DEVELOPMENT AND EVALUATION OF A POLOXAMER- AND CHITOSAN-BASED IN SITU GEL-FORMING INJECTABLE DEPOT

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ABSTRACT

Objective: The present study is intended to investigate the applicability of poloxamer- and chitosan-based temperature induced in situ injectable gelling depot for once for a week therapy as an intramuscular injection employing olanzapine as a model drug.

Methods: The thermosetting gel was prepared by admixture of a solution of poloxamer P127 and a solution of olanzapine and chitosan in aqueous acetic acid. The resultant formulation was characterized for gelation temperature, gelation time, viscosity, syringeability, pH, drug content, and in vitro drug release. The in vitro release of olanzapine from the gelled depot was followed using USP paddle type II apparatus in conjunction with a dialysis bag. The gel was injected ex vivo into chicken muscle and observed by subsequent dissection.

Results: The formulation was designed to have a phase transition temperature of 34°C and gelled in <10 s at 37°C. Addition of chitosan imparted favorable rheological properties to the poloxamer gel and resulted in a pseudoplastic mixture with low viscosity in the sol state and higher viscosity post gelation. The preparation had a pH of 5.4, appropriate drug content and readily passed through a 20 gauge needle. The release of olanzapine was unhindered by the dialysis bag. Following an initial burst, a sustained, zero-order release of the remainder of drug was observed up to 9 days. The injectable was found to form a compact depot when evaluated ex vivo.

Conclusion: The developed system showed several features which make it a suitable vehicle for sustained intramuscular delivery of drugs.

Keywords: Poloxamer, Chitosan, In situ injectable gels, Olanzapine.

INTRODUCTION

The "in situ gel" system is emerging as a promising drug delivery option for parenteral depot formulations due to its special characteristic feature of "sol to gel" transition which helps it to sustain and control the release of an incorporated active over a period of time. The preparation exists in liquid state before administration and post-injection, once in the physiological milieu, converts to a near solid implant. The sol to gel transformation may be brought about by any of several triggers such as change in temperature or pH, solvent exchange, ultraviolet (UV) radiation, and presence of specific molecules or ions. Different classes of natural, semi-synthetic, and synthetic polymers have been used to design in situ gelling systems including pectin, gellan gum, chitosan, alginic acid, guar gum, Carbopol, xylodac, xanthan gum, hydroxypropyl methylcellulose, poloxamers, and biodegradable polyesters such as poly (lactide-co-glycolide) (PLGA) and copolymers of PLGA with polyethylene glycol.

Of all the triggers employed to bring about in situ gelation, the temperature-based gelling systems present a convenient option. The triblock poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) copolymer PEO-PPO-PEO (poloxamers) have shown gelation at body temperature when highly concentrated polymer solution >15% w/w is employed [1]. A number of in situ gelling formulations have been reported based on poloxamers alone [2] or in combination with other hydrophilic materials such as Carbopol 934P, hydroxypropyl methylcellulose, and sodium carboxymethyl cellulose [3]. These are formulated for drug administration through the ocular, transdermal, buccal, rectal and vaginal routes, and also as vehicles for injectable depots for intramuscular injection. An early study on the toxicological evaluation of poloxamer vehicles after intramuscular injection in rabbits indicated that muscle toxicity was proportional to the lipophilicity of the poloxamer. Poloxamer 407 (tested as a 25% w/w solution) showed gross lesions and elevations in creatinine phosphokinase comparable to normal saline and was considered an acceptable intramuscular vehicle [4].

The reported duration of drug release from different poloxamer based formulations varies from few hours to about 4 days. However, in the case of the design of intramuscular depots, a longer duration of release is suitable justifying the injection route. Combining the temperature-based gelling properties of poloxamers with other biocompatible polymers to enable sustained release of the incorporated active for a sufficiently long duration, say, a week, is a feasible strategy provided the gelation characteristics of poloxamer are not significantly interfered with. Chitosan has excellent properties such as hydrophilicity, biocompatibility, biodegradability, antibacterial activity, and ability to sustain release which favored its choice [1]. Further, few studies have demonstrated the use of chitosan in combination with poloxamer to increase the retention time of drugs and to provide a mechanically stronger gel [5]. Furthermore, being insoluble at physiological pH, it may be expected to resist erosion, and also retard the erosion of poloxamer. A study on designing a thermosensitive chitosan/poloxamer system for ocular delivery of ciprofloxacin [6] showed that the addition of chitosan, with its amino groups increased the number of hydrogen bonds holding the gel together and hence, the mechanical strength and retention time of the gel [7].

