International Journal of Pharmaceutics 536 (2018) 73–81

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Nanoparticle-loaded hydrogels as a pathway for enzyme-triggered drug release in ophthalmic applications

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ARTICLE INFO

Keywords:
Cellulose nanocrystals
Chitosan
Fluorescence microscopy
Lysozyme
Polyacrylic acid
Polyvinyl alcohol

ABSTRACT

The aim of this study was to develop nanoparticle loaded hydrogel based contact lenses that could be used for ocular drug delivery. Two potential contact lens platforms for controlled ophthalmic drug delivery were developed by incorporating chitosan-poly (acrylic acid) nanoparticles into polyvinyl alcohol (PVA) hydrogels and in-situ gelled nanoparticles and cellulose nanocrystals (CNC) in PVA lenses. The nanoparticles were shown to disintegrate in a physiological 0.2 mM concentration of lysozyme resulting from the hydrolysis of the chitosan chains by lysozyme. An extended release over a 28-h period was demonstrated once the nanoparticles had been integrated into the composite lenses, with nanoparticle-CNC PVA lenses showing even greater potential for extended release. The platform shows great promise in developing enzyme-triggered ocular drug delivery systems.

1. Introduction

Eye drops in the form of suspensions and solutions are currently the most common preparations for ophthalmic drug delivery, accounting for more than 90% of the formulations on the market today (Ali and Byrne, 2008; Guzman-Aranguez et al., 2013; Tieppo et al., 2014; Xinming et al., 2008). However, one major drawback with these types of topical formulations is their low bioavailability resulting from a short residence time of the drug on the cornea, which varies between 2–3 min (Bengani et al., 2013; Xinming et al., 2008). The small volume of between 7–30 µl of fluid that is able to occupy the surface of the eye is partly to blame for this as the majority of the drug is removed 15–30 s after application by nasolacrimal drainage (Ali and Byrne, 2008; de la Fuente et al., 2010; Hsu et al., 2014). Any drug that remains at the anterior surface of the eye faces further removal from tear turnover, which replenishes at a rate of 0.5–2.5 µl tear fluid per min, and metabolic degradation leading to an effective absorption of only 1–7% of the drug (Ali and Byrne, 2008; Bengani et al., 2013; Guzman-Aranguez et al., 2013; Tieppo et al., 2014). As a result of this, ophthalmic solutions require a high concentration of drug which moreover needs to be applied frequently in order to reach therapeutic concentrations within the eye. This could lead to a lower compliance among certain patient groups but more importantly to toxic side effects not only locally in the eye but in the whole body since a large amount of the drug is absorbed systemically (Ali and Byrne, 2008; Alvarez-Lorenzo et al., 2006; Hsu et al., 2014; Xinming et al., 2008). As such alternative formulations and drug delivery vehicles have been investigated in order to circumvent these problems.

Contact lenses for ophthalmic drug delivery are one such device which has garnered interest among scientist for the last 40 years (White et al., 2011) and which theoretically could lead to an increase in bioavailability of up to 50% as a result of a prolonged drug residence time on the surface of the eye (Hsu et al., 2014). The lens could also minimize the systemic side effects of the drug as it would have an occlusive effect (Alvarez-Lorenzo et al., 2006; White et al., 2011). One of the first drug loaded contact lenses were developed by Sedlacek in 1965 who was able to load an ocular paralytic by soaking hydrogel lenses in a diluted solution of the drug. The drug was successfully released and showed an improved effect compared to conventional eye drops when tested on patients (White et al., 2011). Many studies have since been done over the years employing the soaking method of contact lenses in a concentrated drug solution or a solution of prodrug, which upon being released into the eye would degrade into the pharmaceutical active form (McDermott and Chandler, 1989; White et al., 2011). One major disadvantage with this strategy, however, was the initial burst release that was observed during the first hour when the lens was applied, in which the majority of the drug was released. As such the formulation was only able to supply the drug for a couple of hours at most (Alvarez-Lorenzo et al., 2006; Hsu et al., 2014).

