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RESEARCH ARTICLE

Evaluation of TRI-726 as a drug delivery matrix

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Abstract
Background: The TRI-726 polymeric drug delivery matrix is a newly-developed biocompatible hydrogel exhibiting *in situ* reverse-thermal gelling, mucoadhesivity, and sustained-erosion properties.

Methods: Using two model drugs, clindamycin hydrochloride and acetaminophen, we determined the gelling temperatures, *in vitro* release profiles, kinetics of matrix erosion, rheological properties, mucoadhesive strength, microbiological activity of released clindamycin, and biocompatibility when in contact with cells.

Results: It was demonstrated that none of the excipients contained in the TRI-726 polymer matrix caused any loss in clindamycin's antimicrobial activity following incorporation into the polymer matrix. Thus, the new patent pending TRI-726 drug delivery matrix was both inert and non-reactive toward the incorporated clindamycin in terms of chemical degradation (<10% degradation under accelerated conditions over 6 months) and antimicrobial activity.

Conclusions: This new drug delivery matrix is capable of releasing a wide variety of water-soluble drug compounds over an approximate 10-day period, due primarily to protracted dissolution/erosion of the three-dimensional polymer matrix in an aqueous-based biophase. Additionally, TRI-726 exhibits excellent mucoadhesive properties that would allow a candidate drug/TRI-726 formulation to adhere and remain at a potential application site for an extended period of time. Lastly, the biocompatibility tests affirmed the non-toxic and biocompatible nature of TRI-726 when in contact with cells, which suggests its suitability and versatility as a drug delivery matrix for the targeted administration of a wide range of pharmaceutical compounds where *in situ* gelation, protracted release of the active, and mucoadhesion of the formulation are desired.

Keywords: Hydrogel, reverse-thermal gel, mucoadhesive, biocompatible, sustained-release

Introduction

Prevention and treatment of conditions, especially in poorly-vascularized sites like bones and hard-to-reach areas, such as muscles, remain difficult with conventional systemic therapy. Research has shown that drug delivery is one of the key factors for optimizing therapy in such conditions, and the ideal matrix for local delivery should show controlled release, biocompatibility, and good adhesion[1,2]. New delivery systems for local administration are thus essential and have been an area of constant research. Various types of drug delivery devices, such as osmotic pumps, transdermal patches, hydrogels, fast disintegrating tablets, and liposomes, have been developed for achieving ease of use, better tolerability and therapy compliance. Currently, a plethora of technologies and platforms exist, with the list being exhaustive for delivery essentially through any route—oral, nasal, rectal, vaginal, transdermal, and ocular[3].

TRI-726, introduced by TriLogic Pharma, is one such innovative hydrogel-based technology for local administration. The matrix, TRI-726, focuses on three logics or features; namely (a) *in situ* gelation, (b) sustained-erosion/release, and (c) adhesion and bioresorption. The system is designed to take advantage of body temperature to undergo sol-to-gel transition and can be utilized in many different states, for example, as a liquid (viscous or dilute), a semi-solid (gel/paste), a spray, or as a foam, to suit the various therapeutic requirements. In addition, a number of different drugs can be incorporated to treat various conditions. TRI-726 meets the

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biocompatibility testing requirements of ISO/USP and a Patent Cooperation Treaty patent application covering the formulation has been published[4]. In brief, tri-block copolymers, such as poloxamers, have been extensively studied and used in drug delivery to various parts of the body due to their gelling behavior. Despite all of its interesting and useful physical properties, the biggest drawback of the poloxamer gelled matrix, fast erosion, has not been suitably addressed in past years. Rapid matrix erosion leads to reduced retention of the matrix and the amount of drug that is ultimately absorbed into the body. This may lead to pre-mature termination of drug delivery, resulting in sub-optimal pharmacotherapy. The invention describes poloxamer formulations with a reduced rate of matrix erosion so as to obtain longer retention in the body. This is made possible by the incorporation of commonly used pharmaceutical excipients. TRI-726 consists of a combination of tri-block copolymers and a natural polysaccharide. Its composition and development are described in detail elsewhere[4].

