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Novel preparation techniques for alginate–poloxamer microparticles controlling protein release on mucosal surfaces

Katrin Moebus [a], Juergen Siepmann [b], Roland Bodmeier [a,*]

[a] College of Pharmacy, Freie Universität Berlin, Kelchstrasse 31, 12169 Berlin, Germany
[b] College of Pharmacy, Univ. Lille Nord de France and INSERM U 1008, Controlled Drug Delivery Systems and Biomaterials, 3 Rue du Professeur Laguesse, 59006 Lille, France

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ABSTRACT

The objective of this study was to develop novel preparation techniques for protein-loaded, controlled release alginate–poloxamer microparticles with a size range suitable for pulmonary administration. Bovine serum albumin (BSA)-loaded microparticles were prepared by spray-drying aqueous polymer-drug solutions, followed by cross-linking the particles in aqueous or ethanolic $CaCl_2$ or aqueous $ZnSO_4$ solutions. The microparticles were characterized with respect to their morphology (optical and scanning electron microscopy), particle size (laser light diffraction), calcium content (atom absorption spectroscopy), alginate content (complexation with 1,9-dimethyl methylene blue) and in vitro drug release (modified Franz diffusion cell). The spray-dried microparticles were spherical in shape with a size range of 4–6 μm. Aqueous cross-linking led to a significant size increase (10–15 μm), whereas ethanolic cross-linking did not. The substantial drug loss (~50%) during aqueous $CaCl_2$ cross-linking could be avoided by using aqueous $ZnSO_4$ or ethanolic $CaCl_2$ solutions. Protein release from microparticles cross-linked with ethanolic $CaCl_2$ solutions was much faster than in the case of aqueous $CaCl_2$ solutions, probably due to the lower calcium content. The salt concentration and temperature of the cross-linking solutions also affected the composition of and drug release from the microparticles. Cross-linked alginate–poloxamer microparticles can be produced in a size range appropriate for deep lung delivery and with controlled protein release kinetics (time frame: hours to days) with these novel preparation techniques. The systems offer an interesting potential for the controlled mucosal delivery of protein drugs.

1. Introduction

Advances in biotechnology have led to the availability of many new peptide and protein drugs (Walsh, 2003; Pavlou and Reichert, 2004), which are usually applied via the parenteral route. The search for a more patient convenient way of administration led to alternative routes [e.g. rectal, buccal, nasal, pulmonary (Cleland et al., 2001; Motlekar and Youan, 2006)], among which the lung appears to be very promising (Adjei and Gupta, 1994; Patton et al., 1994; Agu et al., 2001). Most peptides and proteins are rapidly eliminated from the systemic circulation. To reduce the administration frequency, microparticulate carrier systems providing extended delivery have been developed (Schwendeman et al., 1996; Sinha and Trehan, 2003). Synthetic poly(lactides) and poly(lactides-co-glycolides) have been extensively studied as biodegradable carrier materials. However, the encapsulation of peptides and proteins within these polymers remains difficult. The use of organic solvents during drug encapsulation (e.g. w/o/w emulsion solvent extraction and evaporation methods), the polymer lipophilicity and the potential formation of an acidic microclimate during release due to polymer degradation can affect protein stability and biological activity (Schwendeman et al., 1996; van de Weert et al., 2000; Estey et al., 2006; Jorgensen et al., 2006). Furthermore, the degradation times of these polymers (several months) are too long for nasal, buccal or pulmonary administration.

A promising attempt to overcome these restrictions is the use of hydrogel-based microparticles as carriers. They offer a preferable environment for peptide and protein drugs due to their hydrophilic nature and generally very mild encapsulation procedures and have been shown to stabilize the complex structure of protein drugs (Gombotz and Hoffmann, 1986; Gombotz and Wee, 1998; Peppas et al., 2000; Jensen et al., 2002; Hennink et al., 2004; Young et al., 2005). Kim et al. (2005) presented sodium hyaluronate-based microparticles as release formulation for recombinant human growth hormone. However, adequate control of protein release from these highly swollen networks is challenging due to their generally very high permeability and short diffusion pathways within microparticles.

