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Original article

Novel in-situ gel for intravesical administration of ketorolac

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ABSTRACT

The urinary bladder stores urine until the time of urination. Systemic administration of drugs to treat bladder diseases faces several limitations. Therefore, intravesical drug delivery is a promising alternative route of administration. An in-situ gel is used to form a gel inside the bladder cavity and ensure continuous release of the drug even after urination. The objective of the present study was to optimize an in-situ gel formulation of poloxamer and chitosan for intravesical delivery of ketorolac tromethamine. The gelling temperature of the prepared combinations ranged from 20.67 to 25.8 °C. In-vitro release of KT was sustained for up to 7 h using a poloxamer concentration ranging from 17% to 19% and a chitosan concentration ranging from 1% to 2%. Design-Expert® 10 was used to select the optimized formulation (poloxamer/chitosan 17/1.589% w/w) which significantly ($p < 0.05$) extended the drug release more than each polymer alone. An *ex-vivo* study showed the ability of the optimized formulation to sustain drug release after emptying two times to mimic urination. Furthermore, the formed gel adhered to the bladder tissue throughout the time period of the experiment. Intravesical administration of the optimized formulation to rabbits via catheter showed no obstruction of urine flow and continuous release of the drug for 12 h.

1. Introduction

The urinary bladder has an important role storing urine formed by the kidney and preventing systemic reabsorption of urine components from the bladder cavity until urination (Lewis, 2000). Different diseases such as interstitial cystitis (Davis et al., 2014), overactive bladder syndrome (Geoffrion, 2012), urinary tract infection (Zacche et al., 2015), and bladder cancer (Kamat et al., 2017) affect the bladder's normal function. Patients with these diseases have symptoms that affect the bladder and cause discomfort such as urinary storage problems and pain. Treatment of urinary bladder diseases with systemic drug administration suffers from several limitations such as poor bioavailability and first pass metabolism leading to a low drug concentration in bladder tissue and the subsequent need for high drug doses which may increase side effects (Tyagi et al., 2006).

Intravesical drug delivery systems (IDDS) can be delivered via urethral catheter as an alternative route of drug administration. Intravesical administration of drugs leads to high drug concentrations in bladder tissue which increases the efficacy of treatment (GuhaSarkar and Banerjee, 2010). However, the drug will be washed out within 2 h after intravesical administration by urination (Lin et al., 2016). Repeated administration of the drug via frequent catheterization increases risk of infection and causes patient discomfort. Therefore, developing an IDDS that can be retained in the bladder cavity even after urination is important to ensure continuous release of the drug (Tyagi et al., 2016).

One of the recent IDDS approaches is using an in-situ gel to increase drug residence time after intravesical administration (Tyagi et al., 2016). A vital advantage of the in-situ gel is that it possesses low viscosity during storage and forms a gel only after intravesical administration. These systems are designed to form the gel in response to different stimuli such as a change in temperature, pH, and ion concentration (Nagarwal and Pandit, 2008).

Among various in-situ gel systems, thermosensitive polymers have been widely used. Thermosensitive polymers, such as poloxamers, are available as a solution before and during administration and form a gel only in response to body temperature. Poloxamers are composed of poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) copolymers (Klouda and Mikos, 2008). Using poloxamer 407 in the formulation of in-situ gels extended the release of different intravesical drugs compared

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with drugs administered without poloxamer 407 (Tyagi et al., 2004). In a previous study, the poloxamer-based formulation for intravesical administration had an *in vitro* release time of up to 3.5 h for adriamycin (Lin et al., 2016). In another study, it was found that poloxamer alone suffered from rapid erosion. Upon addition of HPMC, the release time of adriamycin was extended up to 10 h (Lin et al., 2014). Previous studies support the need of adjacent polymers to be added with poloxamer to form a firmer gel after intravesical administration.

Another IDDS approach is the use of mucoadhesive polymers. In a previous study, an in-situ gel formulation containing gellan as a mucoadhesive polymer for intravesical administration was developed. Gellan was able to adhere to the bladder wall after administration and was superior to drug solutions in increasing the concentration of the drug in bladder tissues (GuhaSarkar et al., 2017).

Therefore, using a combination of poloxamer and mucoadhesive polymers would has the potential to solve the aforementioned limitation of poloxamer. In the current study, chitosan was utilized as a mucoadhesive polymer because it is biocompatible and biodegradable. Chitosan has been widely used in ophthalmic (Gratieri et al., 2010) and nasal (Ravi et al., 2015) in-situ gel formulations. The proposed combination of poloxamer and chitosan should avoid rapid erosion of poloxamer with minimal risk of urinary obstruction.

Ketorolac tromethamine (KT), a nonsteroidal anti-inflammatory drug, has been used to study intravesical therapy (Williams et al., 2014). For patients undergoing urethral stenting, a study showed that pain reduction due to ketorolac was superior to lidocaine and oxybutynin (Beiko et al., 2004).