The objective of this study was to evaluate, *in vitro*, the matrix, TRI-726, for its three features—*in situ* gelation, sustained-erosion/release, and adhesion properties. Since the drug delivery system TRI-726 is new and novel, the data from these tests helped in determining the applicability and robustness of the matrix. Clindamycin hydrochloride (CLD HCl) was chosen as a model drug for evaluation purposes.

Materials and methods

Materials

CLD HCl, citric acid, sodium citrate, mono- and di-basic potassium phosphate, acetonitrile, sodium hydroxide, and hydrochloric acid were purchased from Spectrum Chemicals. Acetaminophen was purchased from Sigma-Aldrich (St. Louis, MO). The poloxamers (F127, F108 and F68) and xanthan gum were procured from BASF and CP Kelco, respectively. All excipients were used as received without further purification.

Methods

Preparation of formulations

The composition and preparation of the formulation(s) have been described elsewhere[4]. CLD HCl was chosen as a model drug for evaluation purposes only. Briefly, all ingredients, except the poloxamers, were dissolved in deionized water. Poloxamers were then added to this solution by the "cold method" of incorporation[5]. The pH of the final formulation was adjusted to 5.5–6 using either HCl or NaOH.

Measurement of the gelling temperature

Approximately 2 mL of the formulation was added to a disposable glass tube (diameter = 12 mm, length = 75 mm) and placed in a temperature controlled water bath. The temperature of the bath was gradually increased until the formulation gelled. The formulation was equilibrated at a particular temperature for at least 20 min before determining the gelation temperature of the formulation. If the formulation did not flow when the tube was inverted, then it was considered to have gelled and the temperature at which this behavior was noted was deemed as the gelling temperature ($T_{sol \rightarrow gel}$).

In vitro drug release

The *in vitro* drug release kinetics of the formulations were studied in a high-performance liquid chromatography (HPLC) autosampler vial (1.5 mL, 12 × 32 mm) using a membraneless system. Approximately 1 mL (~1 g) of the formulation was allowed to gel and equilibrate at 37°C for 15 min. At the end of the 15-min period, 0.5 mL of pre-equilibrated phosphate-buffered saline (PBS (pH = 7.4)) was added on top of the gel as the receptor phase. At pre-determined time points, the entire portion of the receptor phase was sampled and replaced with fresh pre-equilibrated (37°C) PBS. The concentration of CLD HCl in the receptor phase was determined by the HPLC (Shimadzu LC-2010A) method described in the USP monograph for CLD HCl[6].

Briefly, 6.8 g of monobasic potassium phosphate was dissolved in 1 L of deionized water and adjusted to a pH of 7.5 with 8 N potassium hydroxide. The buffer was filtered and degassed. The mobile phase consisted of this buffer and acetonitrile in a ratio of 55:45. A C18 column (250 × 4.6 mm; particle size 5 µm) was used to detect CLD HCl at 210 nm at room temperature. The flow rate was 1 mL/min.

Matrix erosion

The rate of matrix erosion was determined simultaneously with the *in vitro* release study. As mentioned above, the entire receptor phase (0.5 mL) was replaced at each time point during the release study. As the matrix eroded/dissolved with time, the volume of receptor phase removed gradually increased to slightly >0.5 mL. The weight of eroded/dissolved matrix at any time point was determined by subtracting the weight of the added receptor phase from the weight of the collected receptor phase. It was assumed that the densities of the receptor phase (PBS) and the formulation were each ~1 g/cc for this determination. On further testing, the densities of the formulations were found to be in close agreement to 1 g/cc.

Rheological measurements

Viscosity studies were carried out on a Brookfield viscometer using a Helipath stand with a #96 spindle at a speed of 1 rpm and performed as a function of temperature. The temperature of the formulation was controlled with a circulating water bath and the viscosity of the formulation was measured at 25, 30, and 40°C by taking an average of several viscosity readings over 2–3 min.

