde or formaldehyde treatment giving rise to the formation of non-soluble networks. However, the use of cross linkers can lead to reduced biocompatibility, due to the presence of residual cross linking agent and to unwanted reaction between drug and cross-linker [35].

3.2. Synthetic polymers

Synthetic polymers like aliphatic polyesters and polyanhydrides have also been extensively studied for the protein drug delivery [36, 37]. Their biodegradation is the result of cleavage of labile bonds by a non-enzymatic hydrolytic process. Among aliphatic polyesters, the bulk erodible polylactic and polyglycolic acid based polyesters (PLGA) have been most commonly investigated [14]. During protein release from PLGA systems, deleterious effects including an acidic microenvironment and strong hydrophobic interactions occur, which are significant sources for irreversible physical and chemical inactivation of protein drugs [38]. Polyanhydrides differ from polyesters in their erosion mechanism as they exhibit surface erosion, which may prevent covalent aggregation by reducing water penetration into the device. However, these materials are more hydrophobic enhancing interactions between polymer and protein, which may result in non-covalent aggregation [36].

3.3. Lipids

The term lipids refer to a family of products with diverse physical and chemical

properties, including e.g. oils, fats, waxes and fatty acids, triglycerides and phospholipids [39]. The lipids exhibit tremendous variety in acid chain length and saturation and can be relatively easily formed into particles or implants, facilitating the design of the desired release profile [40]. They are degradable and generally recognized as safe [41]. Triglycerides as an important representative revealed great potential for the controlled release of protein drugs. Their use for the preparation of implants, microparticles or nanoparticles has shown good results for the incorporation and sustained release of proteins [42-52]. Triglyceride matrices avoid the pH-changes occurring with PLGA materials which are critical for protein drugs [41, 53, 54]. The drug release mechanism differs from the commonly used polymers. It is mainly controlled by diffusion of drug molecules through aqueous pores created by the release buffer upon penetration into the matrix and dissolution of water soluble drugs, which is neither accompanied by hydrolysis-induced erosion nor by swelling phenomena [55]. This makes lipid materials a promising candidate as an alternative material to the polymers for the design of parenteral protein drug delivery systems.

3.4. Silica

Mesoporous silica has become a promising drug vehicle due to its unique mesoporous structure, high surface area, large pore volume, tunable pore diameter and narrow pore size distribution [56]. The silica materials cause no adverse tissue reactions and may become slowly degraded [57-60]. The features of high chemical and thermal stability, surface functionality and biocompatibility contribute to the controlled release and target drug delivery of drugs [61, 62]. The pores within the silica take on host molecules, sheltering them from the external environment until

unloading conditions are met. The majority of drug release from silica is controlled by the diffusion of drug molecules through the pores within the silica and simultaneous matrix degradation whereas silica materials do not swell in water [58, 62]. This functionality has proven particularly useful in the delivery of small drug molecules [63]. Small proteins with hydrodynamic diameters less than the pore size can also be loaded and delivered [64]. However, entrapment of large molecular weight proteins in the silica gel for parenteral application has been rarely explored. Thus, the incorporation of large molecular weight proteins into mesoporous silica is of high interests.

4. Parenteral sustained release systems for proteins

In virtue of the advantages of triglycerides and silica materials they have been widely recognized as attractive carriers for protein drug delivery in parenteral application. Various types of sustained release systems can be designed for protein drug delivery based on the triglycerides or silica materials. The most commonly used systems are implants, nanoparticles and microparticles.

4.1. Implants

Due to poor water solubility and harsh processing conditions of conventional precursors, silica implants are mainly applied for the small molecule delivery [65, 66]. But incorporation of protein drugs in triglycerides implants has been developed for long-term release application over days to months [45-50, 67-69]. Lipid implants are usually prepared using direct compression, hot melting extrusion, injection molding

or compression molding [46, 67, 70]. The use of organic solvents can be avoided. No water/organic solvents interfaces are created, which might affect protein integrity. As a result of implantation, a tissue response may occur in the form of an encapsulation, or the development of tissue edema or inflammation. In addition, granuloma formation or increased vascularization may occur [71]. Furthermore, if surgical resection is to be avoided after drug exhaustion, complete biodegradability has to be guaranteed *in vivo* [72].

4.2. Nanoparticles

Mesoporous silica nanoparticles as a drug delivery agent have been explored in the past two decades but they have only recently been further modified as a potential agent for the delivery of proteins [61, 62, 64, 73]. Emulsion chemistry is commonly used to prepare silica nanoparticles [74]. The mesoporous structure makes them ideal nanovehicles for protein delivery and release because of their large pore volume ($\approx$1 cm^3 g^{-1}) and tunable pore diameters (2-10 nm), which allows for the loading of substantial amounts of protein [62]. However, the pore size of typical mesoporous silica nanoparticles is the limiting factor for the effective delivery of proteins in particular. Although the pores can be expanded to accommodate larger host molecules, there still remains a size limit. Only small proteins can be successfully loaded, since larger ones may not fit in the pores [61, 62].

It has been demonstrated that the physicochemical parameters of lipid-based nanoparticles (size, surface charge, morphology, surface chemistry, stability) may easily be adjusted as to satisfy the requirements for improved drug safety,

appropriate drug release kinetics, and possibility for scaling-up manufacturing [75]. Preparation methods for lipid nanoparticles include high-pressure homogenization (hot and cold homogenization), solvent emulsification/evaporation, and microemulsion techniques [76]. In those methods, lipid usually requires high temperature, high pressure, organic solvent or ultrasound, which may lead to protein drug denaturation [77]. Additionally, incorporation of sufficient amounts of drug is troublesome due to the hydrophobic nature of the lipids [78].

4.3. Microparticles

Compared to silica implants and nanoparticles, microparticles provide enhanced flexibility in both fabrication methods and release rate. Currently, many techniques are available for the manufacturing of silica-based microparticles such as emulsification-solvent evaporation, solvent displacement, self-assembly systems, supercritical fluid processing or spray drying [79-81]. The larger size of mesopores offers silica microparticles with highly accessible internal surface areas for high loading with large protein drugs. The release rates can be controlled by tailoring the internal structure of the microparticles for a desired release profile based on size of protein drugs. An ideal microparticle formulation should have reasonably high yield, drug-loading capacity and efficiency, stable protein structure, adjustable release profiles, low burst release and provide sustained release of biologically active proteins [79, 80]. Spray drying has been implemented commercially and offers the advantages of rapid production, controllable particle size, shape and density, all crucial features with regard to drug delivery [82]. Recently, spray-drying technology has been successfully applied for different heat-sensitive protein drugs due to fast

drying (seconds or milliseconds) and relatively short exposure time to heat [83-86]. Silica-based microparticles incorporating protein drugs have also been synthesized via self-assembly of surfactants followed by solvent extraction or calcination of the templating agents [16, 87, 88]. However, the protein drug-loaded silica microparticles fabricated by spray drying for sustained release application are rather unexplored. Hence, in this study, a new silica precursor (TMEOS) exhibiting high compatibility with protein drugs is presented to produce microparticles for sustained release application.

Due to better biodegradability, less tissue response, higher drug loading and flexible fabrication methods, lipid microparticles have been recognized as a potentially more suitable and preferred sustained release system compared to lipid implants and nanoparticles. They can be prepared through melt emulsification, solvent emulsification-evaporation, solvent emulsification-diffusion, double emulsion (w/o/w), spray congealing, supercritical fluid-based methods spray drying or fluid bed coating [54]. Fluid bed coating has been widely used to achieve a desired release profile in pharmaceutical products for many years because the process can be applied for coating cores of various sizes starting from small particles (theoretically 50 µm) to considerably large size objects like tablets and capsules (few centimeters) [89]. The process is characterized by the high drug loading capacity and efficiency, as well as the intensive heat and mass transfer between the gas stream and the solid particles. This comes with an efficient drying or coating which enable moderate temperatures to avoid damage of sensitive protein drugs [89]. The drug release properties can be tailored by changing of coating material and level [90].

These features make fluid bed systems particularly suitable for producing protein-loaded microparticles for sustained release application with functional coating. The development of microparticles with lipid coating keeps special challenges due to the extremely small size of particles and the tackiness of the lipid. The most commonly used method is hot melt coating process [91]. But protein drugs may be degraded at higher temperature since the coating agents normally used in hot melt coating have high melting points [92]. This problem can be overcome with organic solvent spray coating method as the process temperature is much lower. But the use of organic solvent bares a higher risk of residual solvents which also lead to protein damage. Therefore, this study is to investigate the possibility of using organic solvent spray coating to form the lipid-coated microparticles without agglomeration at modest temperature for sustained drug release of protein drug in a fluid bed coater.

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CHAPTER 2

Objectives of the thesis

Many protein drugs exhibit short half-lives *in vivo* and multiple dosing schemes and frequent injections are necessary to achieve therapeutic drug levels, which results in poor patience compliance. Due to the good biocompatibility of triglycerides and silica materials, the objective of this work was to develop protein loaded microparticles for sustained release application based on triglycerides and silica (TMEOS) carriers. It included two main parts, which were lipid coating of protein carrying beads in a fluid bed coater and silica particle fabrication via spray drying. Particular goals were:

a) to optimize the parameters for the lipid coating of microparticles in the fluid bed coater and to achieve a sustained release of model drugs over weeks as well as to investigate the effects of coating level, lipid type, size of starter bead and drug type on model drug release (**chapter 3**);

b) to stabilize IgG1 during spray loading of the initial starter beads and to control its sustained release by varying lipid type and coating load, while ensuring the stability of IgG1 after release (**chapter 4**);

c) to understand gel formation by TMEOS and to optimize the parameters for silica microparticle preparation as well as to fabricate the silica microparticles by spray drying. Subsequently a sustained release of high molecular weight model

compound was to be achieved and the effects of amount and molecular weight of additive as well as pH of precursor solution on drug release profile were to be analyzed (**chapter 5**);

d) to investigate the compatibility of TMEOS with IgG1 and lysozyme, and study the effects of additive on the protein release profile (**chapter 6**).

CHAPTER 3

Formation of polyol core microparticles for sustained release with lipid coating in a mini fluid bed system

Abstract

Biodegradable polymeric materials for parenteral controlled release systems are associated with various drawbacks for biopharmaceuticals. Sustained delivery system based on lipids such as implants or microparticles present an interesting alternative. The goal of this study was to prepare sustained release microparticles for methyl blue and aspartame as sparingly and freely water-soluble model drugs by lipid film coating in a Mini-Glatt fluid bed, and to assess the effect of coating load of two of lipids, hard fat and glyceryl stearate, on the release rates. 30 g drug-loaded mannitol carrier microparticles with average diameter of 500 or 300 µm were coated with 5 g, 10 g, 20 g and 30 g lipids, respectively. The model drugs were completely released *in vitro* through pores which mainly resulted from dissolution of the polyol core beads. The release of methyl blue from microparticles based on 500 µm carrier beads extended up to 25 days, while aspartame release from microparticles formed from 300 µm carrier beads was extended to 7 days. Although glyceryl stearate

exhibits higher wettability, burst and release rates were similar for the two lipid materials. Polymorphic transformation of the hart fat was observed upon release. The lipid-coated microparticles produced with 500 µm carrier beads showed slightly lower burst release compared to the microparticles produced with 300 µm carrier beads as they carried relatively thicker lipid layer based on an equivalent lipid to mannitol ratio. Aspartame microparticles showed a much faster release than methyl blue due to the higher water-solubility of aspartame. With the present study appropriate formulation and manufacturing parameters for the design of sustained release microparticles by lipid coating in a mini fluid bed were established, which could subsequently be transferred to biopharmaceuticals.

Keywords: Methyl blue, Aspartame, Mannitol, Hard fat, Glyceryl stearate, Fluid bed coater, Microparticles, Sustained release, Lipids, Coating

1. Introduction

Many biopharmaceutical drugs require frequent parenteral administration to guarantee a therapeutic level due to their short half-live *in-vivo,* which leads poor patient compliance. Consequently, sustained release formulations are of high interest [1-3]. For the parenteral sustained release of biopharmaceuticals, the most commonly used carrier materials are polylactide (PLA) and poly(lactide-co-glycolide) (PLGA) since they can provide sustained release for a range of times from days up to months and are well established for small molecule and peptide drugs[4, 5]. However, lactic and glycolic acid result upon PLGA degradation, leading to an increase in osmotic pressure and a significant pH drop within the micro-environment, which can result in a loss of activity with biopharmaceuticals [6, 7]. Additionally, harsh microparticle manufacturing conditions like high temperature, high shear forces and organic solvent, may result in detrimental effects on the structure and the activity of protein drugs [6, 7]. Triglycerides, which are Generally Recognized As Safe (GRAS), biocompatible, biodegradable and not inherently immunogenic [8], have been successfully used to control sustained release of proteins in form of implants, nanoparticles, as well as microparticles, which are the most suitable and preferred system till today [9-17].

Fluid bed coating for microparticle preparation has been widely used in pharmaceutical industry to control oral drug release [18, 19]. The most widespread coating materials, cellulose and polyacryl acid derivates are hardly appropriate for parenteral drug delivery systems as they are not biodegradable or available at

adequate quality . An interesting alternative is to utilize lipid coated microparticles as parenteral controlled delivery systems. Lipid film coatings provide several noteworthy advantages: (i) they are plastically deformable and form homogenous films without cracks during the coating process; (ii) the amount of excipient required is generally appropriate; (iii) usually only one lipid is required simplifying the formulation and hence the registration of the drug product with regulatory authorities; and finally (iv) they are relatively inexpensive [8, 20, 21].

Typically, hot-melt and organic solvent spray coating are used for lipid coating [21, 22]. Both high temperature and organic solvent may be critical for use with sensitive biopharmaceutical drugs. In general, also the amount of coating that can be deposited on the surface of the smaller cores compared to oral dosage forms is limited [21, 23]. Furthermore, sustained release microparticles are normally suspended in a suitable vehicle and injected by using a conventional syringe with a 18 or 20 G gauge needle, which requires free flowing microparticle powders of less than 250 µm in diameter, ideally less than 125 µm [24]. Lipid coating of such small microparticles is challenging due to the high tendency to agglomerate upon coating with the tacky lipid [25]. Additionally, a small scale process is required for development due to the high costs of protein drugs. Consequently, a new small scale lipid coating process with the potential for manufacturing of sustained release microparticles for biopharmaceutical drugs is highly desirable.

The main objective of this work was to investigate the possibility of using organic

solvent fluid bed spray coating to form the lipid-coated microparticles without agglomeration at modest temperature for sustained drug release. Successful loading of polyol and sugar spheres with protein drugs has been demonstrated before and this renders a mini fluid bed system very interesting for loading and lipid coating of starting beads [26]. Mannitol beads were selected as the carrier core providing high water solubility and biocompatibility. Drug release from the lipid-coated microparticles may occur through pores in the coat, which form upon dissolution of the polyol core beads. The target release profile was expected to reach a few weeks. Two kinds of lipids with different lipophilicity and wettability, hard fat (HF) and glyceryl stearate (GS) were tested. Furthermore, the effect of the core bead size on processing and release was studied. Two model compounds, methyl blue (MB) and aspartame (ASP) with different solubility were loaded to investigate the effect of drug type on the release. The resulting process parameters could be subsequently transferred to protein loaded core beads.

2. Materials and Methods

2.1. Materials

Pearlitol® 500DC-Mannitol and Pearlitol® 300DC-Mannitol (MAN) were kindly provided by Roquette Corporate, Darmstadt, Germany. Methyl blue (MB) and the reagents used for MAN determination were purchased from Sigma-Aldrich, Munich, Germany. Aspartame (ASP) was kindly provided by Salutas Pharma GmbH, Barleben, Germany. Witepsol® E85 (hydrogenated coco-glycerides, HF) and Imwitor® 900

(glyceryl stearate with a monoester content of 40–55%, GS) were kindly provided by Sasol GmbH, Hamburg, Germany. Isopropanol (99.7%) was supplied by the reagent center of the University of Munich, Germany.

2.2. Methods

2.2.1. Preparation of drug-loaded starting cores

0.45 g MB or 1.50 g ASP was dissolved in 45 mL or 150 mL deionized water to get the model drug solution (1.0%, W/W). 30 g MAN microparticles were loaded with the model drug solution in the Mini-Glatt fluid bed (Wurster insert, Glatt GmbH, Binzen, Germany). The detailed operation conditions were as follows: T_{inlet}: 40 °C; $P_{process}$: 1.0 bar; $P_{atomizing\ air}$: 1.0 bar; spray rate: 1.0 mL/min; spray nozzle diameter: 0.3 mm. After coating, the microparticles were dried for additional 15 min at 40 °C in the fluid bed.

2.2.2. Preparation of lipid-coated microparticles

Lipid was dissolved at 2% w/v in hot isopropanol (70 °C). 30 g drug-loaded microparticles were coated with the lipid solution in the Mini-Glatt fluid bed at: T_{inlet}: 40 °C for GS and 30 °C for HF; $P_{process}$: 0.7 bar; $P_{atomizing\ air}$: 0.5 bar; spray rate: 7.0 mL/min; spray nozzle diameter: 0.5 mm. After coating, the microparticles were dried for additional time of 15 min at the same conditions.

2.2.3. Determination of drug loading of lipid-coated microparticles

200 mg of lipid-coated microparticles were dispersed in 50 ml of hot deionized

water (70 °C). Approximate 3 mL slurry was filtered through a 0.2 µm filter to remove the lipid after it was cooled down to room temperature. Filtrate was analyzed for drug content present at a 588 nm for MB and 258 nm for ASP using an Agilent 8453 UV-Vis spectrophotometer (Agilent, Waldbronn, Germany). Each experiment was performed in triplicate.

2.2.4. Microparticle morphology

The morphology of microparticles was analyzed with a light optical microscope (Olympus BX50 F4, Olympus, Tokyo, Japan) equipped with a digital camera (HVC 20, Hitachi, Maidenhead, GB). Additionally, the microparticles were visualized by scanning electron microscopy (SEM) using a Jeol JSM-6500F instrument (Jeol Ltd., Tokyo, Japan) with Inca Software (Oxford instruments, Oxfordshire, UK).

2.2.5. Mannitol determination

The MAN release was determined by a colorimetric method [27]. 10 µL release medium was diluted with 990 µL deionized water, mixed with 1 mL potassium periodate (0.015 mol/L in 0.12 mol/L HCl solution), incubated for 10 min at room temperature, and reacted with 2 mL 0.1% L-rhamnose and 4 mL Nash reagent. The mixture was placed in a water bath at 53 °C for 15 min. After cooling to room temperature the MAN content was quantified at 412 nm using an Agilent 8453 UV-Vis spectrophotometer. Each experiment was performed in triplicate.

