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Evaluation of a newly developed HPMC ophthalmic insert with sustained release properties as a carrier for thermolabile therapeutics

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ABSTRACT

A novel drug delivery system (DDS) with sustained release properties was developed to allow ocular protein delivery. The DDS developed is aimed at overcoming stability issues during preparation such as denaturation of proteins caused by shear forces applied or due to elevated temperatures and air entrapment potentially causing oxidation of the molecule. The rod-shaped HPMC inserts were loaded with lysozyme and several HPMC types were studied and compared. An aqueous colloidal HPMC solution (hydrogel) was prepared and subsequently dried at 150 °C to dehydrate the polymer solution. This partially dehydrated polymer cylinder was loaded with an aqueous glycerol/lysozyme solution at 2 °C. A 2^4 full factorial design was set up to evaluate the effect of the different preparation parameters on water uptake and release properties. As a result, four out of sixteen formulations revealed homogenous distribution for lysozyme in both duplicates. The change in water uptake over time was dependent on the type of HPMC polymer used but not between the chosen HPMC percentages. After 240 min, 50% of lysozyme loaded was released depending on the chosen formulation. Lysozyme molecules exhibit slower release from a K100M matrix compared to E10M inserts, albeit the overall effect is relatively limited.

1. Introduction

The pre-corneal drug delivery has always been a challenging task for researchers. Eye drops such as aqueous solutions are the most frequently used drug delivery system (DDS). These classical dosage forms are generally well accepted by patients. However, the bioavailability of topically applied drugs in solution is poor due to rapid eye blinking, induced lachrymation caused by an external stimulus and followed by drainage of the excess fluid to the nasolacrimal duct. The barrier function of the cornea and conjunctiva reduces ocular availability even more (Davies, 2000). To overcome these inherent drawbacks, frequent instillation is required to obtain an adequate concentration at the site of action. A high frequency of instillation resulting in pulsed dose may lead to undesirable side-effects caused by high systemic absorption of the drug applied and a reduced therapeutic effect (Lee and Robinson, 1986; Salminen, 1990). Researchers have put great effort to overcome these problems through various strategies such as the development of liposomes, muco/bioadhesive dosage forms, collagen shields, drug loaded contact lenses, ocular inserts, nanoparticles, ocular iontophoresis and intra-ocular injections (Ebrahim et al., 2005; Ludwig, 2005; Saettone and Salminen, 1995; Baeyens et al., 1998; Zimmer and Kreuter, 1995; Kaur and Kanwar, 2002; Aburahma and Mahmoud, 2011; Achouri et al., 2013). One of the most important strategies involves the use of viscosity-enhancing agents as drug carriers in order to prolong the residence time of the medication at the site of action and thereby slowing down the drainage of the drug (Urtti and Salminen, 1993). The improved residence time of the drug in the conjunctival sac enhances its ocular availability and thus leads to less side-effects and fewer administrations required. Moreover, ocular inserts allow for a more accurate dosing as opposed to classical eye drops.

In recent years, research has been focusing on the administration of proteins for eye diseases including wet age-related macular degeneration (Barakat and Kaiser, 2009), uveitis (Guly and Forrester, 2010) and corneal neovascularisation (Chang et al., 2012). Their sensitivity to elevated temperatures, pH changes, mechanical shear forces and oxidation by air complicates the manufacturing process of ocular drug delivery devices loaded with proteins (Vogt, 1995; Di Stasio and De Cristofaro, 2010; Torosantucci et al., 2014). For the development of the present drug delivery system, special attention was paid to the development of a method that could potentially minimise the risk of stability issues of (thermo)labile molecules such as peptides and proteins during

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preparation. Therefore, shear forces should be minimised and air entrapment as well as heating should be avoided. In order to improve eye-comfort, the foreign-body sensation should be reduced. This can be achieved by making rod-shaped, semi-solid inserts which are flexible when pressure is applied by the lower eyelid. The insert is entirely water dispersible, eliminating the need to remove the insert from its site of application after drug delivery.

Peptides and proteins require specific attention during the development and processing of ophthalmic inserts. Most peptides and proteins are sensitive to elevated temperatures. In general, the homogenous dispersion of highly viscous polymers in water, such as cellulose derivatives, is performed in hot water to avoid air bubble entrapment and dispersing the polymer molecules homogenously. After being fully wetted and dispersed in the solvent, a non-thermolabile drug can be added simultaneously with the polymer in the solvent at high temperature. The viscous dispersion can be cooled down to room temperature or below, enhancing the formation of hydrogen-bonds and structure build-up. Of course, this procedure cannot be employed for protein molecules as they require specific treatment during formulation.