For evaluation of the proposed poloxamer – chitosan in situ gelling injectable, olanzapine was chosen as a model drug. Olanzapine is an antipsychotic agent used in the treatment of psychiatric conditions such as schizophrenia [8]. Long-acting injectables of antipsychotics offer number of advantages compared with oral medication, including not having to remember to take drugs daily, reducing the risk of
unintentional or deliberate overdose, transparency of adherence, and superior control over the disease state. Further, when patients stop the medication, plasma levels decrease more slowly than with oral formulations, giving time for the health-care professionals to intervene at an early stage. Olanzapine is available as a long-acting suspension based on its palmitate salt. It is sparingly soluble in water and aqueous buffers. However, being a weak base with pKa values of 5.0 and 7.4 [9], it is soluble in acidic media, making the chitosan dissolved in dilute acetic acid an ideal reservoir for the drug. Due to the rationale for long-term therapy and the acid solubility, olanzapine was considered suitable as a model drug for investigation of the poloxamer – chitosan thermoreversible in situ gelling depot for intramuscular once a week therapy.

METHODS

Olanzapine was a gift sample from Zhejiang Langhua Pharmaceutical Co., Ltd., China, Poloxamer 407 (Kolliphor P407®) was kindly gifted by IMCD, India. Chitosan (Low molecular weight, degree of deacetylation minimum 90%) was obtained from SRL, Pvt. Ltd., India, and glacial acetic acid was purchased from Loba Chem, India. Dialysis tubing (12000–14000 Dcutof) was purchased from HiMedia, India. All other reagents were of analytical grade.

Preparation of the in situ gelling depot

Each dose of the in situ gel was prepared as follows. Initially, the poloxamer 407 gel was formed using the cold method [2]. The polymeric gelling agent was weighed (2.3 g) and placed in a flat-bottomed vial carefully tared for 10 ml at 20°C. About 0.6 ml of water was added, and the vial was placed at 4°C overnight until the poloxamer was completely dissolved, and a clear solution was obtained. The solution was then maintained at 20°C and the volume was made up to the 10 ml mark (23% w/v solution). Separately, 75 mg olanzapine was weighed using an analytical balance and transferred to a different vial and was dissolved by adding 0.8 ml of a 0.5% w/v chitosan solution prepared in 4% glacial acetic acid. This drug solution was added to 3.8 ml of the cold poloxamer 407 solution prepared earlier and mixed well to yield the in situ gelling olanzapine depot. For determination of gelation characteristics and for the viscosity studies, blank gel was prepared in an identical manner, except that the addition of olanzapine was omitted.

Evaluation of the in situ gelling depot

Measurement of the gelling temperature and time to gelation

Glass vials containing 5 ml each of blank poloxamer sol, poloxamer chitosan sol with and without the drug, and a mixture of poloxamer solution with equivalent volume of acetic acid solution was placed in a cold water bath. A thermometer with an accuracy of ±0.5°C was immersed in each vial. The temperature of the water bath was gradually increased at a constant rate, and the vial was tilted and examined for gel formation after every half-degree rise in temperature. The temperature at which the mixture stopped flowing was regarded as gelling temperature. Once the gelling temperature was determined, the formulation at 20°C was again placed into the bath maintained at the gelling temperature, and the time taken for it to gel was regarded as gelling time. The result was the mean of triplicate measurements.

Syringeability

The in situ gelling injectable formulation (4.5 ml) cooled to 20°C was withdrawn into disposable syringe sequentially fitted with each of the following: 18, 19, 20, 21-, and 22-gauge needles and was then passed through. The needle of minimum size through which the formulations could be readily withdrawn and extruded was selected as the one showing adequate syringeability for the formulation.

pH

The pH of the formulation was determined at 25°C using a standardized pH meter by immersion of the probe into the sol.

