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Cubic phase-forming dry powders for controlled drug delivery on mucosal surfaces

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ABSTRACT

The purpose of this study was to prepare and physicochemically characterize protein-loaded, glycerol monooleate (GMO)-based dry powder systems, which can be used for the controlled mucosal delivery of macromolecules (e.g., nasal, buccal, pulmonary). Bovine serum albumin (BSA)-loaded powders were prepared by spray-drying, freeze-drying and/or spray-freezing using different types of carrier materials, including mannitol, polyvinyl pyrrolidone (PVP 25) and polyethylene glycols (PEGs). The systems were characterized by optical and polarized light microscopy, X-ray powder diffraction, gel electrophoresis and diffusion studies. The type of carrier material strongly affected the resulting particle size and shape. The presence of GMO effectively slowed down BSA release. Importantly, broad ranges of release patterns could be achieved by varying the type of preparation method and composition of the dry powders. In all cases, the primary structure of the BSA remained intact. GMO, which is a wax solid at room temperature, has been successfully converted into dry powder formulations that offer potential for the controlled mucosal delivery of proteins.

1. Introduction

Most peptide and protein drugs have to be administered by injection, since the preferred oral application is often not feasible due to significant drug degradation in the gastro-intestinal tract (GIT), the low permeability of the GIT mucosa for macromolecules as well as first pass metabolism. The increasing number of available recombinant peptide and protein therapeutics [1,2] has motivated the search for alternative administration routes to parenteral applications, (e.g. rectal, buccal, nasal, pulmonary) [3,4]. Furthermore, most peptides and proteins are rapidly eliminated from the systemic circulation. In order to reduce the application frequency, microparticulate carrier systems providing sustained delivery have been developed [3,4]. For example, synthetic polylactides poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic-coglycolic) acid (PLGA) have been studied extensively as carrier materials for parenteral depot formulations [5,6]. However, PLA/PGA/PLGA degradation times of weeks to months render them unsuitable for administration on mucosal surfaces. Furthermore, the use of organic solvents during drug encapsulation (e.g. using w/o/w emulsion solvent extraction and evaporation methods), the polymer hydrophobicity, and the potential formation of acidic microclimates upon polymer degradation within the delivery system can affect protein stability, resulting in a loss of the biological activity of the drugs [3,7,8].

Glycerol monooleate (GMO) is known to spontaneously form liquid crystalline cubic phases in excess water, consisting of bicontinuous lipid bilayers extending in three dimensions, separating two networks of water channels [9,10]. Providing this unique structure, cubic phases are able to incorporate and control the release of drugs of various molecular weights and polarities [11–16]. It was shown that different peptides and proteins retain their native conformation and bioactivity within cubic phases, being protected against chemical and physical inactivation [17–21]. In the early 80s, Ericsson et al. [17] presented the ternary phase diagram of a monoacylglycerol/lysozyme/water system and examined the thermal stability of incorporated lysozyme by differential scanning calorimetry. The protein molecules are located within the water channels of the cubic phase. Cubic phase also protects oligopeptides (e.g. desmopressin, somatostatin, renin inhibitor) against enzymatic degradation [19]. Furthermore, bovine hemoglobin (BHb) has been successfully incorporated in GMO-based cubic phases and shown to maintain its biological activity [18]. Cubic phases are also able to protect insulin from agitation-induced aggregation/precipitation [20]. In an in-vivo follow-up study, insulin maintained its biological activity in agitated cubic phase gels, whereas its hypoglycemic activity was completely lost upon agitation in solution [21]. Interestingly, cubic phases can be bioadhesive [22], rendering them highly attractive for mucosal applications. The significant inherent tortuosity allows for the diffusion-controlled release of incorporated molecules [23,24]. In addition, GMO is a metabolite formed during digestions of oleic triglycerides, thus, being nontoxic, biodegradable, biocompatible and is Generally Recognized as Safe (GRAS status of the FDA) [25]. All these properties render cubic phases an interesting and promising vehicle for advanced drug delivery systems for proteins.

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Generally, cubic phases are applied directly as bulk materials, in form of liquid precursors or dispersed within particulates (e.g., cubosomes). One major obstacle for the direct use of bulk cubic phases as drug delivery systems is their high viscosity. The administration of a drug that is suspended within a GMO matrix is often not convenient due to the latter's semisolid/sticky character. A possibility to overcome this restriction is the use of liquid precursors. For example, a low viscosity lamellar phase can be used [21] or the GMO-water phases can be modified in order to lower the systems' viscosity by the addition of high drug concentrations and/or polar co-solvents [e.g., ethanol, PEG 400 or N-methyl-2-pyrrolidone (NMP)] [26]. Upon contact with excess water, the systems absorb water and spontaneously convert into the cubic phase. Alternatively, the cubic phase can be dispersed into nanostructured (usually submicron-sized) particles (e.g., cubosomes, United States Patent and Trademark Office registered trademark of GS Development AB Corp. Sweden) using polar lipids or surface-active polymers (e.g. poloxamer 407, [27–29]). However, due to their extremely small size (and the resulting short diffusion pathways) cubosomes are unlikely to offer similar opportunities to control drug release as bulk cubic phases do. Boyd et al. [30] studied drug release from cubosomes using the pressurized ultrafiltration method and observed only burst release in aqueous media. In addition, the large amount of water present during cubosome formation render the incorporation of water-soluble drugs difficult [31].

