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Research paper

Alginate–poloxamer microparticles for controlled drug delivery to mucosal tissue

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ABSTRACT

Purpose: The aim of this study was to prepare and characterize novel hydrogel-based delivery systems allowing for the controlled release of drugs to mucosal surfaces. *Methods:* Terbutaline sulfate and bovine serum albumin (BSA)-loaded alginate–poloxamer microparticles were prepared by a w/o-emulsion- and external gelation method. The microparticles were characterized by optical and scanning electron microscopy, laser light diffraction, atomic absorption spectroscopy, energy-dispersive X-ray analysis, via complexation with 1,9-dimethyl methylene blue and using dialysis bags as well as modified Franz diffusion cells for in vitro drug-release measurements. *Results:* Using heptane as organic phase, homogeneous and almost spherical microparticles were obtained with a high-loading efficiency (>90%). The resulting drug-release patterns could effectively be adjusted by varying the "alginate:poloxamer" blend ratio. In addition, the particle size, morphology, calcium and chloride content as well as alginate-release rates could be altered. Erosion was the predominant release mechanism for BSA. Special attention needs to be paid to the microparticle recovery procedure, which can significantly affect key properties such as the resulting drug-release patterns. *Conclusions:* The novel hydrogel-based microparticles offering mild conditions for incorporated drugs (e.g., proteins) provide an interesting potential as controlled delivery systems for mucosal surfaces.

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1. Introduction

Most peptide and protein drugs have to be administered by injection, since the preferred oral application is often not feasible due to degradation, low permeation and significant first pass metabolism. The increasing number of available recombinant peptide and protein therapeutics [1,2] has enhanced the search for alternative administration routes to parenteral applications (e.g., rectal, buccal, nasal, pulmonary [3–5]). Peptides and proteins are generally rapidly eliminated from the systemic circulation. To reduce the application frequency, microparticulate carrier systems providing sustained delivery have been proposed [6,7]. For example, polylactides [poly(lactic acid), poly(glycolic acid), and poly(lactic-co-glycolic acid)] (PLGA) have been studied extensively as carrier materials for depot formulations [8,9]. However, the PLGA degradation times (several weeks to months) are not suitable for all administration routes, e.g., via mucosal surfaces. Furthermore, the use of organic solvents during drug encapsulation (e.g., in w/o/w emulsion solvent extraction and evaporation methods), the polymer hydrophobicity, and the potential formation of acidic microclimates upon polymer degradation often results in a loss of the biological activity of the protein [6,10,11].

The use of hydrogel-forming polymers (e.g., alginates and poloxamers) as encapsulation materials presents a promising alternative to overcome these restrictions. These polymers swell and gel in water, and retain a significant fraction of water in their structure without dissolving [12,13]. Often, hydrogels are highly biocompatible. They usually possess a shorter duration of release and degradation compared to, e.g., PLGA-based formulations. Due to their aqueous, hydrophilic nature and mostly mild preparation procedures, they offer a preferable environment for peptide and protein drugs and have been shown to stabilize the complex structure of protein drugs [13–16]. However, the appropriate control of drug release from these highly swollen networks is challenging.

Poloxamers/Pluronics are a series of synthetic block copolymers of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) (PEO–PPO–PEO) with varying molecular weights and block ratios. They are non-ionic surfactants possessing 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 ($B = 30\%$), which is transformed from a low-viscosity solution to a semisolid gel upon heating from 4 °C to room or body temperature in aqueous solutions at concentrations of $\geqslant 20\%$ [17].

This (reverse) thermal gelation and the low toxicity are properties that have made poloxamer 407 an attractive matrix-former in in situ gel-forming, controlled drug-delivery systems [18–20]. Such injectable depot formulations have enhanced the stability and sus-

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tained the delivery of biologically active peptide/protein drugs, e.g., interleukin-2 [21], urease [22], deslorelin and GnRh [23], insulin [24], human growth hormone [25] and the MSH-analog melanotan-I [26]. Furthermore, the suspension of protein precipitates in a poloxamer 407 gel matrix was reported to result in an enhanced protein stability [27]. Poloxamer 407 has also been investigated as release-sustaining additive in buccal, nasal, ophthalmic and rectal delivery systems [18,19,28,29]. However, due to the dissociation of packed poloxamer 407 micelles in an excess of water, the gel integrity does not persist during prolonged periods of time. Most formulations show sustained release kinetics only during several hours (e.g., 8 h for IL-2 [21], and 4 h for melanotan-I [26]). And most of the described systems are liquids (injectable at low temperature). However, with respect to long-term stability, dry powder systems would be preferable. Also, evidence for the safety of poloxamer for delivery to mice lungs was provided [30].

Alginates are naturally occurring, linear unbranched polysaccharides which contain various amounts of 1,4́-linked β-D-mannuronic and α-L-guluronic acid units arranged as blocks along the chain, where homopolymeric regions are interdispersed with regions of alternating structure. They are able to form water-insoluble gels by cross-linking with divalent cations (e.g., Ca^{2+}). Due to this mild gelation process, a relatively inert aqueous environment within the matrix and the high biocompatibility of alginate, this polymer has been widely used as a matrix-former for the microencapsulation of bioactive peptides and proteins as well as living cells [13,15,31,32]. However, alginate gels degrade and precipitate in 0.1 M phosphate buffer (as the calcium ions are removed and a calcium phosphate precipitate is formed), leading to rapid drug release. Furthermore, alginate gels show a high porosity resulting in high-diffusion rates. The formation of stable complexes of alginate with polycations such as poly(L-lysine), chitosan or polyethyleneimine has therefore been proposed. The calcium alginate gels are, thus, protected against Ca^{2+} chelators. The porosity is reduced, resulting in a more sustained drug delivery [33–35]. However, an additional coating step with the polycations is required.