Viscosity

A Brookfield Dial Viscometer (DV-III+Pro) was used to measure the viscosity of the preparation. Drug-free chitosan – poloxamer formulation was used for the measurements. In addition, the corresponding poloxamer solution was also subjected to viscosity measurements. The viscometer was initially standardized using viscosity standard fluid (mineral oil) and then each test sample was subjected to measurement of viscosity. The spindle 62 was used for the study. Preparations were held in 100 ml beakers placed in water bath and care was taken to center the spindle. Measurements for each preparation were made from 17°C upward. The temperature probe of the viscometer was used to monitor the temperature of the preparation. At each temperature, measurements were carried out both at 60 rpm and at 100 rpm movement of the spindle.

Drug content

The formulation was held in the sol state at 20°C, and 0.5 ml of formulation equivalent to 8.33 mg of drug was pipetted into a 100 ml volumetric flask, and 10% v/v HCl was added. The mixture was shaken well and the volume was made up to 100 ml using 10% v/v HCl. The resultant solution was suitably diluted, and absorbance was measured using UV spectrophotometer at 258 nm and percentage drug content determined. A blank gel was also prepared and subjected to the same procedure to rule out any interference in the analysis from any formulation ingredient.

In vitro drug release studies

The USP apparatus II, i.e., paddle equipment in conjunction with a dialysis bag was used to follow the drug release from the gelled depot. The dialysis bag was allowed to soak overnight in the release medium consisting of pH 7.4 phosphate buffer containing 0.25% Tween 80. Formulation (4.5 ml) was introduced into the dialysis bag with one end sealed, and the bag was placed in an incubator at 37°C. To this gel, 1 ml of release medium was added as inner medium, and then the other end of the dialysis bag was also sealed tightly using clips taking due care to ensure that there were no air pockets inside the bag. A thread was tied tightly around the clips to ensure the absence of any leakage from/into the dialysis bag. The dialysis bag was now promptly placed into 500 ml of the release medium (outer medium), in a dissolution flask pre-warmed to and maintained at 37°C. The release medium was stirred at 50 rpm. At predesignated time intervals, 10 ml sample was withdrawn, and the same amount replaced with pre-warmed release medium. The samples were suitably diluted with 10% v/v HCl solution and absorbances were measured using UV-visible spectrophotometer at 258 nm to estimate the amount of drug released. To adjudge the barrier posed by the dialysis bag, the procedure was also carried out with a solution of 75 mg olanzapine in 4.5 ml of a 1% solution of acetic acid.

Ex vivo studies

A piece of chicken muscle obtained from a local meat vendor was used in this study. Drug-free injectable formulation was prepared and to improve the contrast from the surrounding tissue, a solution of crystal violet dye was incorporated into the sol. The intensely colored sol was then injected into the chicken muscle, which was maintained at 37°C by immersion in a water bath. The tissue was allowed to rest for 10 min and was then dissected open to enable visualization of the formed depot, and a photograph was obtained.

RESULTS AND DISCUSSION

Preparation of the in situ gelling depot

Poloxamer was used in the formulation for providing the temperature sensitive gelling system. In addition, chitosan was also included using a dual rationale: to serve as a vehicle component which could hold the drug and also sustain its release from the gel.

Sol-gel transition temperature is a parameter key to the success of a thermosensitive formulation. At this temperature, the rheological
properties of the system abruptly change resulting in a shift from a liquid-like state to a solid-like state. It is critical to control sol-gel transition temperature for formulations based on the intended application. For injectable drug delivery, the sol-gel transition points for in situ gelling formulations should be close to body temperature, 33–37°C [10].

For the present work, it was rationalized that a gelling temperature slightly lower than body temperature, i.e., about 33–34°C would be an appropriate feature. First, gelling in vivo at 37°C would be a quick phenomenon which may aid in bringing about a rapid consolidation of the sol, minimizing the burst release of the active. Second, poloxamer gels are reported to exhibit an increase in viscosity above the gelling temperature [2]. Hence, material which gels at say 33, would rest as a gel of greater viscosity at body temperature, thereby increasing the applicability as a sustained release depot. Finally, the injection would be a low viscous sol at a temperature of 20–25°C, which would make it amenable to easy injection.