As few products on the ophthalmic drug market at that time, and

Abbreviations: NP, nanoparticle; PVA, poly vinyl alcohol; CNC, cellulose nano crystals; FITC, fluorescein isothiocyanate isomer I

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https://doi.org/10.1016/j.ijpharm.2017.11.053
Received 4 September 2017; Received in revised form 21 November 2017; Accepted 22 November 2017
Available online 24 November 2017
0378-5173/

even today, were unable to supply an extended release of drug for the treatment of ocular diseases, further studies were made in order to enhance and improve both the drug loading and release from contact lenses (Ali and Byrne, 2008). The choice of contact lens material, when designing therapeutic lenses, was an important starting point. The choice of material not only affects drug incorporation and release but also the mechanical stability as well as other properties such as transmittance and oxygen permeability of the lenses. Therefore the choice of either hydrogels, e.g. NVP (n-vinyl pyrrolidone) and HEMA (hydroxyethylmethacrylate), which have high water content and varied oxygen permeability or silicone-based materials, such as silicone acrylates or fluorosilicone acrylates, which contain no water, would have big impact on the development of different therapeutic lenses (Lippman, 1990; McDermott and Chandler, 1989). Molecular imprinting of silicon-based contact lenses was one new way in which an increase in drug loading as well as an extended release was achieved. This was attained by imprinting the drug structure in the polymeric network of the lens by using the drug molecule as a template in the synthesis process (Ali and Byrne, 2008; Alvarez-Lorenzo et al., 2006). The drug would interact with the functional groups in the polymeric network as it diffused through the lens resulting in a slower release. Extended in vitro releases were successful for ketotifen fumarate and timolol with these molecular imprinted lenses (Venkatesh et al., 2008).

Other methods have also been investigated to reduce the diffusion of the drug molecules in the lenses such as creating diffusion barriers by adding vitamin E (Kim et al., 2010; Peng et al., 2010). It was found that soaking silicone lenses in an ethanol solution of vitamin E resulted in the formation of aggregates inside the lens. These aggregates were then be able to act as diffusion barriers for hydrophilic drugs as they would be forced to travel around these aggregates when diffusing through the lens (Hsu et al., 2014; Kim et al., 2010; Peng et al., 2010). Nanoparticle loaded contact lenses have also been shown to be promising platforms for ophthalmic drug delivery (Bengani et al., 2013). A study by Kim et al. showed that nanodiamond gels loaded with timolol maleate crosslinked with chitosan could successfully be imbedded into poly-2-hydroxyethyl methacrylate (pHEMA) lenses. These lenses were then able to release the drug in a controlled fashion in the presence of tear fluid, where the enzyme lysozyme was able to degrade the chitosan chains by hydrolysis of the 1,4-β-glycosidic bond between the subunits leading to release of the drug (Kim et al., 2014). Behl et al. were also able to achieve a sustained release of encapsulated dexamethasone sodium phosphate from chitosan nanoparticles in pHEMA lenses (Behl et al., 2016). However, although nanoparticle formulations, made from dispersed colloidal systems, in general show great promise some of the drawbacks involved the possibility that the incorporated drugs may diffuse from the particles and lens prematurely during storage (Alvarez-Lorenzo et al., 2006; Hsu et al., 2014; Maulvi et al., 2016).

The aim of this study was to develop nanoparticle loaded hydrogel based contact lenses that could be used for controlled ocular drug delivery. As such the goal was to show that the synthesized nanoparticles would disintegrate in the presence of lysozyme as well as show that the particles could be incorporated into and released from the hydrogel lenses in a controlled manner. In order to achieve this aim two types of chitosan-poly (acrylic acid) nanoparticle loaded hydrogel lenses were developed as possible platforms for controlled ophthalmic drug delivery. A controlled extended release from the lenses would be achieved by enzymatic degradation of the nanoparticles in the presence of lysozyme. Cellulose nanocrystal (CNC) reinforced polyvinyl alcohol (PVA) hydrogels were chosen as contact lens material for one of the lenses. Previous studies by Tummala et al. had shown the material to have good mechanical stability, biocompatibility among other properties, such as high water content and transparency, which were comparable or even superior that of commercial contact lenses (Tummala et al., 2016a, 2016b, 2017a). The chemical and physical qualities of the PVA, which has made it a popular material in areas such as biomaterials and the abundance of cellulose in nature coupled with the above mentioned properties make the hydrogel lenses an attractive vehicle for drug delivery, with superior qualities to that of commercial lenses (Tummala et al., 2017b). Pure PVA hydrogel was used as the reference material in the study. The controlled release properties from the lenses were demonstrated by covalently labeling the nanoparticles with a fluorescent marker, used as a model drug substance.