2.2.6. In vitro release

In vitro release was studied in phosphate buffer saline (PBS, pH=7.4) in 37 °C water bath with constant shaking at 30 rpm (Julabo SW21, Julabo GmbH, Seelbach, Germany). Approximate 1.0 g of drug-loaded microparticles suspended in 3 mL buffer were transferred into a dialysis bag with 12 kDa cutoff which was sealed and immersed into a 50 mL disposable plastic tube with 37 mL of phosphate buffered saline release medium containing 0.01% NaN_3. At designated time points, 1 mL release medium was withdrawn and replaced with the same amount of fresh release medium. The model drug content in the release medium was quantified as described above. Each experiment was performed in triplicate.

2.2.7. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis was performed using a Mettler DSC 821e (Mettler Toledo, Giessen, Germany). DSC scans were recorded at a heating and cooling rate of 5 °C/min. The samples were weighted in 40 µL aluminium pans and cooled down from 25 °C up to 0 °C, kept for 2 mins at 0 °C, heated up to 110 °C, kept at 110 °C for 3 mins, cooled again down to 0 °C, kept for 2 mins at 0 °C and reheated up again to 110 °C, kept at 110 °C for 3 mins, cooled down to 25 °C.

3. Results and Discussion

3.1. Methyl blue release from HF-coated microparticles

The lipid-coated microparticles prepared in this study consisted of a MAN starter

core coated with a model compound and subsequently a lipid layer for sustained release of different thickness. To study the influence of the amount of lipid coat on the release of MB, 30 g drug-loaded MAN microparticles were coated with 5 g, 10 g, 20 g or 30 g HF. The lipid-coating of MAN microparticles is illustrated in **Fig. 1.** The uncoated microparticles were irregular in shape (**Fig. 1a**). The model drug MB migrated into the carrier beads exhibited a homogeneous loading (**Fig. 1b**), potentially reducing the contact with organic solvent during the lipid coating process, which is very important for biopharmaceutical drugs. The drug loaded microparticles subsequently coated with lipid maintained their original shape with only minimal agglomeration (**Fig. 1c**). MB diffusion into the retarding lipid coat was not observed (**Fig. 1d**), which may be beneficial to keep potential burst release low

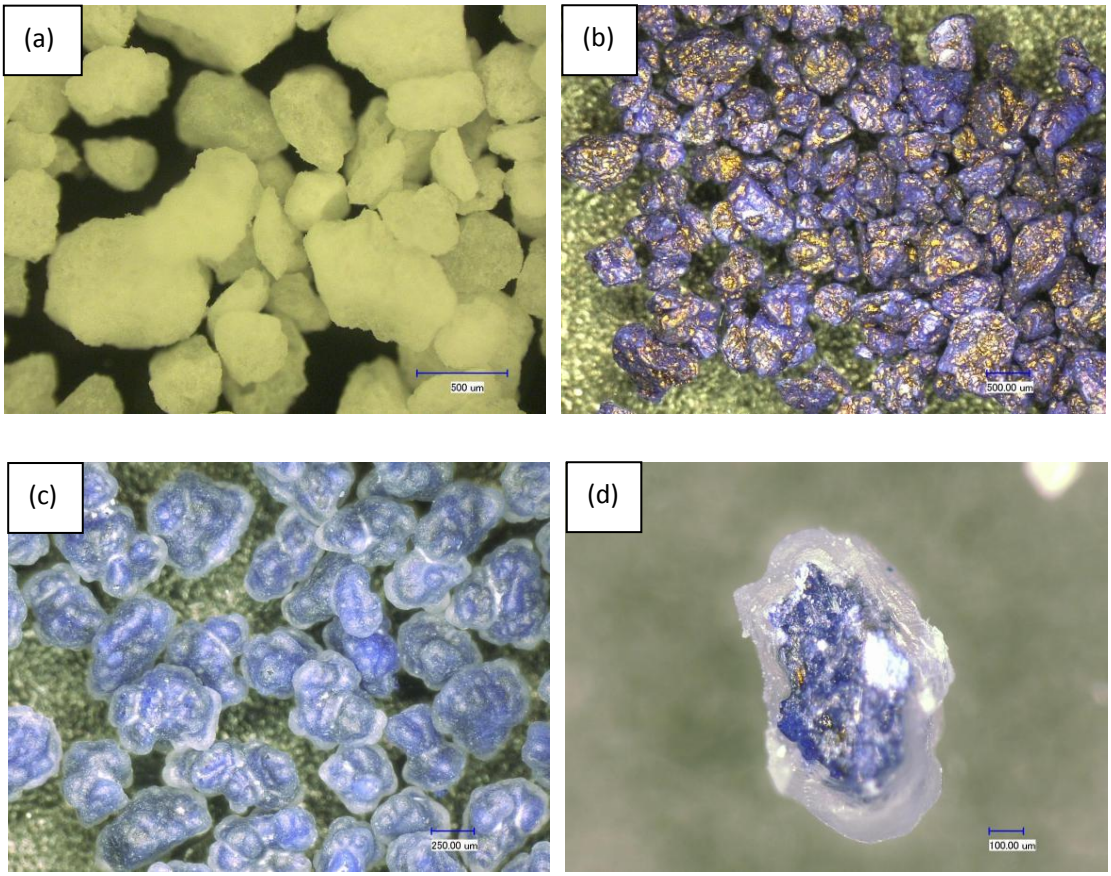


Fig.1. Microscopic images of lipid coating of MAN microparticles, (a) starting MAN beads; (b) MB-loaded microparticles; (c) 30g HF-coated microparticles; (d) cross section of 30g HF-coated microparticle

Fig. 2a shows the MB release profiles from microparticles coated with different amounts of HF. The microparticles coated with 5 g HF show a high burst release of around 50% followed by subsequent MB release over 14 days. More HF lowers the burst and the release rate. The release period is prolonged to 25 days by coating with 30 g HF. The deceleration of release is also found the MAN core material (**Fig. 2b**). In general, MAN shows much higher burst and a faster release than MB. Nearly all MAN is liberated within 1 day from microparticles coated with 5 g HF, while the release is extended to 10 days upon coating with 30 g HF. This is consistent with the particle morphology after release (**Fig. 3**). Almost all of the microparticles coated with 5 g HF are collapsed after the release while this is the case for only a small portion of microparticles coated with more lipid.

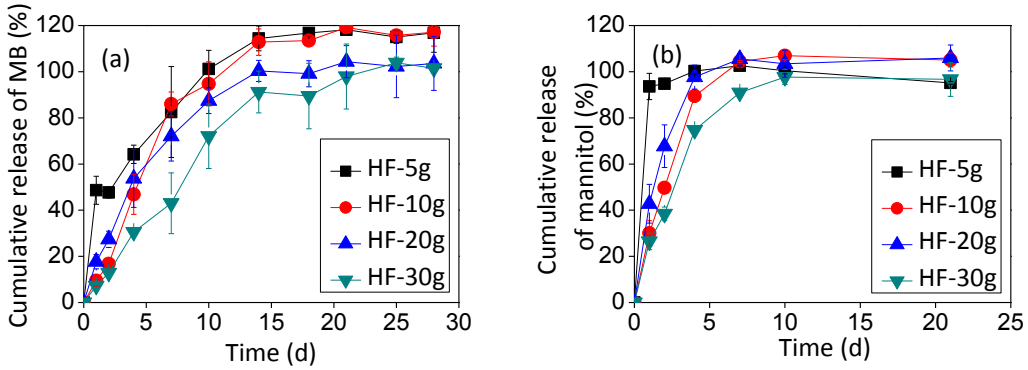
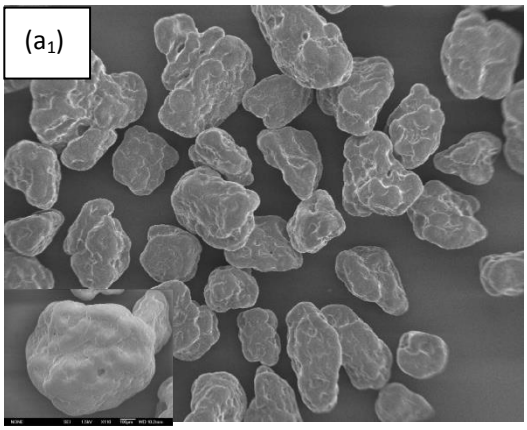
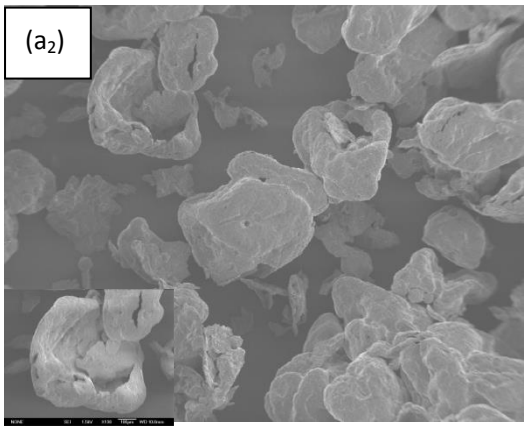


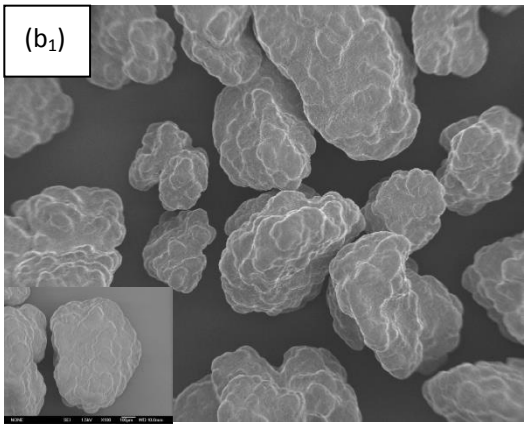
Fig.2. MB (a) and MAN (b) release profiles of microparticles coated with different amounts of HF

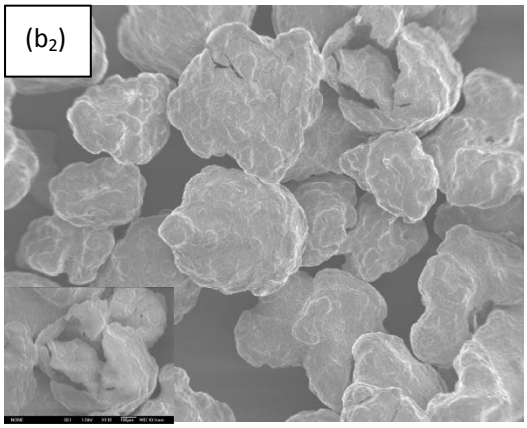
before release

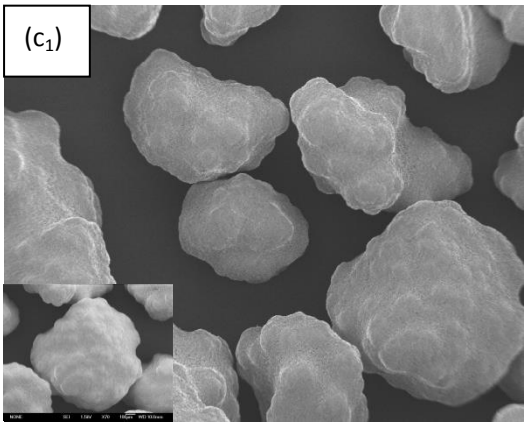
after release

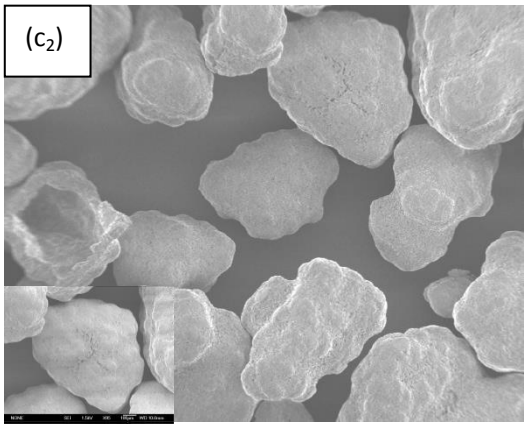












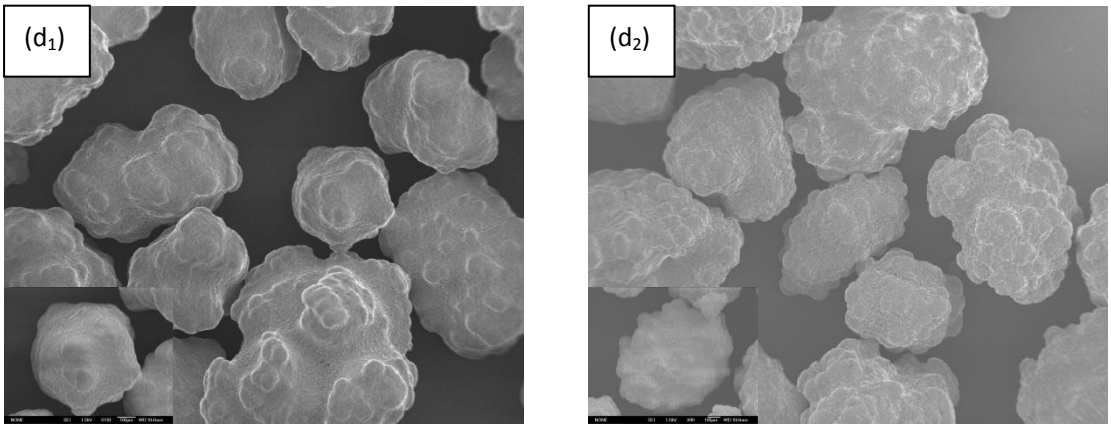


Fig.3. SEM photographs of MAN particles coated with 5g (a), 10g (b), 20g (c) and 30g (d) HF before (left) and after (right) release

For the lipid-coated microparticles, Fickian diffusion is the underlying drug release mechanism, where water: (i) penetrates into the matrix, (ii) dissolves the MAN and drug, (iii) occupies the pores generated by the diffusion of dissolved MAN and drug and (iv) creates channels which enhance matrix porosity and drug mobility, allowing for continuous MAN and drug diffusion out of the microparticle and into the release medium [8]. Microparticles with thin lipid layer became hollow with the dissolution and diffusion of MAN upon contact with the release medium and subsequently collapsed, inducing the burst release. In contrast, microparticles with thick lipid layer maintained their geometry during the release process even after the core MAN was gone, which resulted in sustained drug release. The lipid film thickness of microparticles is influenced by the size and mass distribution of beads in a fluid bed apparatus equipped with a Wurster column. Based on different velocities and fluidization patterns of the various size beads, the larger and heavier beads within a batch coated by this method receive a thicker film and therefore display a

significantly slower release rate when compared to smaller and lighter beads [28].

The highly water soluble MAN (182 g/mol, S_{water} = 216 g/L) [29] present at large quantities in the core acted as pathbreaker. Its dissolution and diffusion resulted in pore formation in the lipid layer. In general, water diffusion into the microparticles is an important release rate controlling factor for the lipid coated beads. The osmotic activity of MAN should stipulate one of the osmotic driving force behind the water influx, which can be expected to hinder the diffusion of dissolved drug through the lipid layer in the opposite direction. Upon dissolution and diffusion would result in that pores form enabling exchange of dissolved molecules by release medium. Thus, MAN and drug concentration gradients form between inside and outside of the lipid layer, decreasing the barrier for drug diffusion and accelerating the release. Occasionally, the microparticle collapse along with MAN release also makes a contribution to drug release if the lipid layer is not thick enough to maintain the integrity of microparticles. Generally, the layer thickness is increased as the coating load of lipid increased, and determines the path-length for drug diffusion. Thus, thick lipid layer not only protects particles from degradation, but also lowers burst and release rate. Compared to MAN, MB exhibits much lower water solubility (800 g/mol, S_{water} = 1 g/L) [30] resulting in much lower burst and release rate.

3.2. Methyl blue release from GS-coated microparticles

The wettability of the lipid is a key factor affecting the drug release properties of lipid matrices [8]. Higher wettability leads to faster drug release. In order to

investigate the effect of lipid type on the drug release profiles, the microparticles were coated with GS. GS is a mixture of 40–50% mono-, ~40% di- and ~5% triglycerides whereas HF is a mixture of 5% mono-, 29% di- and 66% triglycerides esters of fatty acids (C_8-C_{18}) [31, 32]. GS-based microparticles should take up more water due to GS's higher wettability, which may result in higher release rate. However, as shown in **Fig. 4**, comparable burst and release rates were observed for GS and HF-coated microparticles except that the microparticles containing 5 g and 10 g GS showed higher burst release than HF coated particles.

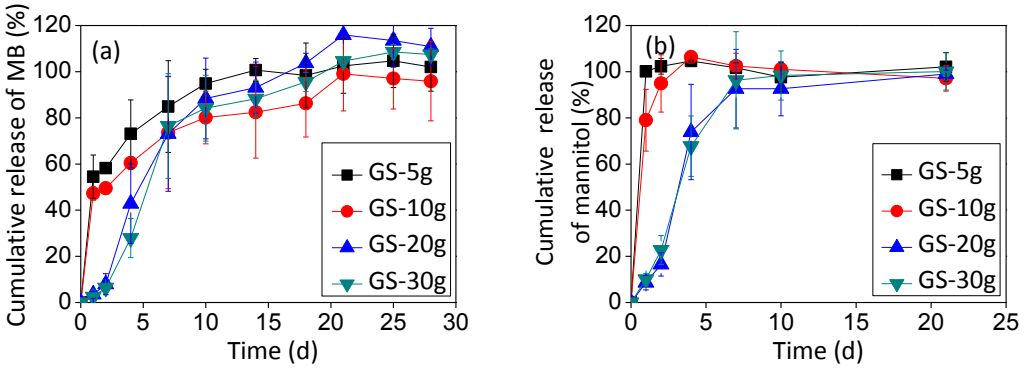


Fig.4. MB (a) and MAN (b) release profiles of microparticles coated with different amounts of GS.