* Corresponding author. Tel.: +49 30 83850643; fax: +49 30 83850692.
E-mail address: bodmeier@zedat.fu-berlin.de (R. Bodmeier).

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Poloxamers are non-ionic synthetic block copolymers, exhibiting excellent wetting, antifoaming and solubilizing properties. Poloxamer 407 (Pluronic® F127) is an ABA-type triblock copolymer consisting of poly(oxyethylene) units (A = 70%) and poly(oxypropylene) units (B = 30%). Aqueous poloxamer 407 solutions (concentration ⩾ 20%) are thermosensitive: At 4 °C, they form low-viscosity solutions, whereas at body temperature they form semisolid gels (Schmolka, 1972). This property of reverse thermal gelation and the low toxicity (FDA approval of poloxamer 407 containing products) render these copolymers attractive as in situ gel-forming matrix materials for controlled protein delivery systems. Recent reviews on the use of thermal gelation for controlled drug delivery have been provided by Hatefi and Amsden (2002) and Jeong et al. (2002). Injectable depot formulations enhance the stability and extend the delivery of peptide/protein drugs, including interleukin-2 (Johnston et al., 1992), urease (Pec et al., 1992), deslorelin and GnRh (Wenzel et al., 2002), insulin (Barichello et al., 1999), human growth hormone (Katakam et al., 1997) and the MSH-analog melanotan-I (Bhardwaj and Blanchard, 1996). Poloxamer 407 has also been used as release sustaining additive in buccal, nasal, ophthalmic and rectal delivery systems. However, protein release is generally controlled for only a few hours [e.g. 8 h for IL-2 (Johnston et al., 1992); 4 h for melanotan-I (Bhardwaj and Blanchard, 1996)].

Alginates are naturally occurring, linear, unbranched polysaccharides, containing 1,4′-linked β-D-mannuronic and α-L-guluronic acid residues. They are able to form water-insoluble gels by cross-linking with divalent cations (e.g. Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}) (Grant et al., 1973) or oppositely charged larger molecules like poly-L-lysine (Gonzales Ferreiro et al., 2002a,b). Due to this mild gelation process and the good biocompatibility (Orive et al., 2005), alginates have been widely used as matrix formers for the microencapsulation of bioactive peptides and proteins as well as of living cells (Gombotz and Hoffmann, 1986; Smidsrod and Skjak-Braek, 1990; Gombotz and Wee, 1998; Ribeiro et al., 2005). Three methods are frequently used to prepare alginate beads/particles: (i) atomisation (spraying of alginate solutions into solutions of divalent cations); (ii) (water-in-oil) emulsification; and (iii) coacervation (with oppositely charged polyelectrolytes). However, alginate gels are not stable in 0.1 M phosphate buffer (calcium ions are removed due to calcium phosphate precipitation), resulting in rapid protein release. Furthermore, alginate gels are generally highly porous, resulting in high diffusion rates. The formation of complexes of alginate with polycations such as poly(L-lysine), chitosan or polyethyleneimine has been proposed to stabilize the calcium alginate gel against Ca^{2+} chelators and to reduce its permeability (Thu et al., 1996; Vandenberg et al., 2001; Ribeiro et al., 2005). However, this requires an additional coating step.

To overcome the restrictions of pure poloxamer and pure alginate-based formulations, both materials were combined for controlled drug delivery (Moebus et al., 2009). The poloxamer gel is hereby reinforced by the calcium cross-linked alginate gel, which hinders the rapid dissociation of poloxamer micelles/molecules. In addition, the poloxamer (upon gelation in aqueous media at body temperature) "fills the pores of the alginate gel" and acts as diffusion barrier for entrapped drug as well as the dissolution medium, thus, slowing down the exchange of calcium ions and consequently the calcium alginate gel degradation. Microparticles were prepared using a w/o emulsion method (Moebus et al., 2009) with organic solvents (which need to be completely removed and can cause protein instability). In addition, if pulmonary administration is intended, it is difficult to produce microparticles in the suitable size range (<5 μm) (high shear energies and/or high concentrations of surfactants potentially affect protein stability). The aim of this study was to develop new preparation techniques for this type of microparticles, allowing pulmonary application and avoiding the use of organic solvents.