Preliminary studies revealed that titration of all formulation variables including strength of chitosan solution, strength of acetic acid solution used to dissolve chitosan, and the ratio of poloxamer to chitosan solutions was necessary to yield a formulation with desirable features such as complete drug dissolution into the formulation, gelling temperature of about 33–35°C, volume as low as possible and restricted to an upper limit of 5 ml and pH in biocompatible range. Such a balance was attained when 75 mg olanzapine was dissolved in 0.8 ml of a 0.5% w/v solution of chitosan in 4% acetic acid, and the resultant solution was mixed with 3.7 ml of a 23% w/v aqueous solution of poloxamer.

Evaluation of the poloxamer- and chitosan-based olanzapine gel Gelling temperature and time

The gelling temperature of the selected formulation was found to be 34°C. This allows for the convenient injection of the preparation when used at 25°C. At the same time, the formulation promptly gelled at body temperature. The time taken by the preparation in the vial to stop flowing when held at 34°C was <8 s, indicating rapid gelation.

The gelling temperature of poloxamer 407, when used alone at a concentration of 23% w/v was found to be about 24°C. Accounting for dilution due to the addition of the chitosan solution, the concentration of poloxamer in the final product was about 18.9% w/v, which is also found to have a gelation temperature of about 27–29°C. Thus, there was a significant upregulation of the gelation temperature of the poloxamer solution on admixture with the chitosan solution.

Excipients in the formulation are reported to influence the sol-gel transition temperature of poloxamers. For example, HCl, propylene glycol, and ethanol are reported to increase the sol-gel transition temperature, whereas sodium chloride, NaHPO₄, and sodium alginate decrease the sol-gel transition temperature [11]. These substances show their action by influencing the micellization of poloxamer and alter dehydration of the polyoxypropylene core [10]. In the present studies, although the inclusion of drugs did not alter the gelation temperature of poloxamer, the addition of chitosan solution led to the introduction of two additives: Chitosan itself and acetic acid which resulted in a significant increase in the gelling temperature. Hence, a relatively more concentrated poloxamer 407 solution (23% w/v) with a low gelation temperature of 24°C was used in the preparation of the gels which yielded a greater scope for upregulation of the gelation temperature even after undergoing dilution.

Poloxamer 407® is an amphiphilic synthetic copolymer consisting of a hydrophobic poly(oxypropylene) (PPO) block between two hydrophilic poly(oxyethylene) (POE) blocks and has a molecular weight of approximately 12,600 Da (70% POE). At lower concentrations, the molecules are hydrated and form unimolecular micelles. However, as the PEO-PPO block copolymer concentration in solution reaches a certain critical micellization concentration (cmc) at a fixed temperature, multimolecular micelles begin to form. Conversely, in solutions containing sufficient amounts of poloxamer, increasing the temperature to the critical micellization temperature (cmt) at a fixed block copolymer concentration also induces micellization. The moderate increase in temperature results in a reduced solubility of the PEO, and especially the PPO blocks in water on heating. This is because the hydrophilic chains of the copolymer become desolvated as a result of the breakage of the hydrogen bonds that had been established between the solvent and these chains. This phenomenon favors hydrophobic interaction among the polyoxypropylene domains. With increasing temperature, micellization becomes more important, and at a certain point, the micelles come into contact and can no longer move. Experimental evidence using ultrasonic and light-scattering measurements, nuclear magnetic resonance, rheology, and fluorescence has all been reported and has also shown the micellar arrangement of block copolymers. In addition, the formation of highly ordered structures, such as a cubic crystalline phase, has been proposed as the driving force for gel formation. Thus, gelation in poloxamers is an endothermic process [12].

Acetic acid is known to hydrogen bond with itself (forms dimers) and also with other molecules such as water and acetone [13]. It is possible that, in addition to water, if acetic acid would also hydrogen bond with poloxamer, establishing the crosslinked and desolvated micellar state would require an increase in energy and therefore would be attained only at a higher temperature. This could be the reason for an increased gelling temperature seen in the poloxamer-chitosan mixtures.