Mucoadhesivity measurements

Mucoadhesivity was assessed in terms of adhesion contact time. This experiment was only performed to rank-order the relative adhesiveness of candidate formulations. Briefly, a glass plate was coated with a micro-thin layer of 10% w/w gastric porcine mucin. Following the drying period (generally 30 min), a stainless steel weight of ~350 g was coated on one side with a test formulation. Next, 500 g of pressure was applied for 30 s to the stainless steel weight to ensure intimate contact of the weight with the mucin-coated glass plate. Lastly, the plate, with the weight, was inverted over a water bath maintained at body temperature (37°C), and the time required for detachment was recorded. For each formulation, a minimum of three measurements were made. The 350 g weight was selected for convenience, because pilot studies had demonstrated contact times that were within an 8-hour window of time when using this mass. As stated above, contact times were determined for the sole purpose of rank-ordering the mucoadhesive strength of test formulations, and no attempt was made to extrapolate an *in vitro* contact time to an estimated, or anticipated, contact time *in vivo*.

Microbiology

The Kirby Bauer disk diffusion method using *Staphylococcus aureus* was performed to evaluate the antimicrobial efficacy of the released clindamycin from the formulation. Antimicrobial efficacy was determined in terms of zone of inhibition. The bacterial strain used in the disk diffusion experiments was isolated from a bacteremic patient[7,8]. Initially, bacteria were cultured overnight in Mueller Hinton Broth. Next, agar (containing beef and yeast extract) was prepared and autoclaved. Exactly 2.5 mL of the bacteria culture was mixed thoroughly with 100 mL of agar, 10 mL of the mixture was poured into a 100 mm petri-dish, and then three sterile disks of 6 mm diameter were placed in each petri-dish in a triangular pattern. Finally, 20 µL of the solution of interest was placed onto the upper surface of a disk. Each aliquot of receptor phase was tested in triplicate and the diameter of the three zones of inhibition averaged and recorded. The petri-dishes were incubated at 37°C for 24 h before any zones of inhibition were measured with a micrometer.

Biocompatibility

The biocompatibility of the formulation was assessed by the direct contact cytotoxicity test as per the method described in ISO 10993/USP <87>[9]. Briefly, mouse areolar fibroblast (L-929) (ATCC cell line CCl 1, NCTC clone 929) cells were cultured in six-well plates at 37°C in a humidified atmosphere with 5% CO_2 using 5% FBS supplemented minimum essential medium. The seeding density was 10^5 cells/mL. Once a confluent (>80%) monolayer was obtained, 50–100 µL of the formulation was applied at the center of a well to cover an area of 100 mm^2. The cells were incubated for 24 h in the presence of the formulations with the above mentioned medium. After 24 h, the cells were washed with Dulbeco's (DPBS (pH 7.4)) and 0.01% neutral red solution in DPBS was added to each well. One hour later, the wells were visually observed to determine the reactivity of the formulations. The reactivity levels were graded from 0 to 4, indicating no reactivity to severe reactivity, respectively. Representative optical micrographs were taken at a magnification of 100×.

Results and discussion

The viscosity of a typical formulation utilizing the patent pending TRI-726 drug delivery matrix is shown in Figure 1. Figure 1 shows that, in general, the viscosity of the formulations increases with an increase in temperature and that the viscosity at body temperature (37°C) is nearly three to four times the viscosity at room temperature. A reduced viscosity at room temperature would allow easy application of the formulation in hard-to-reach areas and avoids the formulation "running out" of the application site. The $T_{sol \rightarrow gel}$ temperature of TRI-726 is 22°C, and is syringeable through a 25G needle.

The *in vitro* release profiles of CLD (two concentrations) and acetaminophen from the patent pending TRI-726 polymeric drug delivery matrix are shown in Figure 2. Figure 2A shows that the duration of release is more than 9 days and the overall shape of the profile for 1 and 2% CLD are similar. On day 8, after collecting the receptor phase, the vials were weighed. The difference in weight between day 8 and day 0, which gives a more accurate estimation of the extent of matrix erosion, demonstrated that nearly 70% of the matrix eroded/dissolved after 8 days.