Lipids usually exhibit three polymorphic forms which are: disordered aliphatic chain conformation (α), intermediate packing (β′) and most dense packing (β) [33]. Changes in the polymorphic forms of lipid may influence the drug release behavior [34]. **Fig .5** shows the DSC profiles of bulk lipid and lipid coated particles before and after release testing. Only one peak which commences from about 40.1 °C could be seen for the bulk HF in the first heating cycle, which is designated

the most stable β-form. A decrease in the melting temperature of bulk HF is observed in the second heating cycle. After coating the β-polymorph could be identified. However, the HF-coated particles show signs of lower melting polymorphs (29.7 °C and 38.8 °C) after the release test. Thus HF partially transformed to the less stable form upon release testing. Since different polymorphic forms differ in their ability to include water and drug molecules in their lattice, the transition from the denser to looser packing form may accelerate drug release [35]. This may explain, why HF-coated microparticles showed comparable release profiles as GS-coated microparticles despite poor wettability of HF. The burst drug release is mainly caused by the breakage of more fragile particles at the beginning. In comparison with HF, GS-based particles took up more water in the first day and subsequently disintegration was more pronounced at the low coating levels of 5 g and 10 g.

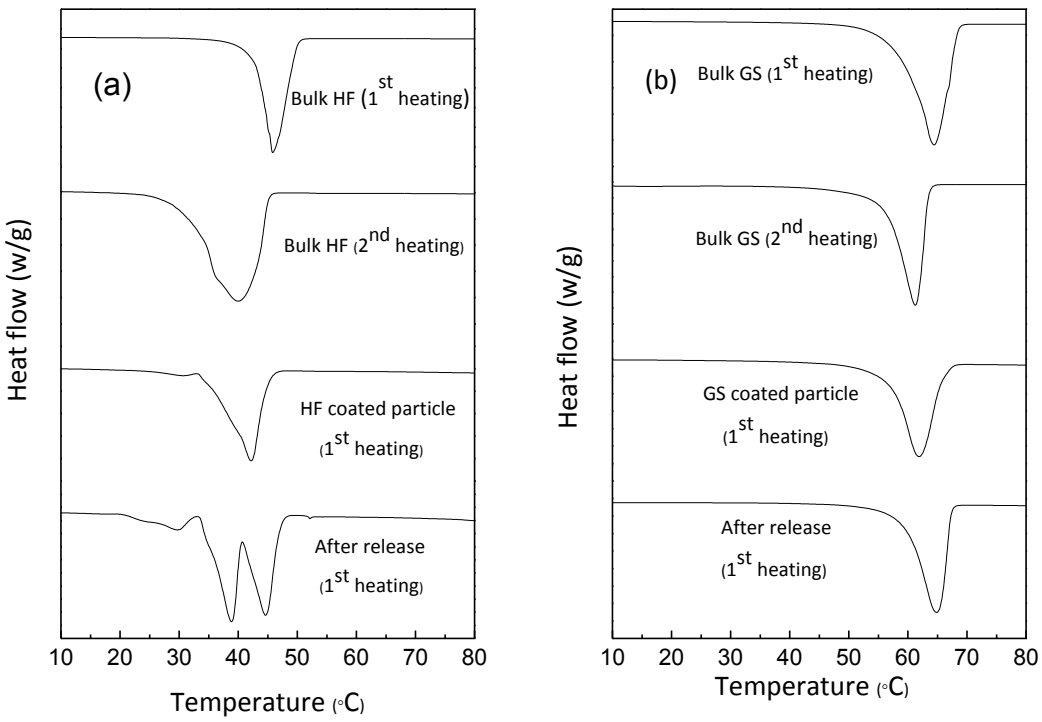


Fig.5. DSC thermograms of HF (a), and GS (b).

3.3. Effect of starting bead size on methyl blue release

Most parenteral suspensions have particle size and content limitations because of syringeability and injectability constraints [36, 37]. Hence, we prepared lipid-coated microparticles based on MAN core beads of 300 µm in diameter to examine the effect of starting bead size on MB release. **Fig. 6** displays MB release profiles of lipid-coated microparticles produced with 300 µm and 500 µm carrier beads. A similar release duration was achieved for both starting beads when equivalent lipid amounts were used, irrespective of lipid type. Generally, the particles produced from 300 µm MAN beads showed a trend to a slightly higher initial release which might be due to the different lipid layer thickness. Smaller carrier beads have a relatively higher specific surface area and thus a thinner coating results when the same absolute mass ratio of coating to core material is used [38]. The thicker lipid layer could protect the microparticles from breakage, decreasing the initial drug release. Additionally, larger microparticles with thicker lipid layer exhibit longer diffusion pathways. Water needs hence more time to penetrate into the system, which delays drug dissolution, pore creation and drug diffusion.

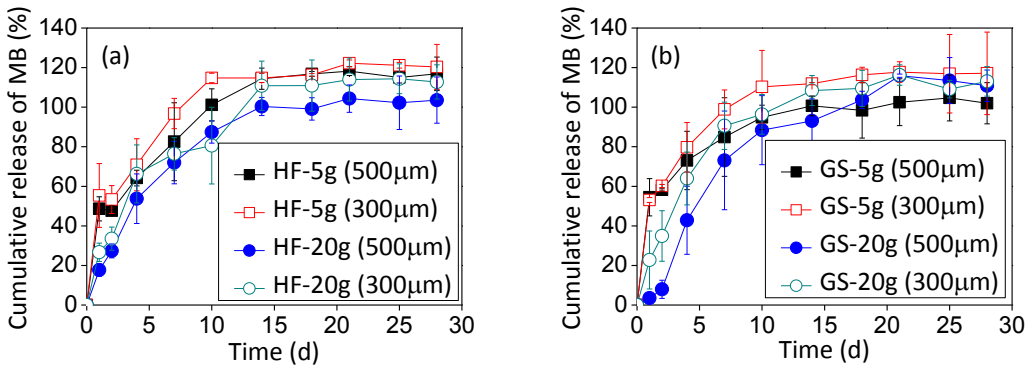


Fig.6. MB release profiles of HF (a) and GS (b) coated microparticles produced with 500 and 300 µm carrier beads

3.4. Aspartame release from lipid-coated microparticles

The aqueous solubility of the drug plays an important role in the formulation of coated particles when the mechanism of release is mainly by transport of the dissolved drug via diffusion through the film or through water-filled pores or channels within the coating [8]. Highly water-soluble drugs are generally released faster than poorly water-soluble compounds. To investigate the effect of the drug's solubility on the release, different model drugs, MB and ASP were loaded on the 300 µm MAN microparticles. These two model drugs of low molecular weights (M_{MB} = 800 g/mol, M_{ASP} = 294 g/mol) differ significantly in water solubility (S_{MB} = 1 g/L, S_{ASP} = 18.6 g/L) [30, 39]. **Fig. 7** displays the release profiles of microparticles loaded with ASP and MB. As expected, particles containing ASP show higher burst and faster release than MB-loaded particles, irrespective of the type of lipid.

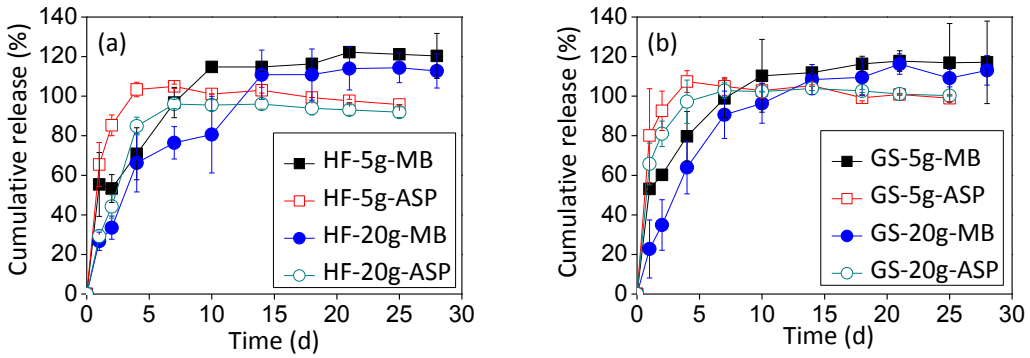


Fig.7. MB and ASP release profiles of HF (a) and GS (b) coated MAN microparticles

As the lipid-coated microparticles are exposed to aquous medium, the liquid penetrates into the microparticles, dissolves the drug to form a saturated solution (as long as undissolved drug is present), and then the drug diffuses out of the microparticles. So it is reasonable to expect a faster drug release from the microparticles loaded with a more soluble drug. Additionally drug solubility could facilitate the hydration process by permitting continuous penetration of water via diffusion and dissolution. Aqueous solubility also affects the osmotic pressure inside coated particles upon contact with the release medium, which impacts drug release. But in our case the osmotic pressure gradient should be unaffected by the drug as the MAN in the major constituent of all lipid coated beads.

4. Conclusion

This study showed that the mini-Glatt fluid bed coater with a Wurster column could be utilized to form lipid-coated microparticles without agglomeration at modest temperature for sustained drug release by using organic solvent spray

coating. Microparticles with a thicker lipid coat were intact after release in contrast to particles with a thinner coating this thicker coating extended the release up to 25 days for MB and 7 days for ASP. Drug release was driven by core bead dissolution and diffusion. HF-coated particles showed a comparable burst and release rate as GS-coated particles. The reduction in size of starting bead had only marginal effect on the drug release behavior. In contrast, the release of the more hydrophilic model compound was much faster than that of the less hydrophilic. Overall, these results provide an encouraging basis for lipid-coated microparticles for sustained release of sensitive biopharmaceutical drugs.

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CHAPTER 4

Lipid-coated polyol core microparticles for sustained release of protein

Abstract

Parenteral sustained release systems for proteins which provide therapeutic levels over a longer period avoiding frequent administration, which preserve protein stability during manufacturing, storage and application and which are biodegradable and highly biocompatible in the body are intensively sought after. The aim of this study was to generate and study polyol core microparticles loaded with a monoclonal antibody IgG1 and coated with lipid either hard fat or glyceryl stearate at different coating levels. The protein was stabilized with 22.5 mg/mL sucrose, 0.1% PS 80, 10 mM methionine in 10 mM His buffer pH 7.2 during the spray loading process. 30 g protein-loaded mannitol carrier microparticles were coated with 5 g, 10 g, 20 g and 30 g of lipid, respectively. Placing more lipid onto the protein-loaded microparticles reduced both burst and release rate, and the particles maintained their geometric form during the release test. The IgG1 release from microparticles covered with a hard fat layer extended up to 6 weeks. The IgG1 was released in its monomeric form and maintained its secondary structure as shown by FTIR. Incomplete release of IgG1 from glyceryl stearate-coated microparticles was observed, which may be due to the small pore sizes of the GS layer or a detrimental surfactant character of GS to protein.

Hence, these hard fat-coated polyol core microparticles have high potential for protein delivery.

Keywords: Protein drug, Monoclonal antibody IgG1, Mannitol, Hard fat, Glyceryl stearate, Fluid bed coater, Microparticles, Sustained release, Stability, Lipids, Coating.

1. Introduction

Proteins play an important role in the treatment of severe diseases like cancer, autoimmune and inflammatory diseases for their high specificity, efficacy and low adverse effects [1]. Specifically, the successful applications of numerous therapeutic antibodies have resulted in an exponential growth in their research and development some of them showing blockbuster [2]. Owing to the inherent instability of protein drugs in the gastrointestinal tract, they are usually administered by parenteral administration [3, 4]. Many protein drugs exhibit short half-lives *in vivo* and multiple dosing schemes and frequent injections are necessary to achieve therapeutic drug levels, which result in poor patience compliance [5, 6]. Incorporation of proteins in a sustained release system for systemic delivery which can maintain therapeutic plasma levels for an extended period is of high interest [7, 8].

Parenteral controlled release system delivering small molecular drugs and peptides are well established for decades. In contrast, their use for proteins is limited due to the protein sensitivity leading to instabilities during manufacturing, storage and application [5]. Lipids such as triglycerides have gained growing attention in this context due to their good biocompatibility and biodegradability [9, 10], which qualifies them to be an interesting alternative to polymeric matrix materials as they do not show the shortcomings of the commonly used PLA and PLGA polymers, such as the acidic microclimate and formation of detrimental polymer degradation products during erosion [11]. Lipid microparticles have been proposed as drug delivery systems for long-term release of peptide and protein drugs over days to months [12-14]. They are often prepared using organic solvent evaporation and melt

dispersion techniques by incorporating a protein solution in the molten/dissolved lipid dispersing the preemulsion in an aqueous phase [15]. The process technologies like spray-drying, extrusion, emulsion systems tested for controlled release protein drugs have shortcomings regarding protein stability, which resulting from high temperature or organic solvent use during the preparation process [16]. Additionally, incorporation of sufficient amounts of drug is troublesome due to the hydrophobic nature of the lipids [17].

An organic solvent fluid bed spray coating technique was developed in **Chapter 3** for the lipid-coated polyol core microparticle preparation. This method featured the advantage of a microparticle production process at moderate temperature which could secure the stability of the protein drug during manufacturing. The model compounds methyl blue and aspartame were released upon dissolution of the polyol core beads over 25 and 7 days, respectively. Furthermore, we previously established successful loading of polyol and sugar beads with protein drugs in a fluid bed system [18] and this renders lipid-coated polyol core microparticles very interesting for sustained release application of protein drugs. Consequently, we intended to develop a new process to load an IgG1 antibody model onto polyol beads and provide them with a release controlling lipid layer in a mini fluid bed system. Protein stability during fabrication and release as well as the duration of release were the main critical parameters of interest. In order to prevent IgG1 unfolding at the air-liquid interface and subsequent aggregation, surfactant was added as stabilizing excipients during spray loading. Turbidity analysis, light obscuration measurement and size-exclusion chromatography (SEC) analysis were carried to evaluate aggregate

formation upon manufacturing and release. Additionally, the secondary structure of incorporated IgG1 was analyzed by Fourier transform infrared (FTIR) spectroscopy.

2. Materials and Methods

2.1. Materials

Pearlitol® 500DC-Mannitol (MAN) was kindly provided by Roquette Corporate, Darmstadt, Germany. A 20.9 mg/mL IgG1 monoclonal antibody solution in 10 mM histidine pH 7.2 was used. Sucrose, polysorbate 80 (PS 80) and L-Methionine were purchased from Sigma-Aldrich, Munich, Germany. Witepsol® E85 (hydrogenated coco-glycerides, HF) and Imwitor® 900 (glyceryl stearate with a monoester content of 40-55%, GS) were kindly provided by Sasol GmbH, Hamburg, Germany. Isopropanol (99.7%) was supplied by the reagent center of the University of Munich, Germany.

2.2. Methods

2.2.1. Optimization of formulation for IgG1 loading

Sucrose was dissolved in 10 mL his buffer (pH 7.2, 10 mM) containing 10 mM methionine and 0.02% PS 80 to final concentrations of 22.5 mg/mL, 45 mg/mL or 90 mg/mL. 30 g MAN particles were loaded with the former solutions in a Mini-Glatt fluid bed system with Wurster insert (Glatt GmbH, Binzen, Germany). The detained operation conditions were T_{inlet}: 35 °C; $p_{process}$: 1.0 bar; $p_{atomizing\ air}$: 1.0 bar; spray rate: 1.0 mL/min; and spray nozzle diameter: 0.3 mm. Based on the optimal sucrose concentration, PS 80 concentration was increased to 0.1% to prevent IgG1 aggregation under the same condition.

2.2.2. Preparation of drug-loaded starting cores

The spray solution (10 mL) containing 3.1 mg/mL IgG1, 0.1% PS 80, 22.5 mg/g sucrose, and 10 mM methionine in 10 mM histidine buffer pH 7.2 was filtrated through an Acrodisc 0.2 µm PES syringe filter. Mannitol particles (30 g) were loaded with the drug solution in a Mini-Glatt fluid bed system with Wurster insert (Glatt GmbH, Binzen, Germany). The detailed operation conditions were T_{inlet}: 35 °C; $p_{process}$: 1.0 bar; $p_{atomizing\ air}$: 1.0 bar; spray rate: 1.0 mL/min; and spray nozzle diameter: 0.3 mm. After loading, the particles were dried for additional 15 min at 35 °C in the fluid bed. The drug loaded particles were collected and kept at 2-8 °C until lipid coating.

2.2.3. Preparation of lipid-coated microparticles

Lipid was dissolved at 2% w/v in isopropanol at 70 °C. 30 g drug-loaded microparticles were coated with the lipid solution in the Mini-Glatt fluid bed with Wurst insert at, T_{inlet}: 40 °C for GS and 30 °C for HF; $p_{process}$: 0.7 bar; $p_{atomizing\ air}$: 0.5 bar; spray rate: 7.0 mL/min; and spray nozzle diameter: 0.5 mm. After coating, the microparticles were annealed for additional 15 min at the same conditions.

2.2.4. High performance size exclusion chromatography (HP-SEC)

HP-SEC was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Germany). The autosampler and the column were controlled at 20 °C and 23 °C, respectively. The samples were centrifuged for 5 minutes at 2000 rpm. For each sample solution, 250 µl supernatant was injected onto a Tosoh TSKgel®

G3000SWXL column (7.8x300 mm) (Tosoh Bioscience, Stuttgart, Germany) using a mobile phase of 100 mM sodium phosphate buffer with additional 100 mM sodium sulfate pH 6.8 at a flow rate of 0.5 ml/min. UV detection at 280 nm was used. The chromatograms were analyzed regarding retention times and area under the curve (AUC) with ChemStation® B.02.01-SR2 (Agilent Technologies).

2.2.5. Determination of drug loading of lipid-coated microparticles

1.0 g of HF-coated particles was dispersed in 10 ml PBS buffer at 45 °C. After cooling down to room temperature, approximate 2 mL slurry were filtered through a 0.2 µm filter to remove the lipid after. Filtrate was analyzed for drug content by HP-SEC. Each experiment was performed in triplicate.

2.2.6. Microparticle morphology

The morphology of microparticles was analyzed by use of a light optical microscope (Olympus BX50 F4, Olympus, Tokyo, Japan) equipped with a digital camera (HVC 20, Hitachi, Maidenhead, GB).

2.2.7. Turbidity

The turbidity of IgG1 solution in formazine nephelometric units (FNU) was determined with a NEPHLA turbidimeter (Dr. Lange, Düsseldorf, Germany), based on light scattering in a 90 ° angle at $\lambda = 860$ nm. The system was calibrated with a formazine standard. 1000 mg of microparticles was dissolved in 6 ml PBS buffer (10 mM, pH 7.4, 2.7 mM KCl, 137 mM NaCl, 0.05% NaN_3). Approximately 2 mL of each sample were used for analysis in triplicate.