And finally, the conclusion that it was the acetic acid rather than chitosan, which was responsible for the increase in gelation temperature, was also confirmed experimentally. A mixture of 3.7 ml of a 23% w/v poloxamer solution with 0.8 ml of a 4% solution of acetic acid was also found to gel at 34°C, even in the absence of chitosan.

The gelation behavior, including gelation temperature and time, was found to be unaltered by olanzapine.

pH

The pH of the final drug-containing formulation was found to be 5.4. Acidic solutions are associated with pain during and after intramuscular injections. However, a pH of 5 was found to be well tolerated by muscle tissue [14]. Furthermore, in the present case, the solution was unbuffered which is an added advantage, since a buffered preparation would be more resistant to a re-establishment of the pH at the site of injection back to the physiological level.

Syringeability

As seen from the subjective evaluation of the ease of syringeability is represented in Table 1, the formulation could be easily withdrawn and extruded through the 20-gauge needle. Hence, syringeability of the formulation was considered to be good when a 20-gauge needle is used.

Viscosity

Poloxamer solutions containing low concentrations of the surfactant (<15% w/v) are reported to be Newtonian systems, and these fail to gel in a temperature-dependent manner [15]. In case of poloxamer solutions at higher concentrations, the rheological properties are altered in a temperature and concentration dependent manner. At a given temperature, the viscosity of the solution increased as a function
of concentration. As the temperature is increased, the systems exhibit pseudoplastic behavior.

In the present studies, the viscosity of the poloxamer as well as the poloxamer-chitosan solutions was monitored from a temperature of 17°C onward. At this temperature and also at 23°C, the solutions were pseudoplastic (Fig. 1). Shear thinning is an advantageous property for viscous injectable systems.

At the gelation temperature of 24°C, the 23% poloxamer solution showed an abrupt increase in viscosity. However, the viscosity of the gel could not be measured with the equipment used in the present case since due to its cohesive nature, the gel formed a vortex and created a void-like space around the spindle so that no more reliable measurements could be made.

As reported earlier [16], the addition of chitosan resulted in a drop in the viscosity of the formulation when tested at identical temperatures (Fig. 2). Studies on gelation behavior of poloxamer-chitosan mixtures have attributed this drop in viscosity to acetic acid rather than chitosan since the latter is reported to cause a negligible change in the micellization behavior of poloxamers [17,18]. Water-soluble additives, including drugs, such as lidocaine hydrochloride, have been rationalized to be located outside the micellar structure and thereby interfere with the gelation behavior and with the physico-chemical characteristics of the formulation [19]. A similar rationale may be applied to the acetic acid present as an additive in the present formulation. In addition, there is also the likelihood of a dilution effect due to admixture of the poloxamer and chitosan solutions. With an increase in temperature, however, the viscosity increased at the same rate as in the case of the poloxamer sol and reached a value of 1000 cps. The higher viscosity of the chitosan-poloxamer mixture at the time of gelation when compared to that of the poloxamer solution is noteworthy.

The presence of the acetic acid-chitosan in the formulation was thus found to impart favorable rheological properties to the formulation: a decreased viscosity at lower temperature, favoring easier flow and injectability and a higher viscosity post gelation, favoring sustained release of the drug.

Drug content
Olanzapine as controlled release olanzapine palmitate based depot is available in several strengths chosen depending on the duration of release desired, stage of treatment, and severity of the disease state being treated. The formulation intended as a maintenance dose is provided as 150 mg for 2 weeks based on which the strength of the present formulation was arrived at as 75 mg for a once a week therapy. The drug content in the formulation was found to be 96.87% w/w of the intended content of 75 mg/4.5 ml.

In vitro drug release studies
Various reports have shown drug release from poloxamer gels to range from few hours [3] to about 3–4 days [20] depending on the physicochemical properties of the drug and the presence of additional polymers and additives in the formulation.