The actual amount of the CLD HCl that was released from the TRI-726 polymeric matrix at different time points is shown in Figure 2B. Figure 2B shows that the amount of released CLD HCl increases with time and reaches a

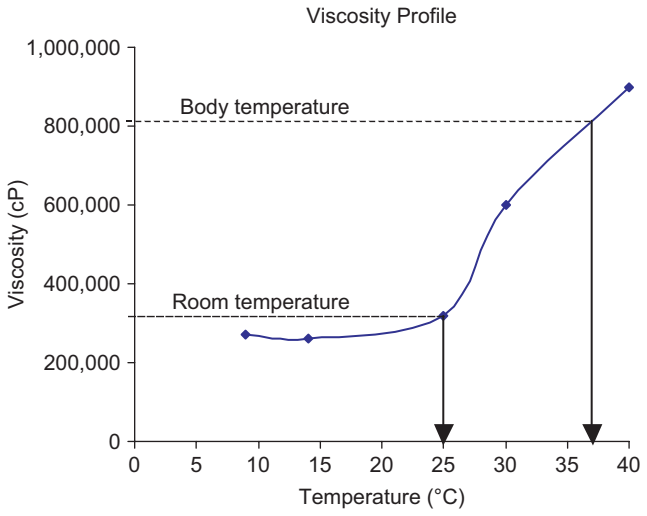

Figure 1. Viscosity profile of a typical formulation utilizing the TRI-726 matrix.