2.2.8. Light obscuration

Light obscuration tests were carried out according to Ph.Eur. 2.9.19. The particle counting of subvisible particles in a size range between 1 and 200 µm was conducted using a SVSS-C instrument and associated analysis software (PAMAS GmbH, Rutesheim, Germany). For each sample (n = 3), three measurements of a volume of 0.3 mL with a pre-run volume of 0.3 mL at fixed fill rate, emptying rate and rinse rate of 5 mL/min were performed. Prior to each measurement the system was rinsed with high purified water until particle counts of less than 30 particles/mL were determined. The obtained results represented the mean value of the particle counts of three measurements, referred to a sample volume of 1.0 mL.

2.2.9. *In vitro* release

Protein release was studied in PBS pH=7.4 on a horizontal shaking incubator at 37 °C (60 rpm). Approximately 1.0 g of drug-loaded particles were suspended in 3 mL buffer, transferred into a dialysis bag with 1 MDa cutoff and immersed into a 15 mL disposable plastic tube with 7 mL of PBS release medium containing 0.01% NaN_3. At designated time points, 350 µl release medium were withdrawn from the tube and replaced with the same amount of fresh release medium. The drug content in the release medium was quantified using a HP-SEC. Each experiment was performed in triplicate.

2.2.10. Fourier-transformed-infrared spectroscopy (FTIR)

The release medium was analyzed using a Bruker Tensor 27 FTIR spectrometer

(Ettlingen, Germany) equipped with a Bio ATR measuring cell and an MCT detector at 25 °C. 35 µL of the sample was spread under dry nitrogen to ensure an equal distribution on the crystal surface and analyzed against PBS buffer as blank. For each experiment, 100 scans were set for the blank and sample with a resolution of 4 cm^{-1} and water vapor correction. The data were analyzed with the OPUS 6.5 software for second derivative spectra and vector normalization.

3. Results and discussion

3.1. Optimization of formulation for IgG1 loading

Generally, temperature, air-liquid interfaces and dehydration are three main stress factors affecting the chemical and physical protein instabilities during spray drying [19]. As the inlet air temperature is lower in the fluid bed system, thermal denaturation during drug loading could be regarded as negligible. However, the tremendous expansion of the air-liquid interface may lead the orientation of hydrophobic amino acid residues towards the nonaqueous environment and subsequent protein unfolding and aggregation [19-21]. Meanwhile, the protein molecules are deprived of the surrounding and protective water, and are thermodynamically destabilized by losing their hydrogen bonding to water molecules [19].

Addition of surfactant e.g. polysorbates (PS) and other excipients e.g. polyols, sugars, salts and amino acids are effective in protecting the stability of a protein drug during spray drying [19, 20, 22, 23]. Surfactants adsorb at the air-liquid interface reducing the appearance of protein molecules at the surface [19, 20]. Small molecule

excipients could be incorporated as "water substitutes" replacing the hydrogen bonding existing in an aqueous environment [19, 23]. Therefore, PS 80 and sucrose were selected as two stabilizers for the drug loading in the fluid bed coater. Although improved protein stabilization has been shown with increasing concentration of sucrose, its addition is limited by the formation of more viscous solutions result in microparticle agglomeration in the fluid bed. Thus, the concentrations of PS 80 and sucrose have to be optimized during the IgG1 loading process. As shown in the **Fig.1**, protein loading with a formulation containing 22.5 mg/mL sucrose did not show agglomeration, whereas large agglomerates were observed at higher sucrose concentrations.

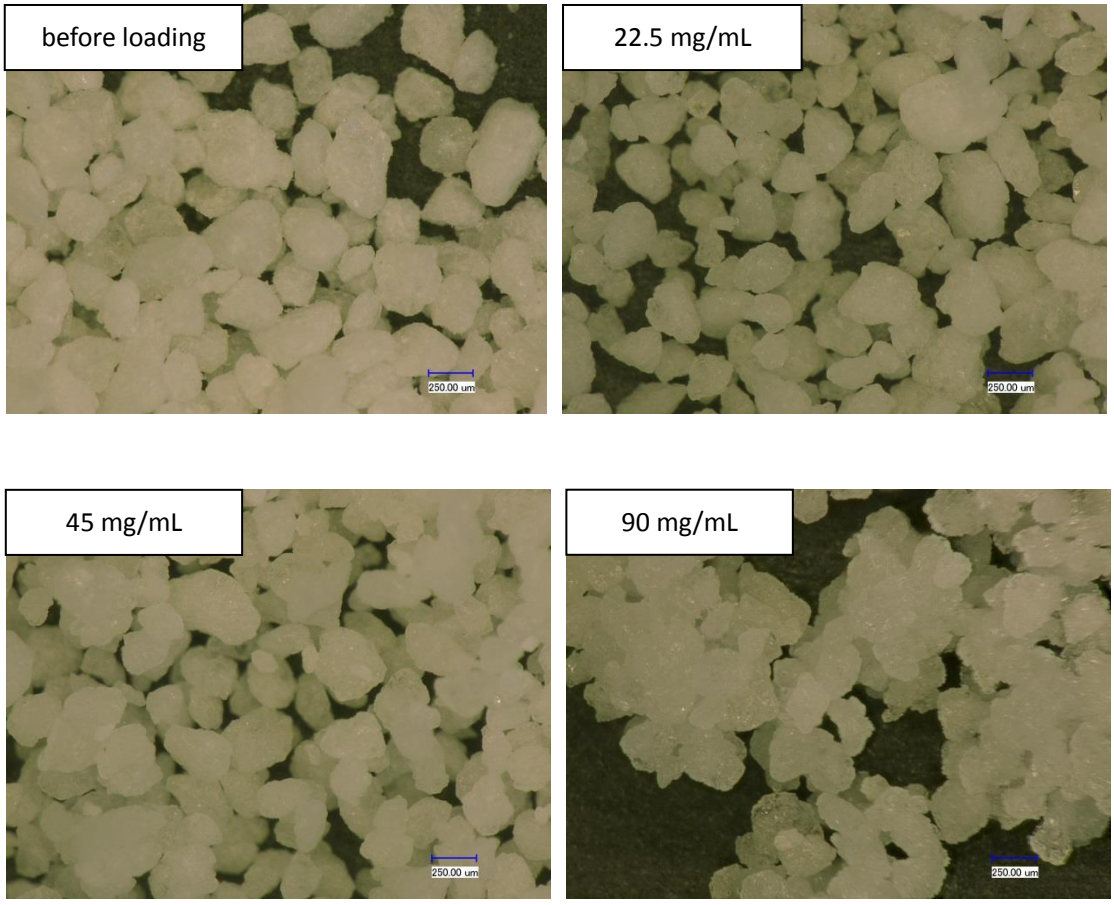
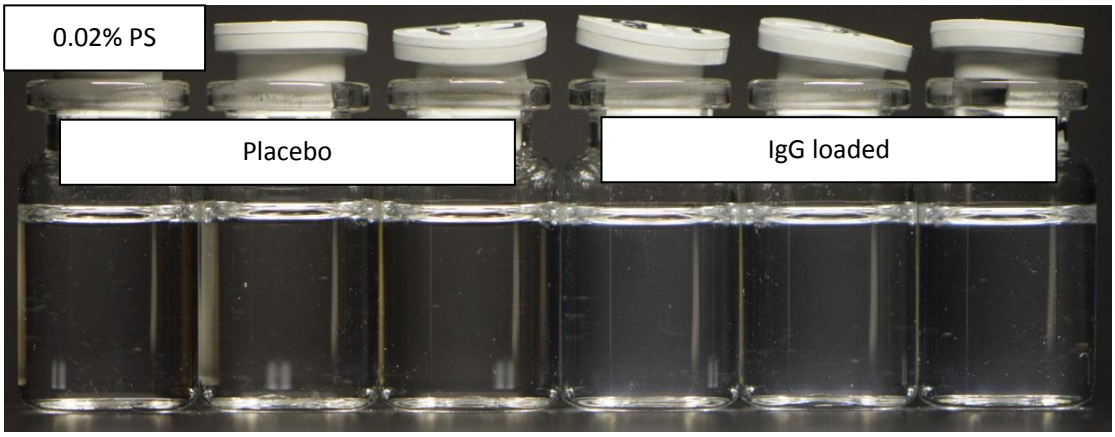


Fig. 1. Visual appearance of mannitol beads after loading with placebo, containing

22.5, 45 or 90 mg/mL sucrose.

The influence of PS 80 concentration on IgG1 stability after drug loading was analyzed via visual inspection, turbidity, light obscuration and HP-SEC. At a level of 0.1% PS 80 in the spray solution, the redissolved IgG1 loaded microparticles did not show any sign of aggregation, neither visually (**Fig.2**) nor in turbidity and subvisible particle concentration compared to placebo samples (**Fig.3**) nor in HP-SEC with 100% monomer recovery. In contrast, at 0.02% PS 80 slight formation of particles (**Fig.2**, **Fig.3**) and 0.4% dimers in HP-SEC were found. According to these results, it was concluded that 22.5 mg/mL sucrose, 0.1% PS 80 in 10 mM His buffer pH 7.2 presented a suitable formulation for IgG1 loading.



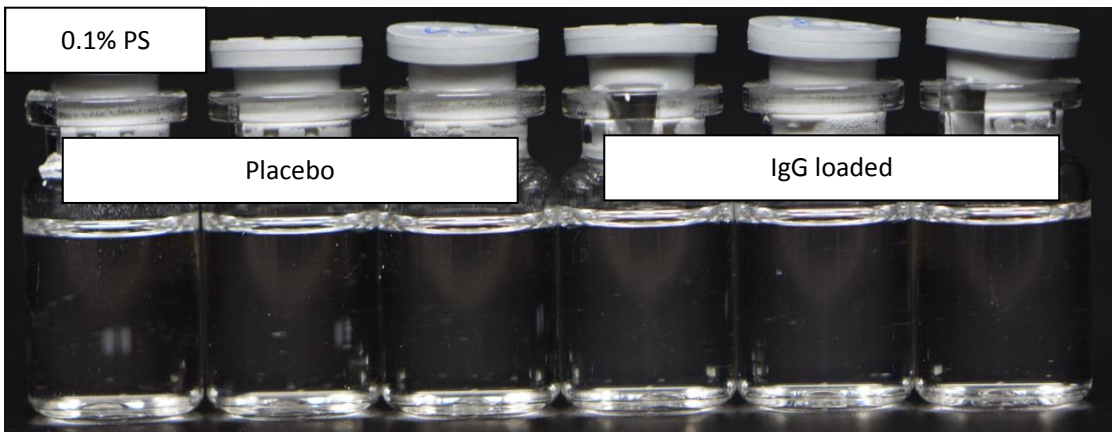


Fig. 2. Visual appearance of reconstitutions of placebo and IgG1 loaded microparticles from a formulation composed of 0.02% or 0.1% PS 80, 22.5 mg/g sucrose, and 10 mM methionine in 10 mL histidine buffer (pH 7.2, 10 mM), 1 g of microparticles were redissolved in 6 ml PBS buffer (10 mM, pH 7.4, 0.05% NaN_3).

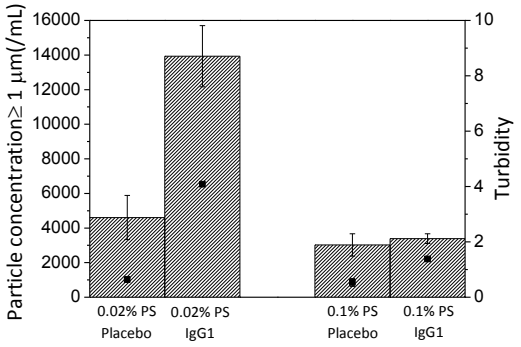


Fig. 3. Results of light obscuration and turbidity of reconstitutions of placebo and IgG1 loaded microparticles from a formulation composed of 0.02% or 0.1% PS 80, 22.5 mg/g sucrose, and 10 mM methionine in 10 mL histidine buffer (pH 7.2, 10 mM), 1 g of microparticles were redissolved in 6 ml PBS buffer (10 mM, pH 7.4, 0.05% NaN_3).

3.2. IgG1 release from lipid-coated microparticles

The obtained *in vitro* release profiles of IgG1 from lipid coated microparticles are illustrated in **Fig. 4**. IgG1 loaded microparticles coated with 5 g HF exhibited an approximate 50% burst release and subsequently nearly complete release (81.9% (SD=16.8%, n=3)) within two weeks. Coating with 10 g lipid reduced the protein release rate. This deceleration of the release became more apparent with the addition of more lipid. For the microparticles coated with 20 g and 30 g lipid, IgG1 was released in a sustained manner over 5 weeks and 6 weeks without initial burst

release. For methyl blue the release period was only extended to 14 and 25 days for 20 and 30 g lipid coat and much shorter for 5 and 10 g (**Chapter 3**). In parallel to this prolongation of the release period, the total amount of drug released differed with the size of drug. Complete release was determined for methyl blue, whereas about 25% of IgG1 antibody remained in the microparticles coated with 20 or 30 g lipid. Compared to HF, GS exhibits a higher wettability and more stable polymorphic structure (**Chapter 3**). **Fig.4b** depicts IgG1 release profiles of microparticles coated with different amount of GS. A long term sustained release of IgG1 was not observed at any GS coating level. After a burst release within the first day, only little amounts of IgG1 were released in the following days.

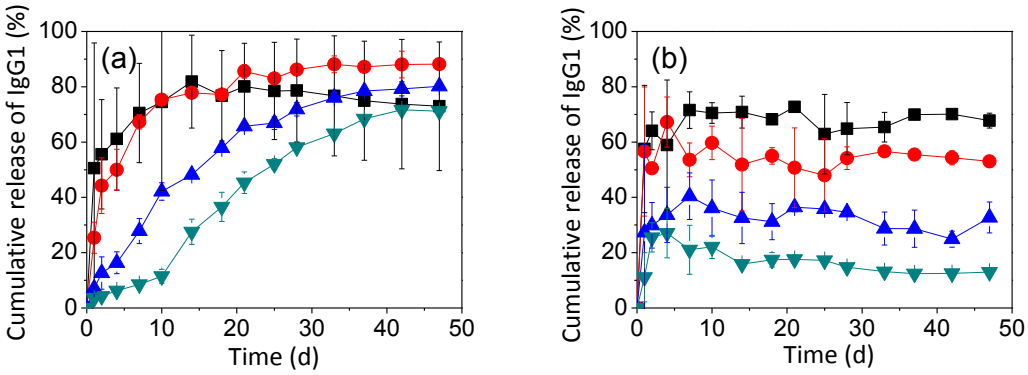
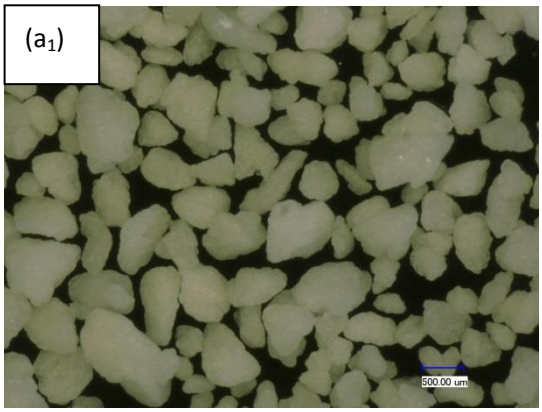


Fig.4. IgG1 release profiles of microparticles coated with different amounts of HF (a) or GS (b), 5 g: ■; 10 g: ●; 20 g: ▲; 30 g: ▼.

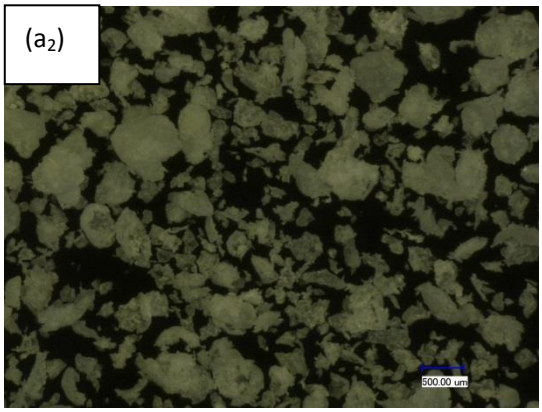
Almost all of the protein-loaded microparticles coated with 5 g HF were degraded after the release test period whereas this was the case for only a small amount of microparticles coated with more lipid (**Fig. 5**). Thus, particle degradation substantially contributes to the drug burst and release of 5 g lipid-coated microparticles. Drug

release from microparticles with a thicker lipid layer, which maintained their geometric form during the release test, was more controlled by Fickian diffusion. Therefore, the microparticles coated with 5 g lipid showed similar release for methyl blue and IgG1. The slower IgG1 release from microparticles with more lipid could be explained by the increase in drug size resulting in a lower diffusion coefficient [24] and limited movement if the sizes of pores resulting from dissolution of the polyol core beads were too small for free diffusion of large molecule IgG1 (150 kDa, hydrodynamic diameter ≈ 11 nm) [25]. Additionally, a small pore size can explain the partial IgG1 entrapment by the lipid matrix leading to incomplete release. The small pores formed by MAN dissolution and diffusion in GS coated microparticles may be too small for the release of large IgG1 molecules in contrast to methyl blue and aspartame. The incomplete release of protein may also be related to the surfactant characters of GS, which may cause the protein denaturation. Consequently, the IgG1 stability after release was analyzed in the following.