In the present case, during the release studies, the gel was found to remain as a compact depot undiluted by the inner medium (Fig. 3a). Neither the limited release medium used within the dialysis bag nor the dialysis membrane posed a significant barrier to drug release since nearly all the drug enclosed in the dialysis bag as a solution in acetic acid was released into the medium in <24 h (Fig. 4). In contrast, the formulation, following an average burst release of about 45% of the drug within the first 24 h, showed a near zero-order release (linear regression coefficient r² = 0.9944) (Fig. 5) from day 1 to day 9 at the end
of which 99.69% of the drug was released. Furthermore, the gel was found to remain intact within the dialysis bag (Fig. 3b), and a significant amount of gel remained even after a 9-day release study (Fig. 3c).

The formulation in the present case provided a longer duration of release of olanzapine which can be attributed to the presence of chitosan, an additional rate control polymer in the system as also to the high concentration of poloxamer in the formulation. Higher concentrations of poloxamer are also found to result in gels which erode more slowly [11].

Chitosan is widely applied as a controlled release polymer in microspheres, implants, etc. A concentration-dependent sustainment of the release of ciprofloxacin from poloxamer-chitosan in situ gelling ocular formulation has been reported [6]. The diffusion of acetic acid away from the chitosan microdomains within the poloxamer gels probably resulted in the formation of a viscous depot of drug dissolved in the chitosan matrix from which the drug gradually diffuses out and then continues diffusion through the poloxamer gel before release.

The injectable formulation needs to achieve effective plasma concentration in a short time to minimize the need for bridging with oral antipsychotic [21]. The high in vitro burst release from the present formulation can serve to establish the initial plasma levels of olanzapine. Although the initial burst seen in vitro is relatively high (45%), reported studies on in vitro in vivo correlation of PLGA microsphere-based depot formulations have concluded that the in vivo burst is lower than that seen in the in vitro experiments [22].

The subsequent release as derived from the slope of the equation describing the zero-order release phase was about 5.12 mg/day. Once released into the surrounding tissue milieu, olanzapine is reported to be rapidly absorbed from intramuscular sites [23]. In vivo studies in animals have also shown the ability of poloxamer gels to release drug for up to a week. For example, effective plasma levels of the antibiotic enrofloxacin in pigs following an intramuscular injection were sustained for more than 6 days [24].

Ex vivo studies
As seen from the image (Fig. 6), the gelled injection was found to remain as a compact depot within the muscle tissue.

CONCLUSION
The chitosan-poloxamer combination offers a biocompatible option for injectable delivery of a suitable drug candidate, especially weakly basic agents soluble in the acidic chitosan solution. The developed formulation presents a novel option as a sustained release depot of olanzapine.

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AUTHORS’ CONTRIBUTIONS
The authors jointly declare that they have both contributed towards the research work. Nazia Begum has conducted the experiments, collected and treated the data under the supervision of Dr. Hema A. Nair. The preparation, corrections, and revision of this manuscript have also been carried out by both authors.

CONFLICTS OF INTEREST
The authors declare the absence of any conflicting interests regarding this work and its publication.

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REFERENCES
1. Dominguez-Delgado CL, Fuentes-Prado E, Escobar-Chávez JJ, Vidal-Romero G, Cruz IR, Díaz-Torres R. Chitosan and pluronic® F-127: Pharmaceutical applications. In: Encyclopedia of Biomedical Polymers and Polymeric Biomaterials. New York, USA: Taylor and Francis; 2016. p. 1513-35.
2. Zhang K, Shi X, Lin X, Yao C, Shen L, Feng Y. Poloxamer-based in situ hydrogels for controlled delivery of hydrophilic macromolecules after intramuscular injection in rats. Drug Deliv 2015;22:375-82.

3. Sindhu SK, Gowda DV, Vishnu Datta S. Formulation and evaluation of injectable in situ gelling matrix system for controlled drug release. Indian J Adv Chem Sci 2014;2:89-92.

4. Johnston TP, Miller SC. Toxicological evaluation of poloxamer vehicles for intramuscular use. J Parenter Sci Technol 1985;39:83-9.

5. Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, de Freitas O, Lopez RF. A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. Eur J Pharm Biopharm 2010;75:186-93.