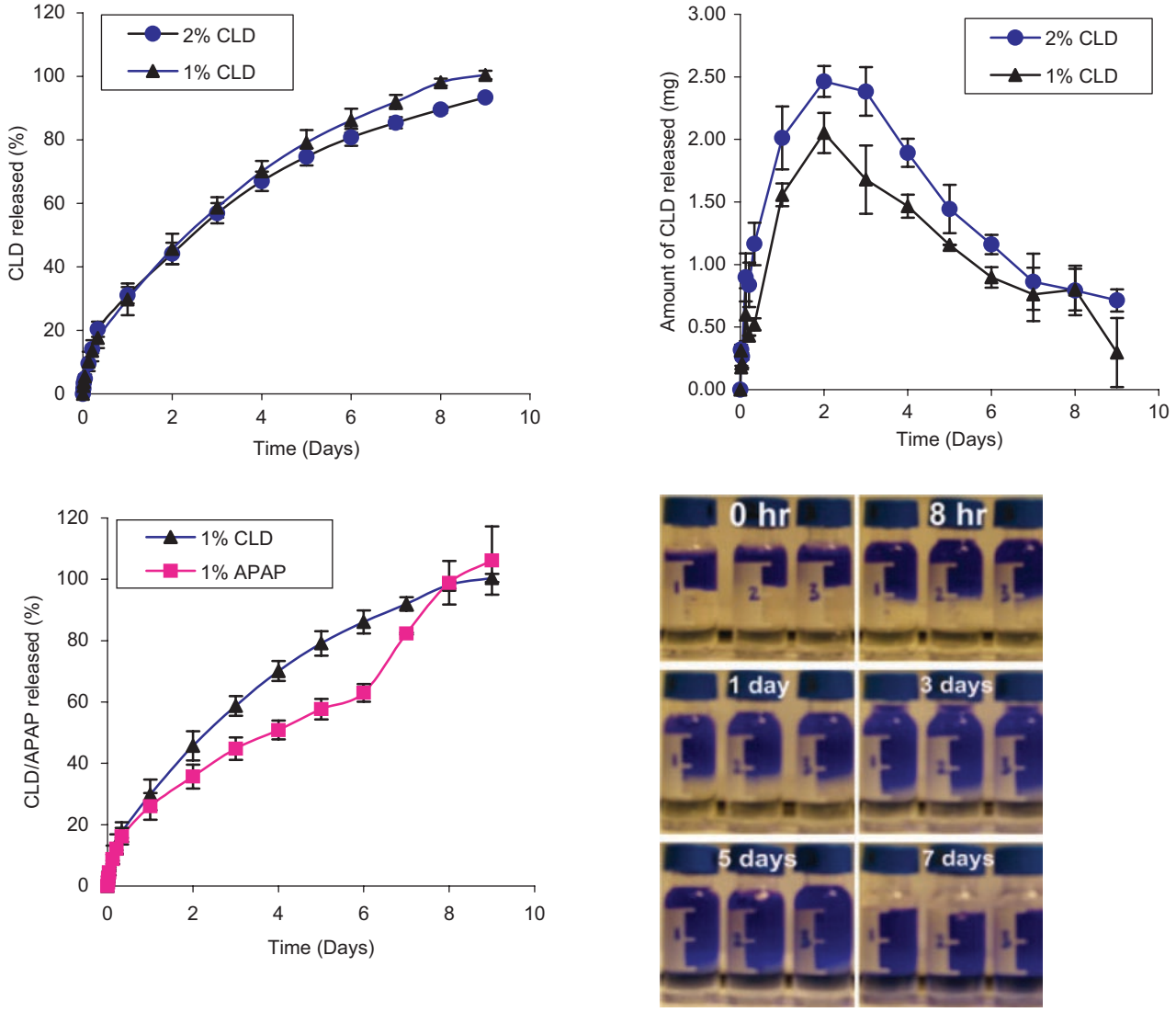

Figure 2. *In vitro* drug (CLD and acetaminophen (APAP)) release profiles of the formulations. (A) Cumulative percent CLD released from TRI-726; (B) actual amount of CLD released from TRI-726 at various time points during the *in vitro* release study; (C) actual percent of CLD and APAP released from TRI-726; and (D) diffusion of a model compound, crystal violet, from the receptor phase into TRI-726 as a function of time.

maximum at days 2 and 3, and then decreases gradually with time. Within the first 2 days, a total of nearly 44% of the drug was released, and by day 3, a total of 57% of the incorporated drug had been released. As the matrix became depleted of drug, both the drug concentration within the gel matrix, as well as the concentration gradient, were reduced, which negatively affected the diffusion of the drug from the gel matrix. For this reason, after reaching a maximum at days 2 and 3, the amount of CLD HCl released from TRI-726 decreased with time.

The release profiles of the formulations containing acetaminophen are compared with those containing CLD HCl in Figure 2C. Figure 2C shows that the *in vitro* release profile of the formulation containing acetaminophen is nearly identical to that containing CLD HCl. This indicates that the release of the active does not depend, to a large extent, on the physicochemical properties of the incorporated active. The slight difference between the particular set of *in vitro* release profiles is most likely due to the difference in aqueous solubility of acetaminophen and CLD HCl and their contribution to gel strength. It should be noted that lipophilicity of the loaded drug may affect its release from the poloxamer gel matrix *in vitro*. Poloxamer gels are considered to consist of micelles and aqueous channels. The loaded drug is partitioned between the micelles and the aqueous channels. Initially, the drug in the aqueous channels is released from the three-dimensional gel matrix. Once the drug in the aqueous channels is released, more drug partitions out of the micelles to the aqueous channels to maintain equilibrium[10,11]. Additionally, the loaded drug may affect gel strength, alter the gelling temperature, and control the rate and extent of drug release *in vitro*. However, in the case of TRI-726, the category of drug incorporated into TRI-726 does not affect its *in vitro* release as significantly as the viscosity of the overall formulation.

How drug molecules diffuse into the gel matrix is shown in Figure 2D. Diffusion was monitored by adding crystal violet to the receptor phase, as it gives a blue color to the gel it diffuses into. At the same time intervals mentioned earlier, the receptor phase was replaced, and representative photographs were obtained. Figure 2D shows that the crystal violet molecules diffuse slowly into the gel matrix. It can be noted that the total volume increases with time and reaches a maximum at day 3, and then gradually decreases over time. This is because the matrix absorbs the receptor phase and swells. Matrix swelling assists with dissolution of the matrix. It can also be noted that even after day 7, the entire matrix has not been invaded by the receptor phase. In other words, crystal violet molecules were not able to reach the other end of the gel matrix. The high viscosity associated with the TRI-726 polymeric gel matrix prevents diffusion of the drug molecules and the ingress of receptor phase. The slow ingress of receptor phase prevents matrix erosion and, thereby, extends the duration of release of an active drug molecule.