2. Material and methods

2.1. Materials

Chitosan low molecular weight (Mw: 50–190 kDa, 50–190 kDa, degree of deacetylation 75–85%) (Sigma-Aldrich, SE), Anhydrous acrylic acid (Sigma-Aldrich co., St. Louis, MO, USA), Potassium peroxydisulfate (97%, $K_2O_8S_2$) (Alfa Aesar, GE), Lysozyme from chicken egg white (M_r ~ 14.6 kDa) (Sigma-Aldrich co., St. Louis, MO, USA), Fluorescein isothiocyanate isomer I (FITC) (Sigma-Aldrich co., St. Louis, MO, USA), Polyvinyl alcohol (M_w 146,000–186,000) (Sigma-Aldrich co., St. Louis, MO, USA), Sodium hydroxide (NaOH) (ACS reagent, pellets) (Sigma-Aldrich co., St. Louis, MO, USA), Hydrochloric acid solution 0.1 M (HCl) (Sigma-Aldrich co., St. Louis, MO, USA), Buffer solution pH 7.00 (potassium hydrogen phosphate/sodium hydroxide) (Sigma-Aldrich co., St. Louis, MO, USA), Saline solution 9 mg/ml NaCl (Fresenius Kabi, DE), Dimethyl sulfoxide (DMSO, ACS reagent) (Sigma-Aldrich co., St. Louis, MO, USA), Sodium chloride ACS, ISO, Reag. Ph Eur (NaCl) (Merck KGaA, DE), Methanol (Merck KGaA, DE), Ethanol (ACS reagent) (VWR International S.A.S, FR), Acetic acid (ReagentPlus) (Sigma-Aldrich co., St. Louis, MO, USA).

All chemicals were used as bought without further purification.

2.2. Procedure

2.2.1. Synthesis of chitosan poly- (acrylic acid) nanoparticles

The nanoparticles were synthesized by template radical polymerization according to Hu et al. (Hu et al., 2002) as follows. 3.00 mmol of chitosan was added to 50 ml acrylic acid solution, containing an equimolar amount of acrylic acid, during magnetic stirring in a 250 ml three-necked round bottom flask. The chitosan was allowed to dissolve under nitrogen stream at room temperature until a clear solution was obtained, at which point 1.00 mmol $K_2S_2O_8$ was added. The solution was allowed to polymerize for 2 h at 70 °C until the suspension turned opalescent. The pH was kept at around 4 during the whole polymerization procedure by addition of 0.1 M HCl or 0.2 M NaOH. The finished nanoparticle suspension was then finally filtered and then dialyzed using a dialysis cellulose membrane with a molecular cut-off of 14 kDa for 24 h in 2000 ml of deionized water with continuous magnetic stirring at 150 rpm.

The synthesis was carried out a further two times using a molar ratio of 1:1.33 and 1.33:1 acrylic acid to chitosan.

2.2.2. Physical stability of nanoparticles

2.2.2.1. Effect of pH and salinity. The chemical stability of the synthesized nanoparticles were investigated in solutions of varying pH; NaOH solution of pH 11, phosphate buffer of pH 6.99, acetic buffer of pH 4.5, deionized water of pH 5.77 and an isotonic solution (0.15 M NaCl) pH of 5.67. The prepared samples contained a 1:50 v/v % of nanoparticle suspension to solution and a NaCl concentration of 10 mM in all solutions apart from the isotonic one. Samples were incubated at room temperature for approximately 30 min prior to each measurement and the average diameter and zeta potential was calculated from three measured samples. The effect of pH on the stability of the nanoparticles was evaluated because the morphology of the particles are dependent on the charge density of the ionizable groups of chitosan and polyacrylic acid.

2.2.2.2. Effect of heating in DMSO. The physical stability of the