before release



after release



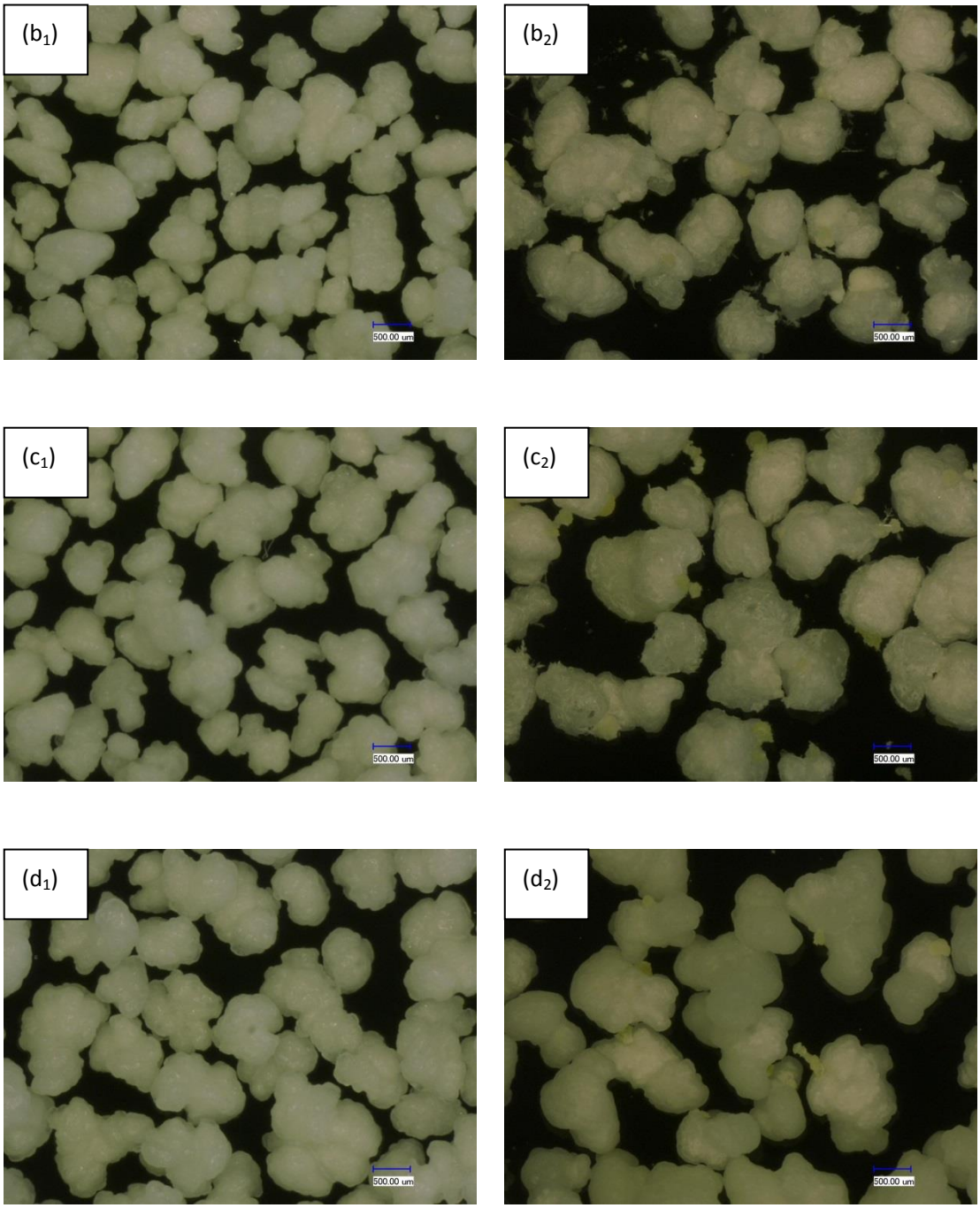


Fig.5. Microscope images of mannitol particles coated with 5g (a), 10g (b), 20g (c) and 30g (d) HF before (left) and after (right) release

3.3. IgG1 stability

The HP-SEC analysis revealed that IgG1 was totally released as monomer over the entire release period without formation of fragments, dimers or higher molecular

weight species. In addition, to determine if the fabrication process and the long term release period induced conformational changes of the IgG1, analysis of the secondary structure was performed by FTIR. Several transmittance bands (e.g., amide I, amide II and amide III) could be used for structure analysis of IgG1 [26, 27]. The IgG1 exhibits major absorption peaks at 1612 cm^{-1}, 1640 cm^{-1}, 1690 cm^{-1}, which correspond to the native β-sheet structure [28, 29]. So **Fig.7** shows the second derivative spectrums of amide I and amide II bands measured in transmission obtained for IgG1 before and after release. For the HF-coated microparticles after release, the second derivatives of the amide I and II spectra showed no significant difference compared to native IgG1. It can be concluded that the loading and the lipid coating process as well as the IgG1 release from HF-coated microparticles did not induce relevant changes in secondary structure. However, for the GS-coated microparticles, formation of a new band at 1633 cm^{-1} was observed. Typically, upon denaturation monoclonal antibodies form anti-parallel β-sheet giving rise to a peak near 1620 cm^{-1} [30]. This change in FTIR spectrum may indicate a structure change of the IgG1 in cause of the release test and could explain the incomplete release from GS-coated microparticles.

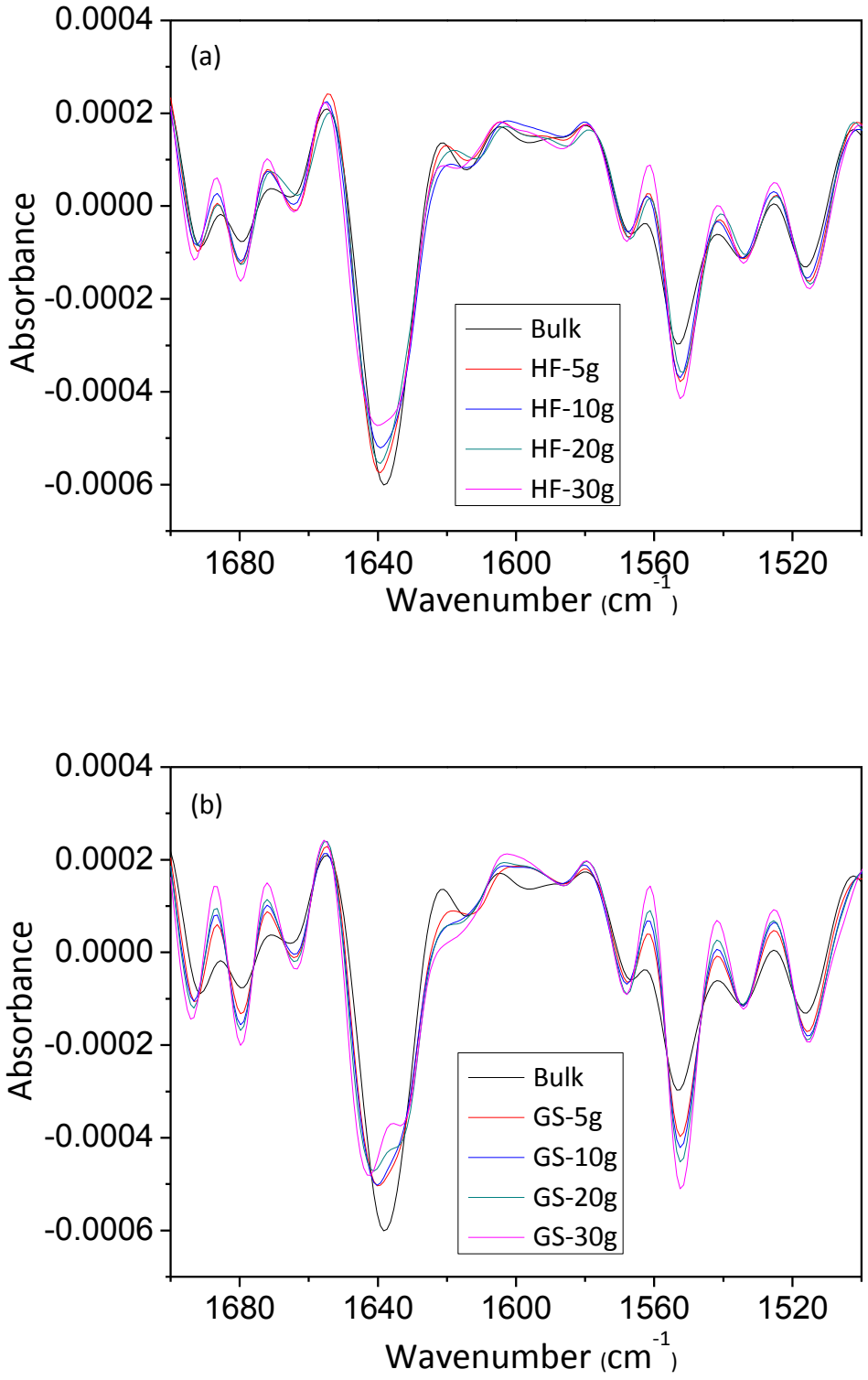


Fig.6. FTIR second derivative spectra of IgG1 after 92 days release from HF (a) and GS-coated (b) particles

4. Conclusion

In this work, mannitol microparticles were loaded with IgG1 and coated with lipid in a fluid bed system to control the sustained release of the protein. 22.5 mg/mL sucrose, 0.1% PS 80, 10 mM methionine in 10 mM His buffer pH 7.2 presented a suitable formulation for IgG1 loading process. An extended release manner over 6 weeks could be achieved by coating 30 g HF. It was shown that IgG1 was totally released as monomer. Furthermore, the particle fabrication procedure and the long release periods did not affect the secondary structures of IgG1 from HF-coated microparticles. Sustained release of IgG1 was not observed from GS-coated microparticles, which may result from the small pore sizes of GS layer or detrimental surfactant character of GS. Thus, HF-coated microparticles developed in this study could be a promising protein delivery systems.

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CHAPTER 5

Spray drying of silica microparticles for sustained release application with a new sol-gel precursor

Abstract

A new precursor, tetrakis(2-methoxyethyl) orthosilicate (TMEOS) was used to fabricate microparticles for sustained release application, specifically for biopharmaceuticals, by spray drying. The advantages of TMEOS over the currently applied precursors are its water solubility and hydrolysis at moderate pH without the need of organic solvents or catalyzers. Thus a detrimental effect on biomolecular drug is avoided. By generating spray-dried silica particles encapsulating the high molecular weight model compound FITC-dextran 150 via the nano spray dryer Buchi-90, we demonstrated how formulation parameters affect and enable control of drug release properties. The implemented strategies to regulate release included incorporating different quantities of dextrans with varying molecular weight as well as adjusting the pH of the precursor solution to modify the internal microstructures. The addition of dextran significantly altered the released amount, while the release became faster with increasing dextran molecular weight. A sustained release over

35 days could be achieved with addition of 60 kD dextran. The rate of FITC-Dextran 150 release from the dextran 60 containing particles decreased with higher precursor solution pH. In conclusion, the new precursor TMEOS presents a promising alternative sol-gel technology based carrier material for sustained release application of high molecular weight biopharmaceutical drugs.

Keywords: TMEOS, Sol-gel, Spray drying, Dextran, Microparticles, Sustained release

1. Introduction

The sol-gel technology is presently believed to be one of the most promising approaches for controlled drug release [1-4]. Its main advantage lies in the fact that the entrapment of drugs in a porous network proceeds without formation of covalent linkages between drug molecules and matrix [1]. As a result, the drug payload is intact, which is specifically important for biomolecular drugs like proteins [5-10]. Additionally, entrapment in a nanostructured amorphous glass matrix can support the long-term and thermal stability of proteins [1, 9-11].

The sol-gel processing includes the use of a precursor, often metal or silicon alkoxides. When an alkoxide is mixed with water, it experiences hydrolysis and the products are involved in condensation reactions leading first to a sol formation followed by cross-linking of sol particles which causes the sol-gel transition and consequently porous network formation [12, 13]. The silica sol-gel process is strongly influenced by additives such as short-chain alcohols [14], electrolytes [15], and hydrophobic solubilizates [16]. Silica as a carrier matrix exhibits several advantages over metal alkoxides, as it is relatively cheap and easy to purify, with excellent physical and chemical stability, good biocompatibility, and biodegradability with favorable tissue responses *in vitro* and *in vivo* [17-19]. Thus, silica-based sol-gel materials are frequently used for drug delivery purpose [1].

Although sol-gel silica materials have many advantages for controlled drug release application, there still exist some disadvantages. Conventional silica

precursors such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS) are insoluble in water. In order to achieve a uniform sol, an organic solvent or surfactant is added and extreme conditions of pH and high temperature are required, which are unfavorable for the encapsulation of biomolecular drugs [20]. Furthermore, in the course of the reaction process, short-chain alcohols such as methanol or ethanol as by-products of the hydrolysis of tetraalkyl orthosilicates are generated, which negatively impact biomolecule resulting in unfolding and aggregation and subsequently restricts their use [21]. In contrast, tetra(2-hydroxyethyl) orthosilicate (THEOS) has been investigated to address the solubility, temperature and pH problems associated with TEOS and TMOS [22]. Moreover, it is known that ethyleneglycol which is produced during THEOS hydrolysis has little effect on surfactant self-assemblies and phase behavior compared to methanol or ethanol [20-25]. However, THEOS alone does not cause the jellification of water at ambient conditions over a period of a month. Additives such as such as polysaccharides are necessary to trigger the sol-gel processes [25].

Those problems may be circumvented by changing the ethoxy, methoxy or ethylene glycoxy groups of the precursor against ethylene glycol monomethylether (EGMM). EGMM with boiling point 124.5 °C is readily removed accompanying the water evaporation. As we found, this new precursor tetrakis (2-methoxyethyl) orthosilicate (TMEOS) is water soluble and the time of water jellification can be controlled from a few minutes to a few hours by adjusting the pH value between pH 6.0 to 8.0 without the need for additives at room temperature.

Furthermore, hydrolysis renders longer chain alcohol which can be expected to show better compatibility with sensitive protein drugs. In the present study, the compatibility of ethylene glycol monomethylether (EGMM) as a by-product produced in course of TMEOS hydrolysis was checked with sensitive IgG1 antibody. Then the effects of pH and ionic strength on TMEOS gelation were examined. A nano spray dryer Buchi-90 was used to produce silica gel microparticles for release application. Prior to protein drug loading, FITC-dextran 150 (FITC-Dx 150) was used as a high molecular weight model compound to regulate the drug release kinetics.dextrans of different molecular weight were incorporated into the silica microparticles. Additionally, the effect of several parameters such as silica/additive ratio, molecular weight of additives and pH of precursor solution were addressed.

2. Materials and Methods

2.1. Materials

Tetrakis (methoxyethoxy) silane (TMEOS) was purchased from Suzhou Chum-Win New Material Science & Technology Co,. Ltd., Suzhou, China, Fluorescein isothiocyanate dextran 150 kDa (FITC:Glucose = 1:160) (FITC-Dx 150) was purchased from Sigma-Aldrich, Munich, Germany and Dextran 1 (Dx 1), Dextran 5 (Dx 5) and Dextran 60 (Dx 60) were purchased from Pharmacosmos A/S, Holbaek, Denmark. A 2 mg/mL IgG1 monoclonal antibody in 10 mM PBS pH 7.2 was used. Ethylene glycol monomethylether (EGMM, 99.5%) was supplied by the reagent center of the University of Munich, Germany. All other reagents used were of

analytical grade. Deionized water (Milli-Q) was used for all precursor preparation.

2.2. Methods

2.2.1. Turbidity

The turbidity of IgG1/EGMM mixtures in formazine nephelometric units (FNU) was determined with a NEPHLA turbidimeter (Dr. Lange, Düsseldorf, Germany), based on light scattering in an 90 ° angle at λ = 860 nm. The system was calibrated with a formazine standard. Approximate 2 mL of each sample were used for analysis.

2.2.2. Light obscuration

Light obscuration tests were carried out according to Ph.Eur. 2.9.19. The particle counting of subvisible particles in a size range between 1 and 200 µm was conducted using a SVSS-C instrument and associated analysis software (PAMAS GmbH, Rutesheim, Germany). For each sample (n = 3) three measurements of a volume of 0.3 mL with a pre-run volume of 0.3 mL at fixed fill rate, emptying rate and rinse rate of 5 mL/min were performed. Prior to each measurement the system was rinsed with high purified water until particle counts of less than 30 particles/mL were determined. The obtained results represented the mean value of the particle counts of three measurements, referred to a sample volume of 1.0 mL.

2.2.3. High performance size exclusion chromatography (HP-SEC)

HP-SEC was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, California, USA). The autosampler and the column were

temperature controlled at 20 °C and 23 °C, respectively. The samples were centrifuged for 5 minutes at 2000 rpm. For each sample solution, 40 µl supernatant were injected onto a Tosoh TSKgel® G3000SWXL column (7.8x300 mm) (Tosoh Bioscience, Stuttgart, Germany) using a mobile phase of 100 mM sodium phosphate buffer with additional 100 mM sodium sulfate pH 6.8 at a flow rate of 0.5 ml/min. The eluted sample was detected by UV absorption at 280 nm. The chromatograms were analyzed regarding retention times and the area under the curve (AUC) with ChemStation® B.02.01-SR2 (Agilent Technologies).

2.2.4. Particle preparation

To investigate the effects of additives, different formulations were prepared (**Table 1**). The total mass content of excipients was set to 5.5% (w/v). In a typical procedure, FITC-Dx 150 solution in 10 mM PBS was mixed with TMEOS to a final concentration of 0.05%. Hydrolysis was performed for 2 hours. The spray drying conditions in the nano spray dryer Buchi-90 were T_{in}/T_{out}: 120 °C /58 °C, flow rate of drying air: 120 L/min, atomizing mesh size: 7.0 µm. Spray solutions were filtered through a0.2 µm PVDF syringe filter prior to spray drying.

Table 1: Formulations of precursors for spray drying

Run number	Silica dioxide	Dextran (%w/v)			pH
		1 kDa	5 kDa	60 kDa	
1	5.5	0.00	0.00	0.00	6.0
2	5.0	0.5	0.00	0.00	6.0

3	4.5	1.0	0.00	0.00	6.0
4	3.7	1.8	0.00	0.00	6.0
5	1.8	3.7	0.00	0.00	6.0
6	5.0	0.00	0.5	0.00	6.0
7	4.5	0.00	1.0	0.00	6.0
8	3.7	0.00	1.8	0.00	6.0
9	1.8	0.00	3.7	0.00	6.0
10	5.0	0.00	0.00	0.5	6.0
11	4.5	0.00	0.00	1.0	6.0
12	3.7	0.00	0.00	1.8	6.0
13	1.8	0.00	0.00	3.7	6.0
14	4.5	0.00	0.00	1.0	6.2
15	4.5	0.00	0.00	1.0	6.4

2.2.5. Particle morphology and size

The particles were visualized with scanning electron microscopy (SEM) using a Jeol JSM-6500F instrument (Jeol Ltd., Tokyo, Japan) with Inca Software (Oxford instruments, Oxfordshire, UK) at an accelerating voltage of 2.0 kV. They were sputtered with carbon. SEM images were further analyzed for particle size by using the integrated software in a Keyence VHX-500FD digital microscope (Keyence, Neu-Isenburg, Germany). Approximately 100 particles were analyzed for each sample.

