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In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery

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ABSTRACT

This study evaluated the use of Pluronic F127 and Pluronic F68 as excipients for formulating *in situ* gelling systems for ocular drug delivery. Thermal transitions have been studied in aqueous solutions of Pluronic F127, Pluronic F68 as well as their binary mixtures using differential scanning calorimetry, rheological measurements, and dynamic light scattering. It was established that the formation of transparent gels at physiologically relevant temperatures is observed only in the case of 20 wt% of Pluronic F127. The addition of Pluronic F68 to Pluronic F127 solutions increases the gelation temperature of binary formulation to above physiological range of temperatures. The biocompatibility evaluation of these formulations using slug mucosa irritation assay and bovine corneal erosion studies revealed that these polymers and their combinations do not cause significant irritation. *In vitro* drug retention study on glass surfaces and freshly excised bovine cornea showed superior performance of 20 wt% Pluronic F127 compared to other formulations. In addition, *in vivo* studies in rabbits demonstrated better retention performance of 20 wt% Pluronic F127 compared to Pluronic F68. These results confirmed that 20 wt% Pluronic F127 offers an attractive ocular formulation that can form a transparent gel *in situ* under physiological conditions with minimal irritation.

1. Introduction

Drugs are commonly administered topically to treat eye conditions. However, the efficiency of traditional ocular formulations is often sub-optimal. This results in substantial drug loss due to poor cornea permeability, nasolacrimal drainage, tear reflex, blinking, and non-productive absorption. Typically, ocular drug retention is poor with less than 5% of the dose reaching the target tissues in the eye (Järvinen et al., 1995; Hughes et al., 2005; Ludwig, 2005; Morrison and Khutoryanskiy, 2014).

Improving pre-corneal drug retention and enhancing corneal tissue permeability are two main approaches to optimise topical ophthalmic drug delivery. *In situ* gelling systems can offer improved pre-corneal retention, by converting from a liquid eye drop formulation to a gel upon ocular administration. This results in enhanced drug bioavailability (Agrawal et al., 2012; Thrimawithana et al., 2012). Gelation in the pre-corneal environment can be triggered by changes in pH, ionic content or temperature. Weakly cross-linked poly(acrylic acid) derivatives with pH-triggered *in situ* gelation properties, such as Carbopols®, have already been exploited commercially as pharmaceutical excipients. However, due to their anionic nature, Carbopols® are likely to have limited utility for cationic drugs, which may form insoluble complexes leading to phase separation (Nurkeeva et al., 2002, 2004). Hence, there is a clear need in the development of *in situ* gelling systems based on non-ionic polymers, where gelling properties will not be affected by the complexation with ionic drugs.

Triblock copolymers of polyethylene glycol-*b*-polypropylene glycol-*b*-polyethyleneglycol, available commercially as Pluronics®, are non-ionic, water-soluble materials that have attracted a lot of interest as pharmaceutical excipients. These polymers have an amphiphilic character, exhibit surface active properties and are capable of interacting with hydrophobic surfaces and biological membranes (Batrakova and Kabanov, 2008). In aqueous solutions, Pluronics® can self-assemble to form micelles, which have found applications in the solubilisation of poorly-soluble drugs (Oh et al.,

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2004; Sek et al., 2006; Kadam et al., 2009). Pluronics® are also known to form gels *in situ* in response to temperature increase (Pandit and McGowan 1998; Ibrahim et al., 2012; Akash and Rehman, 2015). Because Pluronics® are transparent, they do not interfere with normal vision and are therefore most suited for applications in ophthalmology. Several ocular formulations comprising combinations of Pluronic F127 and alginate (Lin et al., 2004), chitosan (Varshosaz et al., 2008), and cellulose ethers (El-Kamel, 2002) have been reported.

Here, we investigate two Pluronics®, i.e. Pluronic F127 (F127) and Pluronic F68 (F68), as excipients to formulate *in situ* gelling systems for ocular administration. The gelation properties of each polymer and their mixtures were evaluated to establish optimal conditions for forming *in situ* gelling systems. The irritancy potential and corneal retention of fluorescein sodium formulated using these polymers were also established both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Pluronic F68 (Lutrol® F68, EO_{80} PO_{27} EO_{80}) and Pluronic F127 (Lutrol® F127, EO_{101} PO_{56} EO_{101}) were acquired from BASF, Germany. Optimal cutting temperature compound (OCT), NaCl and $NaHCO_3$ were purchased from Fisher Scientific, UK. $CaCl_2{\cdot}2H_2O$ was purchased from BDH Laboratory Supplies, UK. Benzalkonium chloride was purchased from Fluka Chemie GmbH, Germany. Ofloxacin and fluorescein sodium were obtained from Sigma-Aldrich, UK. Vectashield mounting medium with DAPI was purchased from Vector Laboratories Inc., UK. T_{zero} pans and T_{zero} hermetic lids for differential scanning calorimetry were purchased from TA Instruments, UK.