TRI-726 can be considered an inert and stable polymer gel matrix. Stability studies of TRI-726 formulations with CLD HCl were conducted under International Conference on Harmonization conditions for zones I & II. The data under accelerated storage conditions (40°C/75% RH) showed that the gel retained its physical properties and the CLD HCl assay was between 90 and 110% at 1, 2, 3, and 6 months (data not shown). From this, a 24-month (2 year) expiry dating can be assigned for the polymeric TRI-726 drug delivery matrix at room temperature. Additionally, the TRI-726 formulations with CLD HCl met the pH and microbiology stability specifications during stability studies (data not shown).

The mucoadhesion experiments were conducted to determine the relative degree of mucoadhesivity, by assessing the contact time to a natural mucin. The average contact time corresponding to the TRI-726 matrices was 5.3 ± 0.5 h. The TRI-726 matrix had the longest contact time compared to matrices containing various poloxamer only combinations. Matrices that contained just one poloxamer, or combinations of two and three poloxamers, all exhibited contact times less than or equal to 2.5 ± 0.3 h (data not shown). It is important to emphasize that the recorded contact times were obtained with a 350 g weight for the sole purpose of rank-ordering the mucoadhesive strength of test formulations and the results indicate that the TRI-726 matrix with active(s) will posses excellent mucoadhesivity *in vivo* at the potential site of application for an extended period of time.

The antimicrobial efficacy of the released clindamycin was confirmed using the Kirby Bauer disk diffusion method. The result of the disk diffusion test is shown in Figure 3. The receptor phases corresponding to different time points were each diluted 100-fold. Each of the receptor phase samples produced a zone of inhibition,