2.2.6. In vitro release

FITC-Dx 150 release was studied in PBS buffer (0.01 M phosphate; 0.138 M NaCl; 0.027 M KCl, pH 7.4) at 39 °C using a shaking incubator (60 rpm). Approximately

100 mg of drug-loaded particles and 1 mL buffer were placed in a 1 MDa cutoff dialysis bag and immersed into a 15 mL disposable plastic tube with 4 mL PBS containing 0.05 % NaN_3. At designated time points, 200 µL of release medium were withdrawn and replaced with the same amount of fresh release medium. FITC-Dx 150 was quantified by fluorescence measurement (Varian Cary Eclipse, now Agilent Technologies, Santa Clara, California, USA; ex. 492 nm; em. 518 nm) in 96-well plates.

3. Results and discussion

3.1. Compatibility of EGMM with IgG1

Hydrolysis of TMEOS generates ethylene glycol monomethylether (EGMM) which can evaporate in the course of the spray-drying process. One of the major requirements in formulation development is the compatibility of the by-product with the incorporated drug. Accordingly, the compatibility was exemplarily tested with an IgG1 antibody. The compatibility of different EGMM concentrations with IgG1 was assessed via visual inspection, turbidity, light obscuration and HP-SEC measurement. These analytical methods were used to identify any aggregation or fragmentation phenomena of IgG1. All IgG1 samples showed visaul turbidity compared to the placebo samples. This was consistent with the turbidity measurement and light obscuration. These results indicated interactions between EMGG and IgG1 leading to formation of layer IgG1 aggregates. However, no soluble aggregate or fragment formation of IgG1 samples was observed by HP-SEC analysis with nearly 100%

monomer recovery except for the sample containing 50% EGMM. Based on these results, it was concluded that at the low EGMM concentrations arising in the particles during gel formation are compatible with IgG1. Therefore, the maximal TMEOS concentration of spray solution was set to 30% (equals to 5.5% silica dioxide), which would yield a safe EGMM concentration of 6.95% for proteins upon hydrolysis. In future studies additional purity analysis of TMEOS will be performed and the impact of protein stabilizing excipients like sugars and surfactants will be tested.

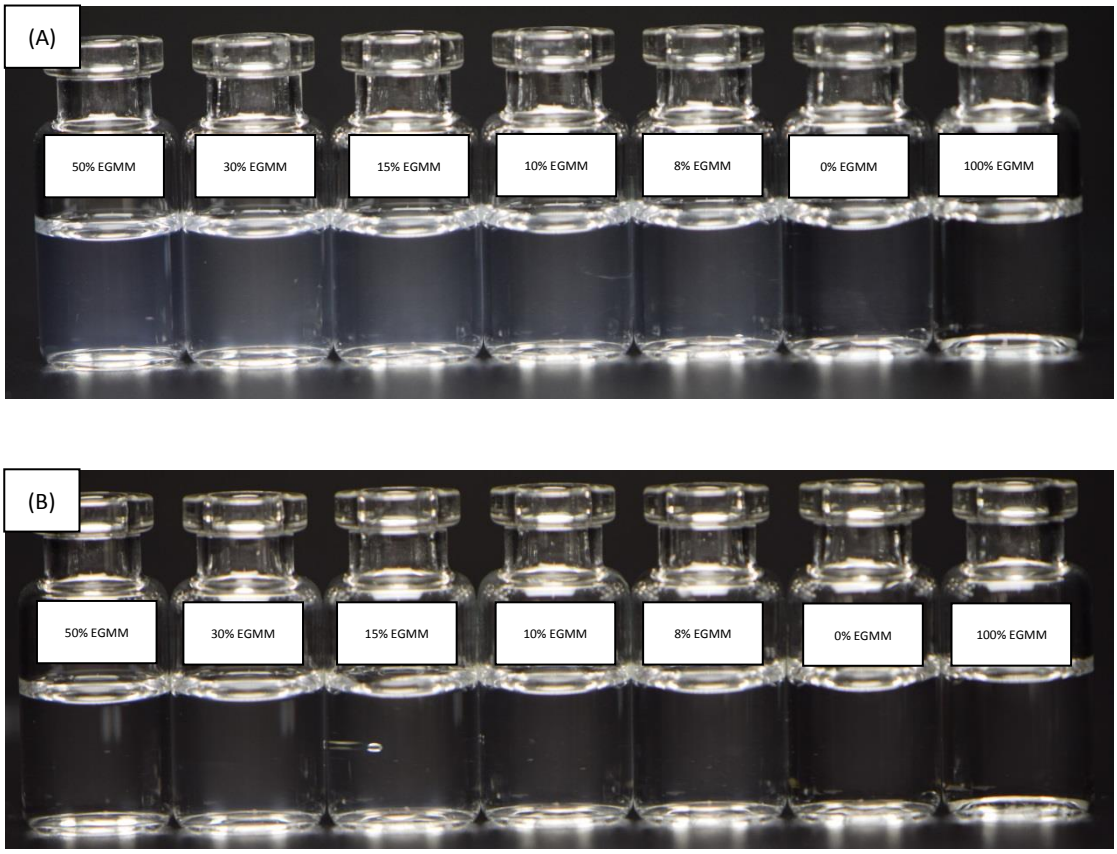


Figure 1. Visual appearance of mixtures of IgG1 (A) or placebo (B) solutions with different EGMM concentration, a 2 mg/mL IgG1 in 10 mM PBS pH 7.2 was used.

Table 2: Analysis of mixtures of IgG1 or placebo solutions with different EGMM concentration by turbidity, particle counter SVSS-C and HP-SEC, a 2 mg/mL IgG1 in 10 mM PBS pH 7.2 was used.

Composition	Turbidity	≥1µm Particle concentration (/mL)	Monomer recovery
IgG1	1.87	9409	
EGMM	0.6	3646	
PBS	0.65	61	
50% EGMM	7.34	10105	0.6%
30% EGMM	17.6	141308	98.2%
15% EGMM	13.76	210226	101.3%
10% EGMM	11.15	162903	100.9%
8% EGMM	9.35	144954	101.0%
Placebo 50% EGMM	2.29	27300	
Placebo 30% EGMM	4.21	75232	
Placebo 15% EGMM	4.05	39533	
Placebo 10% EGMM	3.56	8396	
Placebo 8% EGMM	3.25	3429	

3.2. Effects of pH and ionic strength on TMEOS gelation

Upon spray drying, a pre-hydrolyzed sol-gel solution is atomized into a heated reactor to yield porous particles. It is important to clarify the time t_{gel} required for gelation of the sol to avoid nozzle blockage. Ionic strength and pH are two important parameters impacting the hydrolysis and condensation of silica precursor [12]. The dependency of t_{gel} and these two parameters was studied. For protein drug, the formulation should be at moderate pH. Here, four different concentrations of PBS

were used to investigate the effect of ionic strength on the gelation time between pH 6.0 to pH 8.0. Formation of EGMM during the sol-gel transition could be confirmed is characteristic odour. As shown in **Fig.2**, t_{gel} decreased with higher pH and PBS concentration. The time of water jellification can be controlled from a few minutes to a few hours by adjusting pH and PBS concentration without the need for additives.

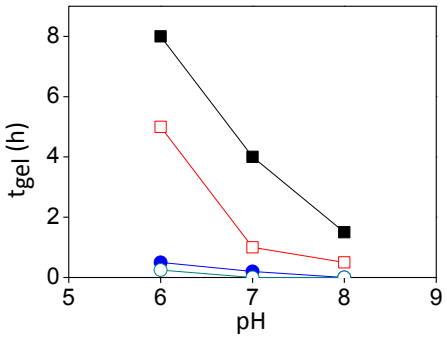


Figure 2. Effects of pH and ionic strength on TMEOS gelation time, 0.6 mL TMEOS was mixed with 1.4 mL four different concentrations (■ : 10 mM, □ : 30 mM, ● :50 mM and ○ : 200 mM) of PBS between pH 6.0 to pH 8.0.

3.3. Particle morphology

Mesh size and formulation parameters like total solid content are predominant parameters affecting the size and morphology of particles spray dried in the Buchi-90 nano [26]. The particles of all the 15 runs showed similar size of 2 µm as they were fabricated with the same mesh size for equivalent total solid content. The absence of the characteristic EGMM odour for the silica particle as powder as well as suspended

in PBS buffer confirmed the EGMM evaporation upon spray-drying. But the microparticles were observed to exhibit different morphologies depending on the composition of the precursor solution (**Fig. 3**). Microparticles spray dried with low sugar content were of spherical shape with smooth outer surface. The incorporation of more sugar 1.8% Si/3.7% Dx induced deformations.

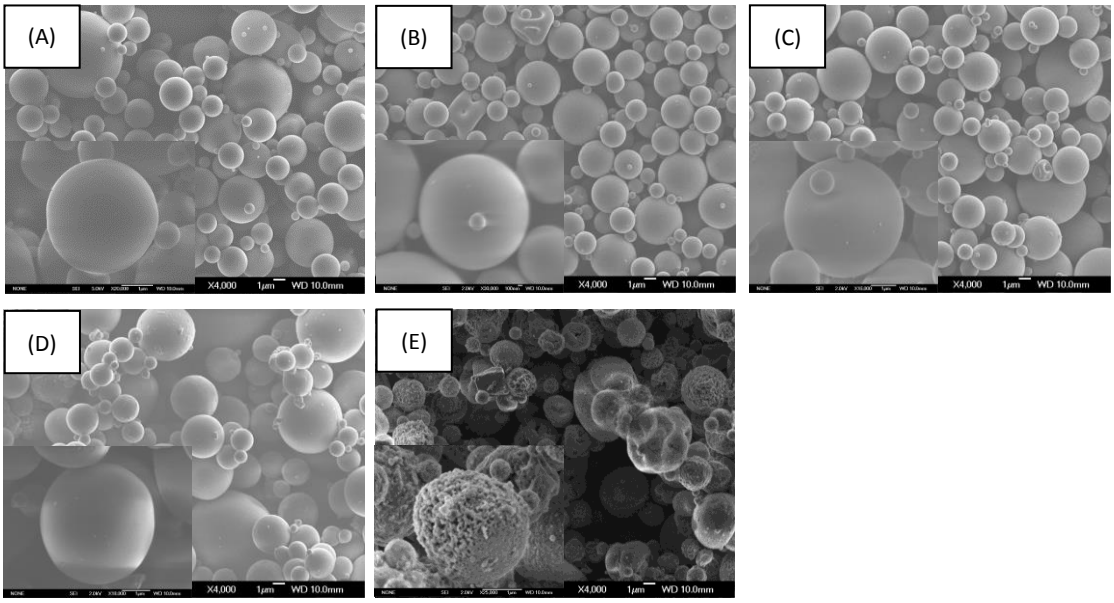


Figure.3. SEM photographs of spray-dried particles with different silica/Dx 5 ratios at pH 6.0, (A) 5.5%:0.0%, (B) 5.0%:0.5%, (C) 4.5%:1.0%, (D) 3.7%:1.8%, (E) 1.8%:3.7%.

The particle formation process during spray drying can be described by solvent evaporation and diffusion of solutes in the droplet because of heat and mass transfer [27, 28]. At the beginning, a moisture-rich droplet shrinks isotropically while the water is evaporated. As the droplet keeps shrinking, a shell is gradually formed on the surface. With continuing evaporation, moisture from inside the droplet exerts compressive capillary stress on the shell, which could induce surface deformation. The formulation composition determines the shell properties, which, in turn, govern

the observed particle morphology. The solubility of the formulation components is probably the most important factor that influences the spray dried particle formation [29]. At the earlier stage of solvent evaporation, the less water-soluble components precipitate, leading to the formation of a solid shell that eventually collapses as drying continues, resulting in corrugated particles [30]. In contrast, highly soluble components, precipitation is expected to appear later in the evaporation process and relatively homogeneously throughout the droplet, resulting in a smooth, spherical particle. Formulation components such as mannitol will tend to crystallize, in spite of its high aqueous solubility, whereas sugars, such as sucrose, raffinose, trehalose, lactose, will tend to precipitate as an amorphous solid, resulting in irregular particle formation [31]. Here, the morphology of particles with relatively low sugar content (1.8 wt% Dx) results from the typical regime with solute. The addition of 3.7% Dx induced a wrinkled morphology. The high Dx concentration led to a high surface viscosity with subsequent early precipitation and shell formation that resisted isotropical shrinkage with further drying.

3.4. Effect of silica/Dx ratio on release rate

The release profiles of FITC-Dx 150 from the spray-dried microparticles with different silica/Dx1 ratios are shown in **Fig.4 (A)**. Pure silica microparticles showed a very slow release with a cumulative release of only around 8% after 4 weeks. Incorporation of Dx 1 enhanced the release. Around 20% of FITC-Dx 150 was released within 2 days for microparticles with the addition of Dx 1 at levels from 0.5% to 1.8%,

while 60% was released in the same period with increasing the amount of Dx 1 to 3.7%. This acceleration of the release became more apparent with the addition of Dx 5 and Dx 60. Within 4 days, up to 25%, 30%, 50%, and 100% of FITC-Dx 150 were released from the microparticles containing 0.5%, 1.0%, 1.8% and 3.7% Dx 5 (**Fig.4 (B)**). Furthermore, sustained release over 35 days and 21 days were achieved by incorporating 1.0% and 1.8% Dx 60, respectively (**Fig.4 (C)**). Generally, increase of Dx molecular weight accelerated the release when the equivalent amount of Dx was added. Since the microparticles showed relatively similar size and morphology with nearly spherical shape and smooth surface except at a silica/Dx ratio of 3.7%/1.8%, the difference in the release behavior could be attributed to the addition of Dx to the matrix.

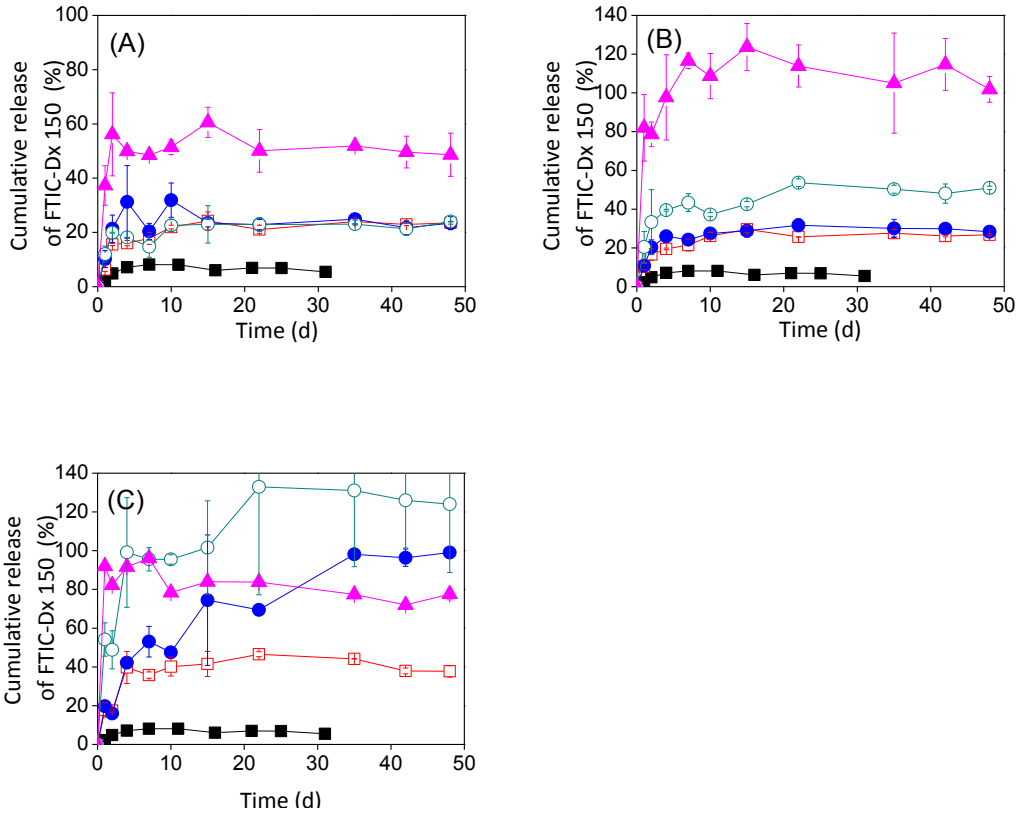
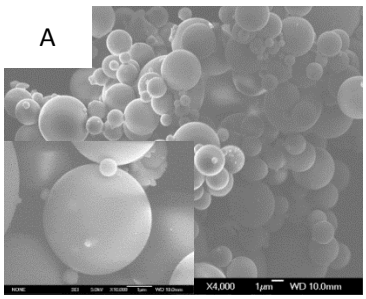


Figure.4. Release profiles of FITC-Dx150 from microparticles of different silica/Dx1(A), Dx5(B) and Dx60(C) ratio prepared at pH 6.0, 5.5%Silica/0.0%Dx: ■ ; 5.0%Silica/1.0%Dx: □ ; 4.5%Silica/1.0%Dx: ● ; 3.7%Silica/1.8%Dx: ○ ; 1.8%Silica/3.7%Dx:▲.

Generally, the silica particles can be considered as matrix systems in which the drug is uniformly distributed within the gel matrix. Liberation of the drug occurs through penetration of solvent into the pores, cracks and interparticular spaces of the matrix. The drug slowly dissolves in the permeating fluid phase and diffuses from the system along the solvent-filled capillary channels [32]. The release from the silica matrix is governed by diffusion and simultaneous matrix degradation [33]. Incorporated of Dxs increased the release due to their high hydrophilicity and water solubility. Dissolution of Dx upon contact with the release medium increases the inner mesopore size of the microparticles and provides more channels for drug diffusion, inducing drug molecules to be released. This is consistent with the particle morphology after release (**Fig.5**). In the absence of Dx, the morphology of pure silica microparticles after release test did not show any change compared to the initial state. With addition of Dx, more sub-micron pores, a looser texture and large cracks appeared on the surface of the particles and even particle degradation was observed. Dx with higher molecular weight and corresponding larger molecular size contributed more to water channel enlargement by its dissolution, resulting in more pronounced acceleration of FITC-Dx 150 release. Concurrently, larger channels resulted in relatively looser inner structure and larger cracks, causing the particles to

disintegrate whereas particles containing Dx1 and Dx5 did stay intact (Fig.5). Meanwhile, another plausible reason for the acceleration effect of bigger Dx on the release could be caused by void space during the particle formation. As described by Vehring, the void space of spray dried particle is increased with a dimensionless Peclet number, $Pe = R^2/\tau_d D_s$, where R^2/D_s is the time required for the solute to diffuse from the surface of the droplet to its center which is controlled by the diffusion rate of the solute and τ_d is the time required for the droplet to dry which is determined by the solvent evaporation rate [27]. All generated droplets incorporated of different Dx ratio were atomized with same mesh and dried at the same conditions. In this case, only the diffusion coefficient of the solute inversely proportional to its hydrodynamic size influences the solute movement during the drying process. Thus, particles containing larger Dx show higher Peclet number, resulting in more void and looser inner structure, subsequently leading to faster drug release. Hence, Dx is a useful regulator in tailoring the release rate from silica microparticles.