2.2. Preparation of pluronic formulations

Pluronic formulations were prepared by dissolving the required amount of Pluronic F127 and/or Pluronic F68 in a cold deionised water, simulated tear fluid (STF) or solution of ofloxacin in STF and stirred overnight at 4 °C. Whilst the concentration of each polymer varied from one formulation to another, the total polymer concentration in each formulation was kept at 20 wt% (Table S1, Supporting Information). After a clear solution was formed, the solutions were stored at room temperature for at least 12 h before experiments commenced. For formulations containing fluorescein sodium salt or ofloxacin as model drugs, a stock solution containing the model drug was used as a solvent for the polymers.

STF was prepared according to Cho et al. (2003). In brief, 6.7 g NaCl, 2.0 g $NaHCO_3$ and 0.08 g $CaCl_2{\cdot}2H_2O$ were dissolved in 1 L of deionised water and stirred at 40 °C until a clear solution was obtained. It was kept at ambient temperature until needed.

2.3. Differential scanning calorimetry

Pluronic solutions were placed in T_{zero}® aluminium pans and hermetically sealed. Differential scanning calorimetry (DSC) was performed using the DSC Q2000 (TA Instruments) at a heating rate of 5 °C/min between 5 and 100 °C under nitrogen atmosphere. The data were analysed using Universal Analysis 2000 software (TA Instruments version 4.5A). The micelle formation temperature (T_m) was determined from the endothermic peak in the recorded DSC thermograms.

2.4. Rheological measurements

Two batches of the binary Pluronics formulations were used where one used deionised water as a solvent and the other used STF containing 0.3 w/v% ofloxacin as a model drug. All samples were kept at 4 °C for at least 48 h to minimise the potential impact of shear history on the rheological behaviour of the tested formulations. The required amount was pipetted out and placed on the pre-cooled peltier plate (5 °C) immediately prior to testing. A solvent trap was used to minimise evaporation and to keep a solvent saturated atmosphere surrounding the sample.

The gelation behaviour was investigated by AR2000ex rheometer (TA Instruments) using TA Instruments' TRIOS software (version 2.2.0.1327). A temperature ramp in oscillatory mode was conducted using 40 mm diameter parallel plate geometry between 5 and 60 °C at 5 °C/min heating rate. The measurement was performed within the linear viscoelastic region (LVR) at 0.1% strain and 1 Hz. The temperature at which elasticity modulus (G') and viscous modulus (G'') had a crossover was considered to be the system gelation temperature.

2.5. Dynamic light scattering

Micelle formation was examined using dynamic light scattering (DLS). Samples were dissolved in deionized water (5 mg/mL). Their micellation behaviour was studied at 10, 25, and 35 °C using the Zetasizer Nano–S (Malvern) and analysed using Malvern's Zetasizer software (version 7.10).

2.6. Slug mucosal irritation test

Limax flavus slugs were collected locally (Reading, UK) and were kept in flat bottom beakers lined with paper towels soaked with STF for 48 h prior to experiments. Slug mucosal irritation test was conducted according to an in-house protocol (Khutoryanskaya et al., 2008). Individual slugs were placed on Petri dishes lined with filter paper pre-soaked with one of the formulations. The slugs were exposed to the formulations for 1 h. Vehicle-only (STF) solution served as a negative control and 1 wt% benzalkonium chloride solution was used as a positive control. The difference in slug weight before (m_b) and after (m_a) the exposure was determined. Mucus production (MP) was calculated according to the following formula:

$$MP = (m_b - m_a)/m_b \times 100\% \quad (1)$$

2.7. Bovine cornea erosion test

Experiments were carried out using fresh bovine eyes acquired from a local abattoir (P.C. Turners, Farnborough, UK). Whole eyes were collected from freshly slaughtered cows and transported in a cold box to the lab to be used immediately.

The testing method described by Mun et al. (2014) was used. Each bovine eye was visually checked for corneal damage and placed in a 150 mL glass beaker. The donor part of a Franz cell was placed on top of the upward-facing cornea and fixed in place with cling film. The beaker was then placed in a 37 °C water bath for 20 min, after which 1 mL of the samples was placed in the donor compartment for 1 h. The eye was taken out and washed with STF. The corneal area in contact with the sample plus 2 mm of surrounding corneal tissue was dissected. The cornea segments were immersed in OCT, frozen using dry ice and stored at −80 °C until use.

Corneal cross-sectional specimens of 10 μm were prepared for microscopy using a microtome within a Bright cryostat (model OTF). They were stained using Vectashield mounting medium with DAPI. Slides were examined using an AXIOCAM MRm 1.3 MP digital camera mounted on a Zeiss AXIO Imager A1 fluorescent microscope with 50 × magnification and a DAPI