The combination of poloxamer 407 and calcium alginate gels in a dry powder microparticulate system could be a promising strategy to overcome the restrictions of the individual polymers. The idea is to reinforce the poloxamer 407 gel via a calcium cross-linked alginate network. Hence, the rapid dissociation of poloxamer 407 micelles should be hindered. In addition, the poloxamer 407 (upon gelation at body temperature) could fill the pores of the alginate gel and act as diffusion barrier for entrapped drug as well as for the dissolution medium, thereby slowing down the exchange of calcium ions, and consequently the calcium alginate gel degradation.

The objective of this study was to prepare drug-loaded calcium alginate–poloxamer 407 microparticles by a w/o-emulsion method, and to investigate the influence of formulation and processing parameters on the encapsulation efficiency, particle size, morphology and in vitro drug-release kinetics of the microparticles. A self-made diffusion cell was used to better simulate the release conditions on mucosal surfaces, where only a small amount of release medium is available.

2. Materials and methods

2.1. Materials

Terbutaline sulfate (Welding, Hamburg, Germany), bovine serum albumin (BSA, M_w 69 kDa; Carl Roth, Karlsruhe, Germany), sodium alginate (low-viscosity grade; Sigma–Aldrich, Steinheim, Germany), poloxamer 407 (polyoxypropylene–polyoxyethylene block copolymer; Lutrol F127, BASF, Ludwigshafen, Germany), sodium hyaluronate (sodium hyaluronate pharma grade 80; NovaMatrix/FCM BioPolymer, Oslo, Norway), hydroxypropyl methylcellulose (HPMC E50; Methocel E50; Colorcon, Dartford, UK), calcium chloride, heptane and peanut oil (Carl Roth), sorbitan trioleate (Span® 85; Merck-Schuchardt, Hohenbrunn, Germany), polyoxyethylene sorbitan trioleate (Tween® 85; ICI Surfactants, Everberg, Belgium), sodium citrate (tri-sodium citrate dehydrate; Merck, Darmstadt, Germany), Coomassie assay (Coomassie Plus Protein Assay Kit; Pierce Biotechnology, Rockford, IL), 1,9-dimethyl methylene blue (DMMB; Sigma–Aldrich).

2.2. Microparticle preparation

Microparticles were prepared by a w/o-emulsion external cross-linking procedure (Fig. 1) adapted from Wan et al. [36], and Chan and Heng [37]. Heptane or peanut oil was used as organic phase:

(1) *Heptane:* Twenty gram aqueous solution of 3% (w/w) alginate, 3% poloxamer 407 and (if indicated) the drug (terbutaline sulfate or BSA, 10% w/w based on total solids) were dispersed in 30 g heptane (w/o volume ratio ~1:2) containing 3.3% w/w Span 85 (1 g) using a magnetic stirrer (1000 rpm) for 10 min. Two gram aqueous solution of 25% Tween 85 (0.5 g, resulting in a ratio of Span 85: Tween 85 = 2:1) was added, and the emulsion was stirred for another 5 min. The system was heated to 40–45 °C under continuous stirring on a magnetic heating plate. Then, 8 g of an aqueous $CaCl_2$ solution (25% w/w) was added dropwise (during ~4 min) using a syringe with needle (Sterican® Gr.1 0.90 × 40 mm; Braun, Melsungen, Germany). Stirring was continued for another 15 min. The system was then allowed to cool down for 10 min (under stirring at 200 rpm). Subsequently, the microparticles were recovered by two different procedures: (i) by vacuum filtration without further washing, or (ii) by centrifugation in 15 mL screw-capped tubes (5 min/1500 rpm, Ecco-Praxa-2, Theodor Karow, Berlin, Germany) and washing of the settled microparticles by redispersion in water followed by another centrifugation step. The obtained microparticles were dispersed in 2 mL water, immediately stored in a −70 °C freezer for at least 1 h and freeze dried for 48 h (Alpha I-5, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany). Parameters such as the drug loading (BSA: 5, 10 and 20% w/w) and the alginate:poloxamer 407 ratio in the aqueous phase (1:5, 3:3, 5:1, 5:0) were varied. Additionally, an alternative method was used to load terbutaline sulfate more efficiently into the microparticles. Blank microparticles were prepared by the same procedure (without drug in the aqueous phase). Five-hundred milligram blank microparticles were dispersed in 2.5 g prewarmed (40–45 °C) terbutaline sulfate solution (0.5, 2.8 or 6.25% w/w, leading to theoretical drug loadings of 2.5, 12.2 and 23.8%) under vortex stirring (1 min, 2500 rpm). Microparticles were recovered by

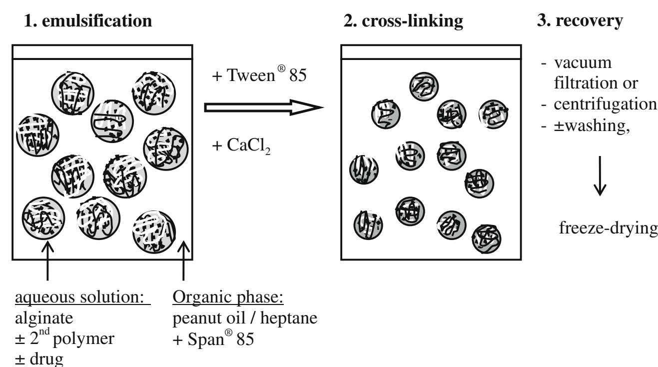

Fig. 1. Schematic presentation of the microparticle preparation techniques.

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