A



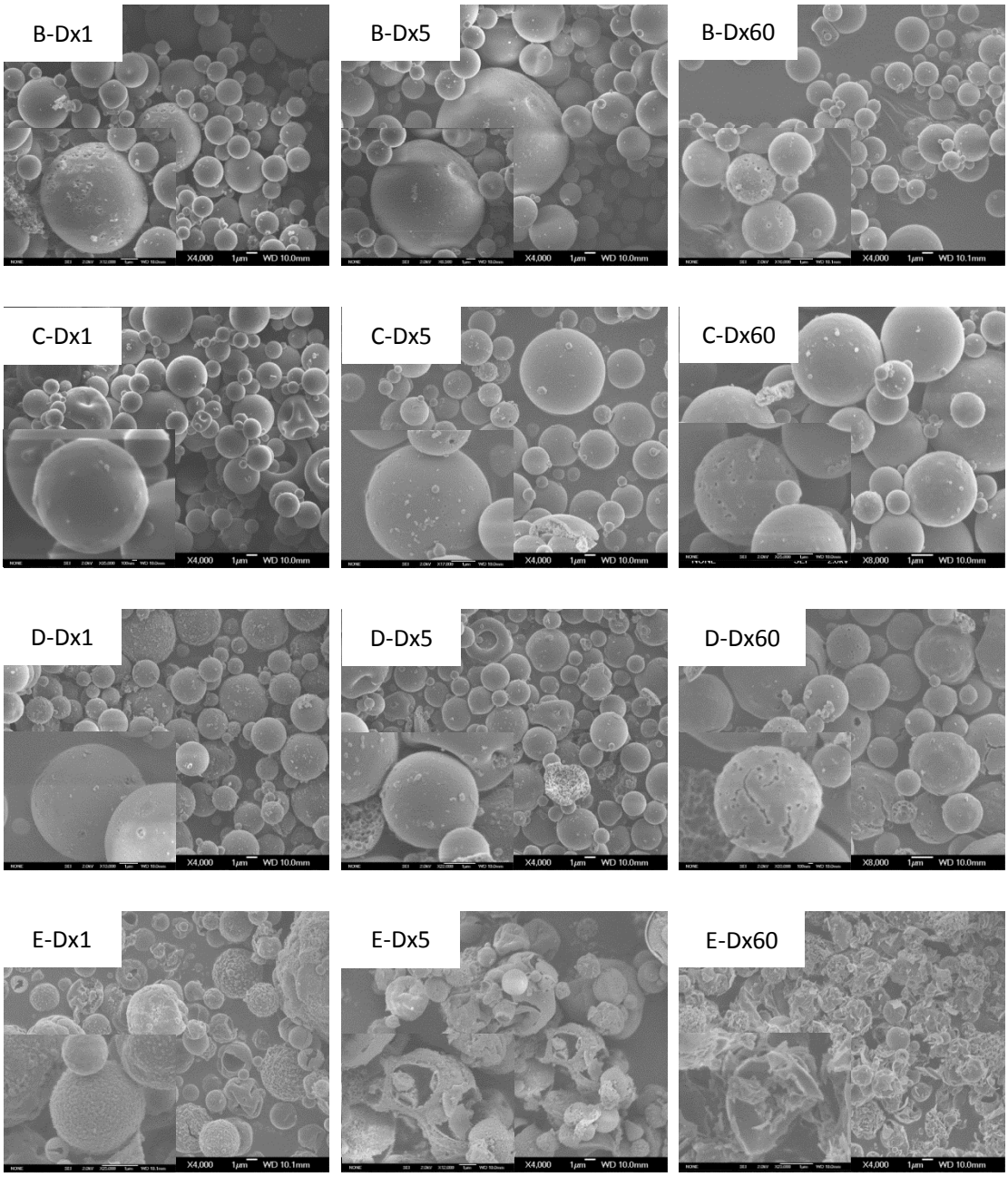


Figure.5. SEM photographs of spray-dried particles with different silica/Dx ratios at pH 6.0 after 7 weeks release test, (A) 5.5%:0.0%, (B) 5.0%:0.5%, (C) 4.5%:1.0%, (D) 3.7%:1.8%, (E) 1.8%:3.7%.

3.5. Effect of pH on release rate

There are two well-known stages of gelation when a silicon alkoxide is used to

create a sol. These two stages, hydrolysis and condensation, are tightly interlocked in certain systems where they occur simultaneously and less so in others where one of the two is relatively fast [12]. Acidic or basic conditions can be used to control the hydrolysis and condensation reaction. The morphology and structure of the silica produced by sol-gel processing can be tailored by controlling the sol-gel reaction kinetics, and in particular, the relative rates of hydrolysis and condensation [12]. To investigate the influence of processing pH on the release rate of FITC-Dx 150, the microparticles containing 4.5% Si/1.0% Dx 60 were fabricated at different pH. The morphology of particles was independent on pH values, and particles with spherical shape and smooth outer surface were formed in all pH conditions tested (**Fig.6**). The effect of pH of precursors on the drug release is shown in **Fig.7**. The initial release for all particles was rather similar. A complete sustained release within 35d resulted from particles generated at pH 6.0 while approximate 30% and 50% FTIC-Dx 150 was not liberated from microparticles prepared at pH 6.2 and pH 6.4, respectively. SEM images of the particles after release showed intact spherical shape with pores on the surface, illustrating the drug release, independent of pH. Possibly pH 6.0 led to the formation of a less dense silica network and consequently complete drug release.

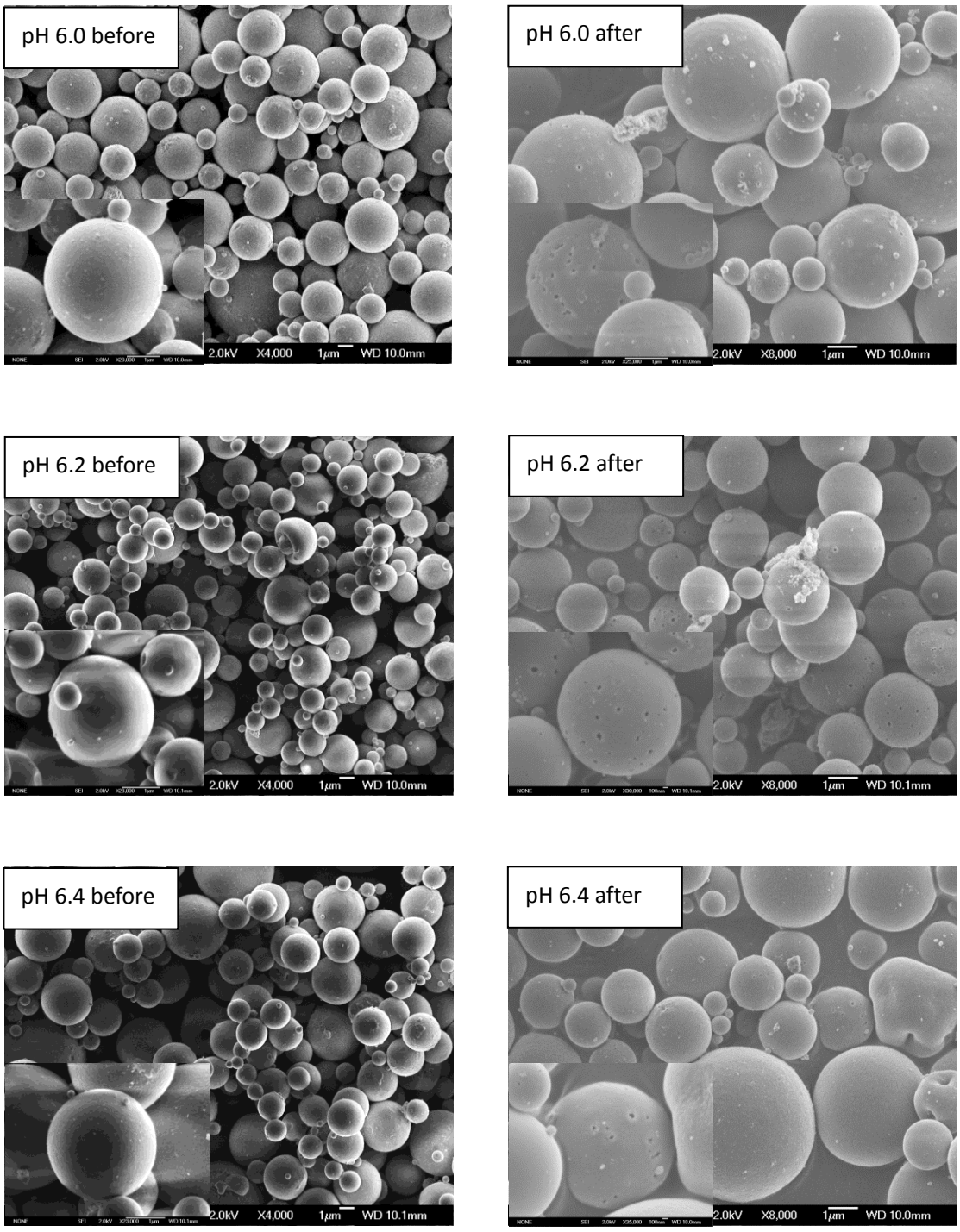


Figure.6. SEM photographs of spray-dried particles with 4.5%silica/1.0%Dx60 at different pH before and after release test

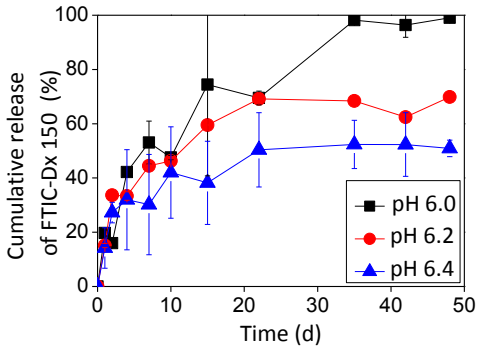


Figure 7. Release of FITC-Dx150 from particles prepared with 4.5%silica/1.0%Dx60 at different pH

4. Conclusion

A new water-soluble precursor (TMEOS) for controlled release application is presented. The spray-dried spherical silica particles can be fabricated at moderate pH and prolonged chemical reaction, organic solvents or catalyzers are not needed. Microparticles with either smooth or wrinkled morphologies are formed, depending on the amount of Dx addition. The addition of Dx significantly alters the microstructure of spray-dried particles, resulting in significantly faster drug release. Dissolution of Dx upon contact with the release medium increases the inner mesopore size of microparticles and provides more channels for drug diffusion, inducing drug molecules to be released. By increasing the Dx molecular weight to enlarge the water channel of silica microparticles, a sustained release of FITC-Dx 150 with 35 days can be achieved by incorporating 1.0% Dx 60. The released amount of particles containing 1.0% Dx 60 decreased with increasing pH of precursor solution. The results demonstrate the potential to control the structure and morphology as

well as the sustained release behavior for high molecular weight compounds of silica microparticles formed via a sol-gel process with a new compatible precursor. The gained understanding should be very useful in designing silica-based microparticles for parenteral application of biopharmaceutical drugs.

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CHAPTER 6

Development of silica microparticles for sustained release of proteins with a new sol-gel precursor

Abstract

Protein entrapment within mesoporous silica microparticles for sustained release application is limited by the poor water solubility and harsh processing conditions of traditional silica precursors. In this work, a new water soluble precursor, tetrakis(2-methoxyethyl) orthosilicate (TMEOS), which exhibits better biocompatibility with proteins, was used to fabricate microparticles to control the protein release via a mesoporous silica network. Two proteins, a monoclonal antibody IgG1 and lysozyme, were employed as the model protein drugs. The by-product ethylene glycol monomethylether (EGMM) from TMEOS hydrolysis showed high compatibility with lysozyme. The silica microparticles were prepared with two different spray drying systems based on two-fluid-nozzle or vibrating mesh nebulization or by cryomilling after a bulk gelation process. Incorporation of various amounts of Dextran, PEG and PVA as additive allowed to modify the protein release. The released amount increased with the amount and molecular weight of the additive. But sustained release of proteins was not achieved. The incomplete release

of proteins may be due to the interactions between the protein and the sol-gel silica matrix or a too dense network.

Keywords: TMEOS, Sol-gel, Spray drying, Milling, Dextran, PEG, PVA, Microparticles, IgG1, lysozyme, Sustained release.

1. Introduction

Protein drugs are generally administrated parenterally and frequent injection may be required [1]. This has elicited the interest in delivery systems that can provide sustained release and protect sensitive protein molecules over extended periods of time.

Entrapment of proteins in microparticles using the silica sol-gel route for sustained release application has been rarely explored [2-4]. Protein drugs get encapsulated in silica microparticles upon hydrolysis and condensation of the silicate sol, which yields a polymeric amorphous oxo-bridged SiO_2 network [5]. This provides high loading capacity, allows to tune pore size and may enhance protein stability [6-9]. However, poor water solubility of traditional silica precursors and harsh processing conditions of the sol-gel transition are the principal drawbacks for the entrapment of protein drugs [10-13]. In fact, so far there are no silica-based drug delivery systems on the market despite that it has shown great promises [14].

Compared to conventional precursor such as tetramethyl orthosilicate (TMOS) or tetraethyl orthosilicate (TEOS) that involve extreme acidic or basic conditions and organic solvent environment detrimental to proteins [15], the new silica precursor, tetrakis(2-methoxyethyl) orthosilicate (TMEOS) proceeds at moderate pH and hydrolysis yields ethylene glycol monomethylether (EGMM) instead of methanol or ethanol, which is compatible with IgG1 at a low concentration. Additionally, EGMM with a boiling point of 124.5 °C is readily removed accompanying the water evaporation. Furthermore, no prolonged chemical reaction or catalyzers are needed

to trigger the sol-gel transition compared to tetra(2-hydroxyethyl) orthosilicate (THEOS). Sustained release microparticles loaded with the high molecular weight model compound FITC-dextran 150 were achieved via spray drying (see **Chapter 5**). Furthermore, protein stability can be assured during spray drying by selection of appropriate formulation and process conditions [16-18]. Consequently, this study aimed to develop protein loaded silica microparticles from TMEOS for sustained release by spray drying. For fast screening a bulk sol-gel process with subsequent cryo-milling was used. A monoclonal antibody IgG1 and lysozyme were employed as the model protein drugs. EGMM is compatible with IgG1 at the low concentrations, and the compatibility of EGMM was checked with another model protein lysozyme. The protein release from silica microparticles was tailored by changing the silica to additive ratio, the additive type and the molecular weight of additives.

2. Materials and Methods

2.1. Materials

Tetrakis (methoxyethoxy) silane (TMEOS) was purchased from Suzhou Chum-Win New Material Science & Technology Co,. Ltd., Suzhou, China. Sucrose, polysorbate 80 (PS 80), PEG 4k, PEG 35k, PEG 1M and PVA 25k were purchased from Sigma-Aldrich, Munich, Germany and Dextran 60 (Dx 60) was purchased from Pharmacosmos A/S, Holbaek, Denmark. Solutions of 20.9 mg/mL IgG1 monoclonal antibody in 10 mM PBS pH 7.2 and 98 mg/mL lysozyme in 10 mM His pH 6.0 were used. Ethylene glycol monomethylether (EGMM, 99.5%) was supplied by the reagent center of the LMU Munich, Germany. All other reagents used were of analytical grade. Deionized water

(Milli-Q) was used for all precursor preparation.

2.2. Methods

2.2.1. Turbidity

The turbidity of lysozyme/EGMM mixtures in formazine nephelometric units (FNU) was determined with a NEPHLA turbidimeter (Dr. Lange, Düsseldorf, Germany), based on light scattering in an 90° angle at λ = 860 nm. The system was calibrated with a formazine standard. Approximate 2 mL of each sample were used for analysis.

2.2.2. Light obscuration

Light obscuration tests were carried out according to Ph.Eur. 2.9.19. The particle counting of subvisible particles in a size range between 1 and 200 µm was conducted using a SVSS-C instrument and associated analysis software (PAMAS GmbH, Rutesheim, Germany). For each sample (n = 3) three measurements of a volume of 0.3 mL with a pre-run volume of 0.3 mL at fixed fill rate, emptying rate and rinse rate of 5 mL/min were performed. Prior to each measurement the system was rinsed with high purified water until particle counts of less than 30 particles/mL were determined. The obtained results represented the mean value of the particle counts of three measurements, referred to a sample volume of 1.0 mL.

2.2.3. High performance size exclusion chromatography (HP-SEC)

HP-SEC was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, California, USA). The autosampler and the column were

temperature controlled at 20 °C and 23 °C, respectively. The samples were centrifuged for 5 minutes at 2000 rpm. For each sample solution, 40 µl supernatant were injected onto a Tosoh TSKgel® G3000SWXL column (7.8x300 mm) (Tosoh Bioscience, Stuttgart, Germany) using a mobile phase of 100 mM sodium phosphate buffer with additional 100 mM sodium sulfate pH 6.8 at a flow rate of 0.5 ml/min. The eluted sample was detected by UV absorption at 280 nm. The chromatograms were analyzed regarding retention times and the area under the curve (AUC) with ChemStation® B.02.01-SR2 (Agilent Technologies).

2.2.4. Spray drying of microparticles

To investigate the effects of additives, different formulations were prepared (**Table 1**). The maximal TMEOS concentration of spray solution was set to 25% (equal to 4.5% silicon dioxide), which would yield an EGMM concentration of 5.7% upon hydrolysis. The total mass content of silicon dioxide and additives was set to 5.5% (w/v). In a typical procedure, IgG1 or lysozyme solution containing sucrose and PS 80 was mixed with TMEOS and additive to a final volume of 10 mL (10 mM PBS, pH 6). Hydrolysis was performed for 2 hours. The spray drying conditions in the nano spray dryer Büchi-90 (Büchi Labortechnik, Flawil, Switzerland) were T_{in}/T_{out}: 120 °C /58 °C, flow rate of drying air: 120 L/min, atomizing mesh size: 7.0 µm. Spray solutions were filtered through a 0.2 µm PVDF syringe filter prior to spray drying. The spray drying conditions in the mini spray dryer Büchi-290 (Büchi Labortechnik, Flawil, Switzerland) were T_{in}/T_{out}: 105 °C /73 °C, flow rate of drying air: 670 L/min, flow rate of feeding: 3 mL/min; nozzle size: 0.4 mm. Spray solutions were filtered through a 0.2 µm PVDF syringe filter prior to spray drying.