2. Materials and methods

2.1. Materials

Terbutaline sulfate (Welding GmbH, Hamburg, Germany), bovine serum albumin (BSA, Mw 69 kDa; Carl Roth GmbH + Co. KG, Karlsruhe, Germany), sodium alginate (low viscosity grade; Sigma–Aldrich Chemie GmbH, Steinheim, Germany), poloxamer 407 (polyoxypropylene-polyoxyethylene block copolymer, Lutrol F127; BASF AG, Ludwigshafen, Germany), calcium chloride (calcium chloride dihydrate) and zinc sulfate (zinc sulfate heptahydrate) (Caesar & Loretz GmbH, Hilden, Germany), ammonium sulfate and sodium citrate (tri-sodium citrate dihydrate) (Merck KGaA, Darmstadt, Germany), Coomassie assay (Coomassie Plus Protein Assay Kit; Pierce Biotechnology, Inc., Rockford, IL, USA), 1,9-dimethyl methylene blue (DMMB; Aldrich-Chemie GmbH, Steinheim, Germany).

2.2. Microparticle preparation

The investigated preparation procedures included the following two major steps (Fig. 1):

(i) Spray-drying of an aqueous alginate solution (3% w/w), containing – if indicated – poloxamer (3% w/w) and/or drug (theoretical loading based on the total solids' content: terbutaline sulfate – 2%, 10% or 20% w/w; BSA – 10% w/w) (Büchi 190 mini spray-dryer; Büchi Labortechnik AG, Flawil, Switzerland) using the following conditions: inlet temperature ~140–145 °C; pump flow 5–7 g/min; spray flow 600 nl/h; aspirator pressure ~50 mbar; outlet temperature 70–80 °C.

(ii) Cross-linking of the microparticles in aqueous and ethanolic solutions: 300 mg microparticles were dispersed with a vortex mixer (30 s, 1200 min^{-1}, Minishaker MS-I; IKA® Werke GmbH & Co., KG, Staufen, Germany) in:

(1) 3 ml 25% w/w or 15 ml 3% w/w (identical Ca^{2+}:alginate ratio) pre-warmed (~45 °C) aqueous $CaCl_2$ solution stirred on a magnetic heating plate (~45 °C) for 10 min, or

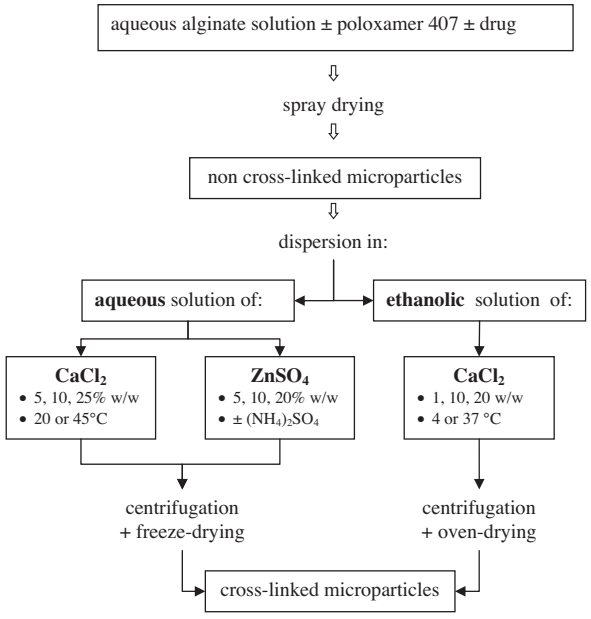

Fig. 1. Scheme of the investigated preparation techniques for alginate–poloxamer-based microparticles.