Firstly, proteins containing thiol groups, such as methionine and cysteine residues, are particularly susceptible to oxidation (Vogt, 1995; Torosantucci et al., 2014). Therefore, as described above, air bubble entrapment in the viscous polymer dispersion should be avoided. Solvent casting is a commonly used method for the preparation of ocular inserts (e.g., ocular films) (Hermans et al., 2014). This method does not require added heat in order to disperse the drug molecules homogeneously. A low-viscous solution is formed by mixing the various components and is poured into an appropriate mould. The solution is dried over an extended period of time at room temperature or above. The exposure of the drug molecules to air and the suboptimal temperatures at which solvent casting is performed, may cause proteins to denature. Oxidation can be avoided by drying in an inert atmosphere and at low temperatures for drug stability reasons. But inherent to these low temperatures, the time required to dry the dispersion will extend and is therefore undesirable.

Secondly, denaturation of peptides and proteins can also occur by applying mechanical stress such as shear forces (Di Stasio and De Cristofaro, 2010). Mechanical agitation such as high shear homogenisers and comparable blending devices can change the conformation of peptides and proteins potentially causing loss of activity.

Thirdly, the most common way of preparing a homogenous gel is by blending the different excipients and active pharmaceutical ingredient (API) together in the same vessel under high shear forces. Due to the desired properties of the gel (high viscosity), the aforementioned method of preparing homogenous gels is not appropriate as lumping of the hydrogel cannot be avoided easily. Additionally, high shear mixing enhances the entrapment of air bubbles. Industrial techniques of extruding inserts are single and twin screw extruders implying the use of high shear and heat.

The goal of the present study is to develop a manufacturing technique that allows proteins to be incorporated into a slow release ocular device without heat or shear forces being applied to the protein molecules and devoid of air bubbles during preparation. The effect of the viscosity grade and percentage of the polymer HPMC on water uptake and release properties of the inserts was evaluated using a 2^4 full factorial design.

2. Materials and method

2.1. Materials

Hydroxypropyl methylcellulose (HPMC) types E10M CR Premium and K100M Premium were obtained from Colorcon (Dartford, UK). Glycerol analytical grade and lysozyme from chicken egg white were provided by Sigma–Aldrich (Steinheim, Germany). By specification, a 2% (w/w) E10M HPMC solution gives an apparent viscosity of 10.000 mPa s (20 °C) while a 2% (w/w) solution of K100M HPMC has an apparent viscosity of 100.000 mPa s under the same measuring conditions. Purified water (18.2 MΩ cm) was used after being filtered over a 0.2 μm cellulose acetate filter from Sartorius (Vilvoorde, Belgium). Sodium chloride (NaCl), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) and disodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$) of analytical grade were purchased from Merck (Darmstadt, Germany) and were used to prepare phosphate-buffered saline solution (PBS, pH 7.4). PBS is an electrolyte solution composed of 8.2 g/l NaCl, 0.3 g/l $NaH_2PO_4{\cdot}2H_2O$, 1.54 g/l $Na_2HPO_4{\cdot}2H_2O$ and purified water. The barrels are syringes of 20 ml and 30 ml with the outer cylinder consisting of polypropylene. The piston is made of polypropylene and polyethylene. The joint of the piston is made of synthetic isoprene with silicone as lubricant (BD Plastipak, Drogheda, Ireland).

2.2. Preparation of ocular inserts

The different rod shaped, ocular inserts consist of varying percentages of HPMC, 3% (w/w) lysozyme as a model protein, 5% (w/w) glycerol as a plasticiser and water as a solvent. The choice of HPMC was based on the commercial availability of pharmaceutical grade HPMC types, admittance for ocular purposes and high viscosity grades. The preparation of this drug delivery system consists of four major phases. A schematic overview of the preparation method is given in Fig. 1.

2.2.1. Phase I: hydrogel-formation

A polymer solution of HPMC 10% (w/w) was prepared by heating purified water up to a temperature of 90–100 °C and adding HPMC to the water under continuous agitation. The HPMC dispersion was steadily stirred (VWR, VMS-C7-2, IKA, Staufen, Germany) and was cooled down to room temperature (22 ± 2 °C) resulting in a highly viscous polymer solution. In addition to this, it was stored at 2 °C overnight in order to obtain full hydration of the polymer chains.

2.2.2. Phase II: dehydration of the polymer solution

The viscous polymer solution was put in a drying oven (Thermo Scientific Heraeus Function Line T12, Langenselbold, Germany) at a

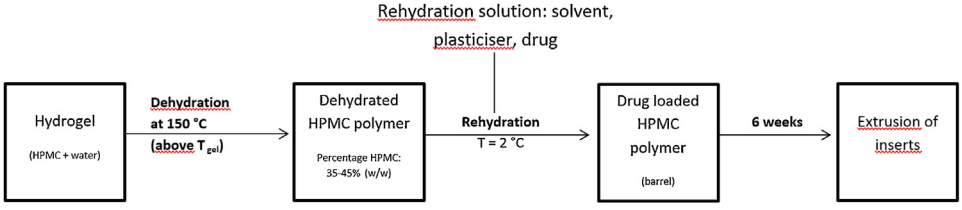

Fig. 1. General overview of the preparation method. T_{gel} stands for thermal gelation temperature.