Table 1: Formulations of precursors for spray drying

Spray dryers	TMEOS (SiO_2) (%w/v)	Dextran 60 (%w/v)	Sucrose (%w/v)	PS 80 (%w/v)	IgG1 (%w/v)	Lysozyme (%w/v)
B-90	25 (4.5)	1.0	0.5	0.1	0.42	0.0
B-90	15 (2.8)	2.7	0.5	0.1	0.42	0.0
B-90	12.5 (2.3)	3.2	0.5	0.1	0.42	0.0
B-90	10 (1.8)	3.7	0.5	0.1	0.42	0.0
B-90	25 (4.5)	1.0	0.5	0.1	0.0	0.49
B-90	15 (2.8)	2.7	0.5	0.1	0.0	0.49
B-90	12.5 (2.3)	3.2	0.5	0.1	0.0	0.49
B-90	10 (1.8)	3.7	0.5	0.1	0.0	0.49
B-290	10 (1.8)	3.7	0.5	0.1	0.42	0.0
B-290	10 (1.8)	3.7	0.5	0.1	0.0	0.49

2.2.5. Microparticle preparation with bulk gelation and milling

IgG1 or lysozyme solution containing sucrose and PS 80 was mixed with TMEOS and additive to a final volume of 10 mL (10 mM PBS, pH 6). The formulations were shown in **Table 2**. The solutions were kept in the oven for 2 weeks at 40 °C until they were completely dry and solidified. Then the dry gels were milled in a swing mill Retsch® CryoMill (Retsch Technology, Haan, Germany). After precooling the system with liquid nitrogen for 10 mins at 5 Hz, the dry gels were ground for 3 mins at 25 Hz. The obtained particles were collected and kept at 2-8 °C until release testing.

Table 2: Formulations of precursors for bulk gelation and milling

TMEOS (SiO2) (%w/v)	PEG 4k (%w/v)	PEG 35k (%w/v)	PEG 1M (%w/v)	PVA 25k (%w/v)	Sucrose (%w/v)	PS 80 (%w/v)	Lysozyme (%w/v)
25 (4.5)	1.0	0.0	0.0	0.0	0.5	0.1	0.49
10 (1.8)	3.7	0.0	0.0	0.0	0.5	0.1	0.49
25 (4.5)	0.0	1.0	0.0	0.0	0.5	0.1	0.49
10 (1.8)	0.0	3.7	0.0	0.0	0.5	0.1	0.49
25 (4.5)	0.0	0.0	1.0	0.0	0.5	0.1	0.49
10 (1.8)	0.0	0.0	3.7	0.0	0.5	0.1	0.49
25 (4.5)	0.0	0.0	0.0	1.0	0.5	0.1	0.49
10 (1.8)	0.0	0.0	0.0	3.7	0.5	0.1	0.49

2.2.6. Particle morphology

The particles were visualized with scanning electron microscopy (SEM) using a Jeol JSM-6500F instrument (Jeol Ltd., Tokyo, Japan) with Inca Software (Oxford instruments, Oxfordshire, UK) at an accelerating voltage of 1.5 or 2.0 kV. They were sputtered with carbon.

2.2.7. *In Vitro* release

The protein release was studied in PBS buffer (0.01 M phosphate; 0.138 M NaCl; 0.027 M KCl, pH 7.4) at 37 °C using a shaking incubator (60 rpm). Approximately 200 mg of drug-loaded particles and 1 mL buffer were placed in a 1 MDa cutoff dialysis bag and immersed into a 15 mL disposable plastic tube with 9 mL PBS containing 0.05 % NaN_3. At designated time points, 1 mL of release medium were withdrawn and replaced with the same amount of fresh release medium. Protein was

quantified at 280 nm using an Agilent 8453 UV-Vis spectrophotometer (Agilent Technologies, Böblingen, Germany). Each experiment was performed in triplicate.

3. Results and discussion

3.1. Compatibility of EGMM with lysozyme

Hydrolysis of TMEOS generates EGMM which can evaporate with the water. It is important to check the compatibility of the by-product with the incorporated drug. We found that EGMM is compatible with IgG1 at the low concentrations. To further study the maximal compatibility of EGMM with proteins, lysozyme was exemplarily tested. All lysozyme samples showed similar visual transparency as the placebo samples (**Fig. 1**). This was consistent with the turbidity measurement and light obscuration. Additionally, no soluble aggregate or fragment formation of lysozyme samples was observed by HP-SEC analysis with 100% monomer recovery. Based on these results, it was concluded that high EGMM concentration (50%) is also compatible with lysozyme.

Fig. 1. Visual appearance of mixtures of lysozyme (A) or placebo (B) solutions with different EGMM concentration. A 1 mg/mL lysozyme in 10 mM PBS pH 7.4 was used.

Table 3. Analysis of mixtures of lysozyme or placebo solutions with different EGMM concentration by turbidity, subvisible particle counting and HP-SEC. A 1 mg/mL lysozyme in 10 mM PBS pH 7.4 was used.

Composition	Turbidity (FNU)	≥1 µm Particle concentration (#/mL)	Monomer recovery (%)
Lysozyme	0.96	5096 ± 730	
EGMM	0.49	616 ± 345	
PBS	0.58	633 ± 37	
50% EGMM	5.14	9586 ± 4089	104.2%
30% EGMM	13.00	103544 ± 11504	102.4%
15% EGMM	9.03	65938 ± 1948	100.7%
10% EGMM	7.14	22388 ± 2773	101.1%
Placebo 50%	3.4	17352 ± 3325	
Placebo 30%	5.45	9239 ± 2444	
Placebo 15%	6.69	2056 ± 565	

Placebo 10%	3.69	2701 ± 423

3.2. Protein release from silica microparticles

Similar as for model drug FITC-Dx 150, the IgG1 or lysozyme loaded silica microparticles were prepared with a Nano Spray Dryer B-90 using different amounts of Dx 60 to investigate the effect of this additive on the release profiles. IgG1 release was not observed from all the silica microparticles even at the highest additive content of 3.7% Dx 60. In contrast 3%, 14%, 31%, and 43% of lysozyme were released from the microparticles containing 1.0%, 2.7%, 3.2% and 3.7% Dx 60 within one week reaching a plateau (**Fig.2 (a)**). Around 15% IgG1 and 20% lysozyme were released within two weeks from silica microparticles of 1.8%silica/3.7%Dx 60 prepared with a Mini Spray Dryer B-290 (**Fig.2 (b)**). As the nozzle design of these two spray dryers is essentially different, the generation and drying process of droplets varies, which led to different morphologies (**Fig.3**) and release profiles. Absence and incomplete release of proteins may be related to the interactions between the protein and the sol-gel silica matrix [19]. Proteins exhibit an abundance of H-bonding groups on the surface, which result in extensive interaction with the silica polymer network. Furthermore, protein molecules may serve as a nucleus for the condensation polymerization and made become tightly entrapped by the silica gel, preventing the release.

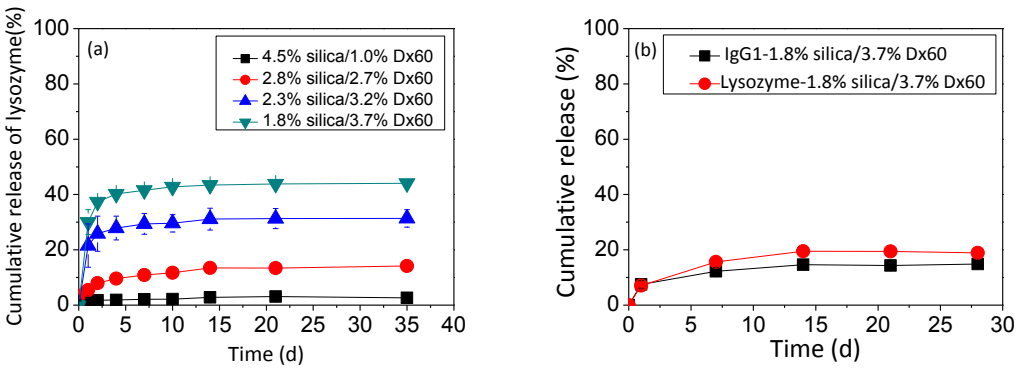

Fig.2. Release profile of proteins from silica microparticles of different silica/Dx 60 ratio prepared with a Nano Spray Dryer B-90 (a) and a Mini Spray Dryer B-290 (b).

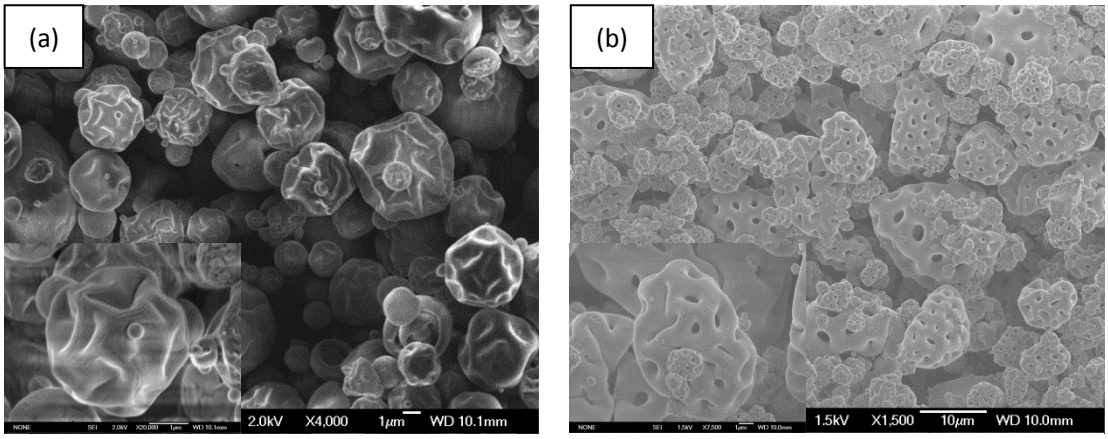

Fig.3. SEM photographs of silica microparticles of 1.8%silica/3.7%Dx 60 prepared with a Nano Spray Dryer B-90 (a) and a Mini Spray Dryer B-290 (b).

Addition of PEG as an alternative was tested to enhance the release. Since PEG exhibits a low melting temperature around 60 °C, which is close to the outlet temperature in the spray drying, a bulk gelation process followed by cryomilling was utilized to fabricate the silica microparticles. The lysozyme release profiles of milled particles are similar to those of spray dried particles as shown in **Fig.4**. The released

amount increased with the amount of additive. An increase of PEG molecular weight accelerated the release when the equivalent amount of PEG was added. Addition of PVA showed a similar effect on lysozyme release. Overall, Dx, PEG, PVA allow to modify the release from the silica network but the capacity to tailor protein release is limited and further studies are necessary.

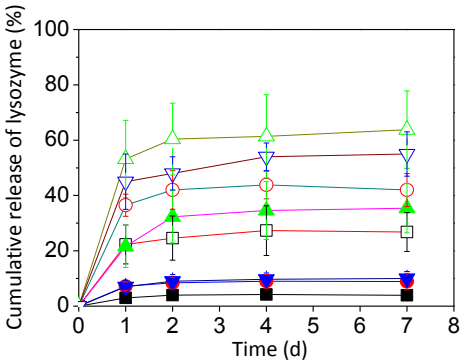

Fig.4. Release profiles of lysozyme from silica microparticles of different silica/additive ratio prepared via bulk gelation and cryomilling from, 4.5%Silica/1.0%PEG 4k ■; 1.8%Silica/3.7%PEG 4k □; 4.5%Silica/1.0%PEG 35k ●; 1.8%Silica/3.7%PEG 35k ○; 4.5%Silica/1.0%PEG 1M ▲; 1.8%Silica/3.7%PEG 1M △; 4.5%Silica/1.0%PVA 25k ▼; 1.8%Silica/3.7%PVA 25k ▽.

4. Conclusion

The by-product EGMM produced in the course of TMEOS hydrolysis exhibited high compatibility with lysozyme and is readily removed accompanying the water evaporation, which holds great promise for the TMEOS applications. The silica microparticles were developed with two spray dryers and a bulk gelation and cryomilling using a monoclonal antibody IgG1 and lysozyme as model proteins.

Incorporation of Dx 60, PEG and PVA into the silica microparticles modified the release but did not render tailor made sustained release. Only a fraction of protein was released within a few days. Generally, the released amount increased with the amount and molecular weight of the additive. The incomplete release may result from the interactions between the protein and the sol-gel silica matrix or a too dense network. Although well controlled release could not be achieved in this study, TMEOS as a potential silica precursor should be further studied for protein drug delivery testing some other additives and reaction conditions.

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CHAPTER 7

Summary of the thesis

Protein drugs have emerged as promising therapeutic agents for treatment of various severe conditions in recent years. They are generally administrated by injection due to rapid degradation by proteolytic enzymes in the gastrointestinal and poor transport across the biological barrier. For treatment of chronic diseases repeated injections are necessary in order to achieve therapeutic drug levels reducing patient compliance. Triglycerides and silica-based microparticles have drawn increasing attention to control the sustained release of protein drugs (**Chapter 1**). In this thesis, triglyceride coated polyol core as well as silica microparticles for sustained release of proteins were formed by fluid bed coating and spray drying, respectively.

Lipid coating of such small microparticles is challenging owing to the high tendency to agglomerate formation upon coating with the tacky lipid. Therefore, in **Chapter 3**, optimal process parameters for hard fat and glyceryl stearate coated microparticle preparation without agglomeration at modest temperature were established in a Mini Glatt fluid bed system. 30 g drug loaded mannitol carrier microparticles were coated with 5 g, 10 g, 20 g and 30 g lipids, respectively. Placing more lipid onto the microparticles reduced both burst and release rate, and the particles maintained their geometric form during the release test. The model drugs methyl blue and aspartame were completely released *in vitro* through pores which

mainly resulted from dissolution of the polyol core beads. The release of methyl blue and aspartame was extended up to 25 and 7 days. Burst and release rates were similar for hard fat and glyceryl stearate. Polymorphic transformation of the hart fat was observed upon release. The reduction in size of starting bead showed only marginal effect on the drug release behavior. In contrast, the release of the more hydrophilic model compound was much faster than that of the less hydrophilic. The formulation and manufacturing parameters for the design of sustained release microparticles were subsequently transferred to a monoclonal IgG1 antibody.

Although lipid coated polyol core microparticles are promising depot formulation for protein drugs, the retention of protein stability during microparticle fabrication is still a major concern. In order to prevent IgG1 unfolding at the air-liquid interface and subsequent aggregation, the protein was stabilized with 22.5 mg/mL sucrose, 0.1% PS 80, 10 mM methionine in 10 mM His buffer pH 7.2 during the spray loading process in **Chapter 4**. Protein loaded mannitol carrier microparticles were coated with the two lipids. An extended release over 6 weeks could be achieved by 30 g hard fat coating. The IgG1 was released in its monomeric form and maintained its secondary structure as shown by FTIR. Sustained release of IgG1 was not observed from glyceryl stearate coated microparticles, which may result from the lack of large pores in the glyceryl stearate layer or detrimental surfactant character of glyceryl stearate.

Owning to poor water solubility of conventional silica precursors, organic solvent and extreme conditions of pH and high temperature are often needed to achieve a uniform sol, conditions which are unfavorable for the encapsulation of protein drugs.

A new water soluble precursor, TMEOS, for controlled release application was presented in **Chapter 5**. The hydrolysis of TMEOS yields ethylene glycol monomethylether (EGMM), which is compatible with IgG1 at a low concentration and readily removed accompanying the water evaporation (boiling point 124.5 °C). The spray dried spherical silica particles were fabricated at moderate pH and prolonged chemical reaction, organic solvents or catalyzers were not needed. Different quantities of dextrans with varying molecular weight were incorporated into the silica microparticles to the tailor release profile of a high molecular weight model compound, FITC-dextran 150. Microparticles with either smooth or wrinkled morphologies were formed, depending on the amount of dextran addition. Dissolution of dextrans upon contact with the release medium increased the inner mesopore size of the microparticles and provided more channels for drug diffusion, inducing drug molecules to be released. The release increased with the amount and molecular weight of added dextran. A sustained release over 35 days was achieved with addition of 1.0% dextran 60 kD. The rate of FITC-dextran 150 release from the dextran 60 containing particles decreased with higher precursor solution pH. The gained knowledge was subsequently applied in the proteins to control the sustained release.

Protein loaded silica microparticles from TMEOS for sustained release application were prepared by spray drying or cryomilling after a bulk gelation process in **Chapter 6**. EGMM exhibited high compatibility with lysozyme. Incorporation of various amounts of dextran, PEG and PVA as additive slightly modified the protein release. The released amount increased with the amount and molecular weight of

the additive. But sustained release of proteins was not achieved. The incomplete release may result from the interactions between the protein and the sol-gel silica matrix or a too dense network.

In summary, this work focused on the sustained release control of proteins based on triglycerides and silica based materials. Hard fat coated polyol core microparticles showed high potential for protein delivery. Although well controlled release of proteins was not achieved from silica microparticles, TMEOS as a potential silica precursor should be further studied by testing some other additives and reaction conditions. Both of them provide interesting and useful information for future protein drug delivery.

Publications and presentations associated with this thesis

Research articles

Bifeng Wang, Wolfgang Frieß; Formation of polyol core microparticles for sustained release with lipid coating in a mini fluid bed system, European Journal of Pharmaceutics and Biopharmaceutics. (Under review)

Bifeng Wang, Wolfgang Frieß; Spray drying of silica microparticles for sustained release application with a new sol-gel precursor, European Journal of Pharmaceutics and Biopharmaceutics. (Under review)

Bifeng Wang, Wolfgang Frieß; Lipid-coated polyol core microparticles for sustained release of protein. (to be submitted)

Bifeng Wang, Wolfgang Frieß; Development of silica microparticles for sustained release of proteins with a new sol-gel precursor. (to be submitted)

Poster presentations

Bifeng Wang, Wolfgang Frieß; Spray drying of silica microparticles for sustained release application with a new sol-gel precursor, 10th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, April 4-7, 2016, Glasgow, UK.

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