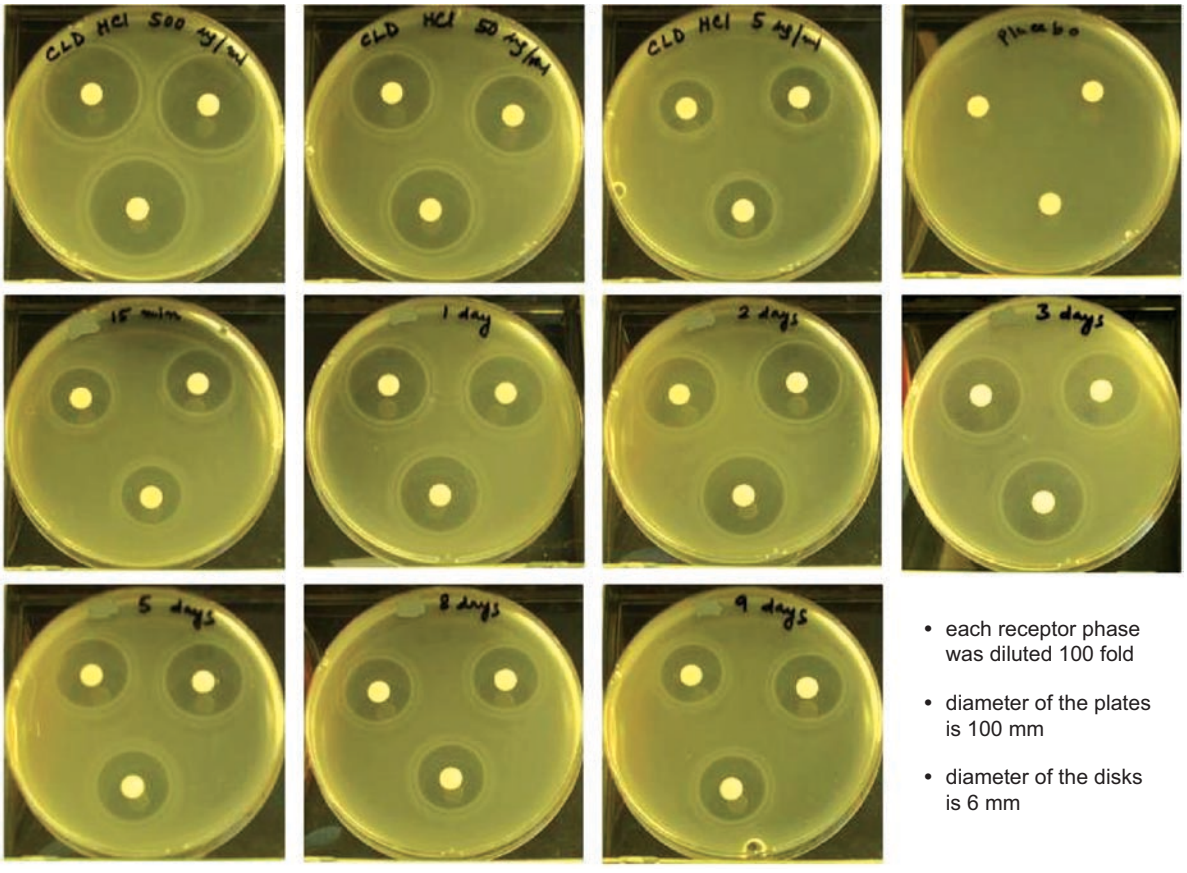

Figure 3. Photographs showing zones of inhibition for receptor phase samples containing CLD released from TRI-726 *in vitro*.

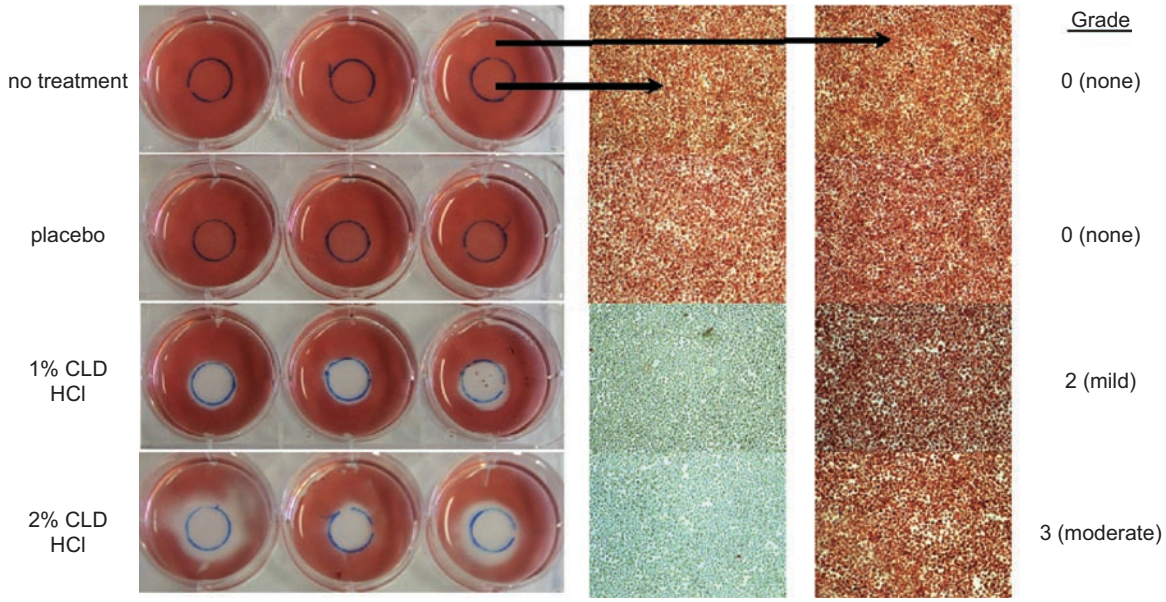

Figure 4. Results of the biocompatibility test. Photographs of the wells, as well as representative optical micrographs of the cells from different regions of the wells are shown. The grades assigned to the formulations are those according to USP <87>.

indicating that the released clindamycin retained its antimicrobial properties and none of the inactive ingredients deactivated the incorporated CLD. Moreover, the concentration of CLD HCl in each of the receptor phase samples was well above the MIC for *Staphylococcus aureus*. The MIC of CLD HCl for the strain of *Staphylococcus aureus* we used is 0.062 μg/mL[12]. Therefore, it is expected that the released clindamycin would be effective *in vivo* as well. Figure 3 shows that as the elapsed time increased from 15 min to 3 days, the size of the zone of inhibition increased, and then subsequently decreased beyond 3 days. This corroborates the observation in Figure 2D, that is, the percent of clindamycin released increases with time, reaches a maximum at days 2 and 3, and then decreases after that.