In the present study, poloxamer 407 and chitosan were used in an in-situ gel formulation containing KT for intravesical administration. This combination has never been used in in-situ gel formulations for bladder diseases. Gelling temperature and *in vitro* drug release were studied to select the optimized formulation using Design-Expert® 10. In addition, *ex-vivo* and *in vivo* release studies were performed to evaluate the optimized formulation.

2. Materials and methods

2.1. Materials

KT was kindly supplied by Amriya Pharmaceutical Industries (Alexandria, Egypt). Poloxamer 407 was obtained from Spectrum (Germany) and chitosan high viscosity grade (M.W. 600,000) was obtained from Winlab (England). All other chemicals were pharmaceutical grade. Freshly isolated bladder tissue of slaughtered sheep was used in the *ex-vivo* study.

2.2. Methods

2.2.1. Experimental design and selection of optimized formulation

Randomized full 3^2 factorial experimental design (Design-Expert® 10, Stat-Ease Inc., Minneapolis, MN, USA) was used to characterize the relationship between poloxamer and chitosan concentration and measured in-situ gel properties by polynomial fitting and analysis of variance (ANOVA). Poloxamer concentration (X_1) and chitosan concentration (X_2) were the two independent variables selected to study their impact on the attributes of the prepared in-situ gels containing KT. Appropriate models were selected by comparing p values and coefficient of determination (R^2) values. The matrix of 3^2 full factorial design for preliminary study of KT in-situ gel formulations is shown in Table 1. Response surface methodology (RSM) was used to investigate the effect of the independent variables (X_1 and X_2) on a range of dependent variables, including gelling temperature Y_1 and percent of drug release at 2 h (D_2 %), 4 h (D_4 %), and 7 h (D_7 %) designated as Y_2, Y_3, and Y_4, respectively. Response surfaces were constructed using the obtained equations and used as an aid in the selection of the optimized formulation (Fopt) based on gelling temperature and percent of drug release.

Table 1
3^2 full factorial design for poloxamer/chitosan combinations.

		Chitosan (X_2)		
		1%	1.5%	2%
Poloxamer (X_1)	17%	F 1	F 2	F3
	18%	F 4	F 5	F 6
	19%	F 7	F 8	F 9

2.2.2. Preparation of in-situ gels

All formulations were prepared based on w/w % calculation. Poloxamer solutions were prepared by dissolving the weighed amount of poloxamer in water and kept in a refrigerator overnight. In-situ gel formulations were prepared by dissolving chitosan in 0.5% v/v acetic acid, then adding the calculated amount of KT (2.5 mg/g of the prepared formulation). Finally, poloxamer was added, and the preparation was kept in a refrigerator until poloxamer completely dissolved.

2.2.3. Determination of effect of different polymer concentration on viscosity

The viscosity of different concentration of poloxamer (17, 18, and 19% w/w) and chitosan (1.0, 1.5, and 2.0% w/w) was measured using a viscometer (SV-10 Vibro Viscometer, Company Limited, Japan) at room temperature of 22 ± 0.5 °C. Before using the viscometer, calibration was done with purified water according to the Japan Calibration Service System (JCSS). All measurements were repeated three times.

2.2.4. Determination of gelling temperature

The gelling temperature of the prepared in-situ gel formulations was determined using a Brookfield viscometer (RVDV-II+; Brookfield, Engineering Laboratories Inc., Stoughton, MA, USA) with small sample adaptor equipped with a water jacket through which water from a Brookfield temperature controller water bath (TC-202) could be circulated. Fifteen mL from each formulation was gradually heated. The gelling temperature was determined from the sharp inflection point in the viscosity versus temperature curve which results from the sudden increase of viscosity accompanying gel formation (Lin et al., 2014).

2.2.5. Assay of drug content

Directly after gel preparation, half gram of formulation was dissolved in 50 mL distilled water in a volumetric flask. Then, 1 mL of dissolved formulation was transferred to 25 mL volumetric flask and further diluted with distilled water. The drug concentration was measured using the developed ultra high-performance liquid chromatography (UHPLC®) method.

2.2.6. In-vitro drug release

Two grams of each formulation was placed on grooved circular disc (5 cm diameter). Each disc was placed in the bottom of dissolution apparatus vessels (USP-II; Caleva, England). In each vessel, 400 mL of the simulated urine fluid (SUF, composed of NaCl 13.75, $MgSo_4$ 1.69, $MgCl_2$ 0.83, $CaCl_2$ 0.67, KCl 0.38, and urea 17.40 gm/L, pH 7.50) (Stolarz et al., 2005) was used as the dissolution medium and maintained at 37 °C. A two mL sample was with-