6. Varshosaz J, Tabbakhian M, Salmani Z. Designing of a thermosensitive chitosan/poloxamer in situ gel for ocular delivery of ciprofloxacin. Open Drug Deliv J 2008;2:61-70.

7. Irimia T, Dinu-Pîrvu CE, Ghica MV, Lupuleasa D, Muntean DL, Udeanu DI, et al. Chitosan-based in situ gels for ocular delivery of therapeutics: A state-of-the-art review. Mar Drugs 2018;16:373.

8. Chokhawala K, Stevens L. Antipsychotic Medications. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2019.

9. Nielsen MK, Johansen SS. Determination of olanzapine in whole blood using simple protein precipitation and liquid chromatography-tandem mass spectrometry. J Anal Toxicol 2009;33:212-7.

10. Chandra GS. The Preparation and Characterization of Poloxamer-based Temperature-Sensitive Hydrogels for Topical Drug Delivery, Doctoral Dissertation. United States: University of Toledo; 2013.

11. Hemelryck SV, Dewulf J, Nieku H, van Heerden M, Ingelse B, Holm R, et al. In vitro evaluation of poloxamer in situ forming gels for bedaquiline fumarate salt and pharmacokinetics following intramuscular injection in rats. Int J Pharm X 2019;1:100016.

12. Escobar-Chávez JJ, López-Cervantes M, Naik A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A. Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations. J Pharm Pharm Sci 2006;9:339-58.

13. Rassing JR. Hydrogen bonding in acetic acid/acetone mixtures. J Chem Phys 1972;56:5225-8.

14. Napaporn J, Thomas M, Svetic KA, Shahrok Z, Brazeau GA. Assessment of the myotoxicity of pharmaceutical buffers using an in vitro muscle model: Effect of pH, capacity, tonicity, and buffer type. Pharm Dev Technol 2000;5:123-30.

15. Tirmakiz F, Robinson JR. Rheological, mucoadhesive and release properties of pluronic F-127 gel and pluronic F-127/polycarbophil mixed gel systems. Pharmazie 2005;60:518-23.

16. Tuğcu-Demiröz F. Development of in situ poloxamer-chitosan hydrogels for vaginal drug delivery of benzylamine hydrochloride: Textural, mucoadhesive and in vitro release properties. Marmara Pharm J 2017;21:762-70.

17. Ur-Rehman T, Tavelin S, Gröbner G. Chitosan in situ gelation for improved drug loading and retention in poloxamer 407 gels. Int J Pharm 2011;409:19-29.

18. Giuliano E, Paolino D, Fresta M, Cosco D. Mucosal applications of poloxamer 407-based hydrogels: An overview. Pharmaceutics 2018;10:159.

19. Ricci EJ, Bentley MV, Farah M, Bretas RE, Marchetti JM. Rheological characterization of poloxamer 407 lidocaine hydrochloride gels. Eur J Pharm Sci 2002;17:161-7.

20. Xuan JJ, Balakrishnan P, Oh DH, Yeo WH, Park SM, Yong CS, et al. Rheological characterization and in vivo evaluation of thermosensitive poloxamer-based hydrogel for intramuscular injection of piroxicam. Int J Pharm 2010;395:317-23.

21. Lindenmayer JP. Long-acting injectable antipsychotics: Focus on olanzapine pamoate. Neuropsychiatr Dis Treat 2010;6:261-7.

22. Andhariya JV, Jog R, Shen J, Choi S, Wang Y, Zou Y, et al. Development of level a in vitro in vivo correlations for peptide loaded PLGA microspheres. J Controll Release 2019;308:1-3.

23. Heres S, Kraemer S, Bergstrom RF, Detke HC. Pharmacokinetics of olanzapine long-acting injection: The clinical perspective. Int Clin Psychopharmacol 2014;29:309-312.

24. Yu Z, Li H, Xiong Y, Guo F, Zhang Z. Pharmacokinetic studies of an in situ forming gel system for controlled delivery of enrofloxacin in pigs. SOJ Pharm Pharm Sci 2018;5:1-5.