Results of the biocompatibility test are shown in Figure 4. The formulations were applied over the cells in the area traced in black ink. The cells are stained with neutral red stain, which concentrates in the lysosomes of the viable cells[13,14]. As a result, viable cells appear red, whereas, dead cells appear colorless. Representative optical micrographs are also shown next to the wells. Figure 4 shows that the cells were viable with cell control and placebo samples. This indicates each of the inactive ingredients is biocompatible and non-cytotoxic. Figure 4 also shows that as the concentration of CLD HCl increases from 1 to 2%, the viability of the cells decreases. Similar observations have been made previously, which may likely be due to the drug's ability to impair DNA synthesis[15]. According to USP classification, while placebo was non-reactive (grade 0), the formulation with 1% CLD HCl was mildly reactive (grade 2), and the formulation with 2% CLD HCl was moderately reactive (grade 3). In addition, the biocompatibility of formulations with 2% CLD HCl were also confirmed through a battery of ISO/USP biocompatibility tests (data not included).

The present investigation is certainly not the first to utilize the reverse-thermal gelling properties of poloxamers for controlled drug delivery. However, the particular composition of TRI-726 imparts some extremely useful properties to this drug delivery matrix. Although poloxamers have long been used for topical ocular delivery of a variety of drug compounds[16–18], the present work was focused on potential applications for mucosal drug delivery. The gastrointestinal, vaginal, intra-oral, and rectal membranes represent mucosal absorption sites that would benefit from a mucoadhesive, controlled-release dosage form. Recently, Lo et al., formulated matrix microspheres for the oral administration of azithromycin[19]. The poloxamer-containing microspheres exhibited a 3 h, first-order release pattern and provided effective taste-masking and good tolerability with regard to gastrointestinal side effects[19]. An example of how the inclusion of water-soluble and water-insoluble polymers in poloxamer matrices are able to modulate drug release was recently demonstrated by Gonzalez and Ghaly[20]. These authors formulated theophylline tablets with varying concentrations of poloxamer and either hypromellose or carbomer. By utilizing these polymers, the authors were able to demonstrate controlled release of the theophylline for over 24 h using a standard tablet dissolution test[20]. The inclusion of carbomer to the theophylline-containing, poloxamer-based tablets not only contributed to the controlled release of the active, but potentially also imparted a slight degree of mucoadhesivity. Lastly, so as to increase the mucoadhesivity and, therefore, retention in the rectum, Park et al., used a combination of poloxamer 407 and polycarbophil to form a matrix that prolonged the release of phenylephrine hydrochloride,

lidocaine hydrochloride, and prednisolone acetate following rectal administration[21]. These authors also used zinc oxide as an astringent, as well as a mucoadhesive-enhancing agent. Inclusion of zinc oxide also caused the formulation to become more syringeable[21].

Conclusions

TRI-726 matrix is a biocompatible hydrogel exhibiting *in situ* reverse-thermal gelling properties, mucoadhesivity, and sustained-erosion properties. The *in vitro* studies indicated that the matrix shows a nearly three- to four-fold increase in its temperature-dependent viscosity at body temperature, possesses sufficient mucoadhesive properties that would allow a candidate drug/TRI-726 formulation to adhere and remain at a potential application site, and slowly erodes over a period of 7–10 days. The stability study of the CLD HCl incorporated into the patent pending TRI-726 drug delivery matrix indicated that the TRI-726 polymer matrix was both inert and non-reactive. The biocompatibility tests affirmed the non-toxic and biocompatible nature of TRI-726, which suggests its suitability and versatility as a drug delivery matrix for the delivery of a wide variety of pharmaceutical compounds where targeted and protracted release of the active is a requisite.

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Declaration of interest

The corresponding author (T.P.J.) acts in the capacity of a consultant to Trilogic Pharma, LLC. Furthermore, the work presented herein was financed by Trilogic Pharma, LLC with a contract to the Johnston Laboratory. Dr. P.M. is a post-doctoral fellow in the Johnston laboratory and is paid from funding supplied by Trilogic Phama, LLC to the Johnston Laboratory. Dr. H.A. is the Vice-President and Chief Scientific Officer of Trilogic Pharma, LLC.

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