For various applications, it is highly desirable to work with dry powders rather than with liquid phase products. Therefore, the preparation of dry powder precursors, which spontaneously form cubic phases upon hydration in situ is an attractive alternative. In addition, the storage stability of protein drugs is likely to be significantly increased compared to water-based systems and a wide range of applications (including inhalation) can be offered. Two types of dry powder precursors were prepared by spray-drying either a dispersion of cubic liquid crystalline particles within an aqueous starch solution or an emulsion based on an ethanol-dextran-monoolein-water system [32]. Both powders formed colloidally stable cubosomes (0.6 μm) upon contact with water. However, no drugs were incorporated into the formulations in these studies. Previously, Shah et al. [33] prepared cubic phase forming powders by spray-drying a solution of GMO in isopropanol containing dispersed diclofenac sodium and the adsorbent/carrier material magnesium trisilicate. Upon contact with water, cubic phase formation was observed and the anti-inflammatory and analgesic activity of the powder precursors were more prolonged and more effective compared to the pure drug. However, this type of delivery system is intended for the oral administration of small molecular weight drugs in capsules or tablets and not for macromolecular drugs to be applied on mucosal surfaces.

The major objective of this study was to prepare and characterize novel protein-loaded, GMO-based dry powders using different preparation techniques and carrier materials for the controlled release of the model protein bovine serum albumin on mucosal surfaces. Carrier materials such as mannitol, lactose, polyvinyl pyrrolidone, poloxamer 407, polyethylene glycol and alginate were selected, which are non-toxic, solid powders at room temperature. Being water-soluble, they should allow for a rapid formation of the cubic phase upon contact with water.

2. Materials and methods

2.1. Materials

Terbutaline sulfate (Welding, Hamburg, Germany), bovine serum albumin (BSA, Mw 69 kDa; Carl Roth, Karlsruhe, Germany), glycerol monooleate (GMO, Rylo™ MG 19, melting point: ~37 °C) and glycerol monolinoleate (GML, Rylo™ MG 13; Pharma; Danisco, Grindsted, Denmark), sodium alginate (low viscosity grade; Sigma-Aldrich Chemie, Steinheim, Germany), lactose (Inhalac® 230; Meggle-Pharma, Meggle, Wasserburg, Germany), mannitol (Roquette Frères, Lestrem, France), polyethylene glycol (PEG, Lutrol® E 1500, 6000, 20,000; BASF, Ludwigshafen, Germany), poloxamer 407 (polyoxypropylene-polyoxyethylen block copolymer, Lutrol® F127; BASF), polyvinyl pyrrolidone (PVP, Kollidon® 25; BASF), Coomassie assay (Coomassie Plus Protein Assay Kit; Pierce Biotechnology, Rockford, IL, USA).

2.2. Powder preparation of cubic phase

The drug-loaded dry powder systems were prepared by the following three steps (Fig. 1): (i) preparation of a drug-loaded cubic phase (GMO, water and BSA/terbutaline sulfate), (ii) dispersion of this preformed cubic phase either into an aqueous solution of the carrier material (mannitol, lactose, PVP 25, poloxamer 407, PEGs) or into dry carrier powder and (iii) conversion of this dispersion into a dry powder by spray-drying, freeze-drying or spray-freezing into liquid N_2 and freeze-drying.

In detail, an aqueous solution of 0.3 g drug (BSA or terbutaline sulfate) in 1.5 g of water was mixed thoroughly with 3.5 g of molten (40 °C) GMO using a spatula (= 30% water w/w, 7.9% drug load w/w based on the total solids' mass). The immediately formed viscous gel was allowed to equilibrate in a tightly sealed, screw capped glass scintillation vial at room temperature for 24 h. The formation of the isotropic cubic phase was verified optically (transparent gel) with a polarized light microscope. Subsequently, the cubic phase

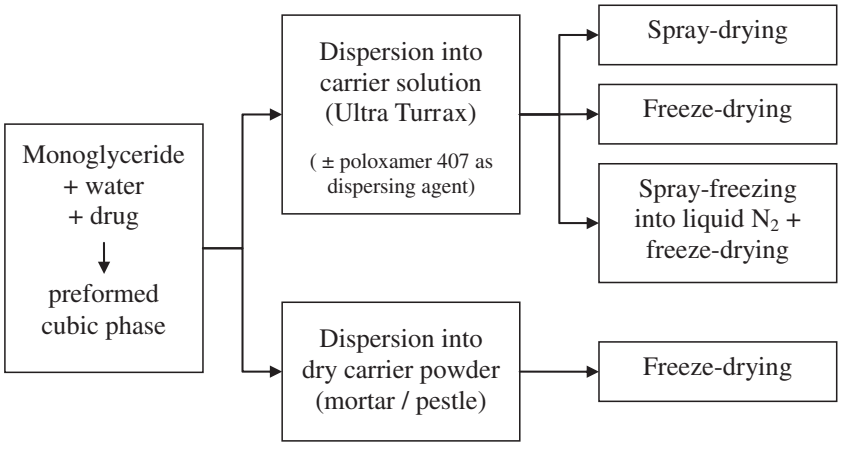

Fig. 1. Schematic presentation of the major preparation steps for drug-loaded, GMO-based dry powders.