Research Article

# Formulation and Evaluation of Thermo reversible *in Situ* Gel of Felodipine

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**ABSTRACT**

The present study focuses on enhancement of bioavailability and sustained release of felodipine by preparation of thermo reversible in situ gel system. Felodipine is an anti-hypertensive drug, has poor bioavailability. To sustain drug release of felodipine in situ gel systems were selected. Thermo reversible in situ gels of felodipine were prepared by the cold method using different concentrations of poloxamer-407 and poloxamer-188. All the formulations were evaluated for gelation temperature, gelation time, rheological properties, syringeability, drug content, invitro drug release and ex-vivo studies. Among different formulations F7 formulation containing poloxamer-407 (20%), poloxamer-188 (5%), felodipine (35mg), DMSO (0.5ml) and Benzalkonium chloride (0.02mg) was selected due to its low viscosity, high drug content and in-vitro drug release. The invitro release of felodipine from formulation F7 showed burst release in the initial 2 hours and the release was sustained for 5days. The drug release from F7 formulation exhibited fickian diffusion mechanism.

**Keywords:** Implants, In situ gels, Thermoreversible poloxamers.

## INTRODUCTION

Oral drug delivery plays a prominent role among all the other routes of drug delivery. By oral administration some drugs may show low bioavailability due to incomplete absorption or first pass metabolism. The drugs with poor oral bioavailability are unable to reach the minimum effective concentration to exhibit therapeutic action[1,2,3]. Currently novel drug delivery systems have increasingly explored to improve therapeutic efficacy and sustained drug release properties while overcoming the problems like poor solubility and low oral bioavailability.

*In situ* injectable gels emerged as one of the best novel drug delivery system which help in sustaining and controlling the release of drugs by its special sol-gel transition. It helps in reducing the frequency of drug administration, improves patient compliance and improves drug bioavailability. Low dose of drug is required and there will be no drug accumulation and side effects. Through this system the residency time of drug will be more due to gel formation and it decreases wastage of drug.

Felodipine is a long-acting 1,4-dihydropyridine calcium channel blocker (CCB). It is used to treat mild to moderate essential hypertension. It belongs to class II under BCS and exhibit low oral bioavailability due to its poor aqueous solubility. It is practically insoluble in water, has half-life (25hrs). Felodipine is commercially available as tablets.

## MATERIALS AND METHODS

### Materials

Felodipine was a gift sample from Aizant drug research solutions Pvt. Ltd, Hyderabad, India Poloxamer 407 (P 407) and poloxamer 188 (P188) were obtained from S.D. Fine-Chem. Ltd, India.

### Preparation of Thermoreversible in situ gel of felodipine

Thermoreversible in situ gels of felodipine were prepared using various ratios of P 407, P 188 using cold method[4,5]. DMSO was used as a solvent and benzalkonium chloride as a preservative. In brief, P 188 and P 407 were dissolved in distilled water at 4°C with gentle stirring. The poloxamer solution was left overnight in a refrigerator until a clear solution was formed. The drug was dissolved in DMSO and added to the poloxamer solutions with continuous stirring and then kept overnight at 4°C. Formulation of *in situ* gel of felodipine is shown in table 1.

### Evaluation of Thermoreversible in situ gels of felodipine

#### *Appearance and Clarity*

The clarity of the formulations before and after gelling was determined to detect the presence of any foreign substances. It was done by visual examination of the formulations under light alternatively against white or black backgrounds.

#### *pH*

The pH of all the thermoreversible *in situ* gel formulations were measured using Digital pH meter.

**Table 1:** Formulation of Thermo reversible in situ gel of felodipine

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| Felodipine (mg) | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 |
| Poloxamer-407 (%) | 16 | 17 | 16 | 17 | 17 | 20 | 20 | 20 | 20 |
| Poloxamer-188 (%) | - | - | 10 | 5 | 10 | 3 | 5 | 10 | 17 |
| DMSO (0.5ml) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Benzalkonium chloride (mg) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Distilled water | 10ml | 10ml | 10ml | 10ml | 10ml | 10ml | 10ml | 10ml | 10ml |

**Gelation temperature**

Gelation temperature is the temperature at which the drug solution gets converted into a gel. It was determined by the tube inversion method. 10 ml of the formulation was transferred into a beaker and placed in a low temperature water bath. A thermometer was immersed into the sample for constant monitoring. The solution was heated with stirring at 100 rpm using a magnetic bar. The temperature at which the magnetic bar stopped moving due to gelation was reported as the gelation temperature (T gel)[6,7]. The experiment was repeated three times.

**Gelation time**

Gelation time is the time taken for injectable gels to change from a liquid state to gel state. It was done at 37±0.5℃ by the tube inversion method as mentioned above. 2 ml of the formulation was taken in a test tube and kept in a water bath maintained at 37±0.5℃. Observe the time taken for the solution to convert into a gel. The test tube was occasionally titled at 90 degrees and flow criteria of the meniscus was observed [6,7].

**Rheological evaluation**

The Viscosity of all formulations was measured using a Brookfield Synchroelectri Viscometer (LVDV-II+P). About 25ml of the sol was taken in a small volume adapter and the viscosity of liquid formulations were measured at different angular velocities of 60, 100 rpm using spindle no.64[6,7].

**Syringeability**

Syringeability is the ability by which a formulation can be drawn and dispensed out of a syringe and injectability refers to the performance of the solution during injection and includes factors such as pressure or force required for injection. These are influenced by the viscosity of the formulations before thermoconversion. Viscosity creates significant challenges in injectability since high viscosity requires a high injection force that leads to increased pressure on injection inevitably causing pain. High viscous products can also deter the completeness of the injection[8] (i.e., the percentage of dose delivered).

To assess the syringeability and injectability, the following scores were given for both these parameters:

- +++Easily passed/injected
- ++Moderate.
- + Difficult.

**Drug content**

Felodipine containing thermoreversible in situ gel (3ml) was taken in a test tube and diluted with DMSO. This solution was analysed using UV visible spectrophotometer at 363nm.

***In vitro* drug release studies**

The *in vitro* drug release studies of Felodipine formulations were performed using modified diffusion apparatus using pH 7.4 buffer as a diffusion medium. The cellophane membrane (previously soaked overnight in the buffer) was tied to one end of a specially designed glass cylinder (open at both ends) having inner diameter membrane of 3.4cm. 2ml of the formulation was placed into the glass cylinder known as donor compartment. The cylinder was suspended in a beaker (receptor compartment) containing 200ml of diffusion medium so that the membrane just touches the surface of the medium. Receptor medium was maintained at a temperature of 37±2℃ with a stirring rate of 50 rpm using magnetic stirrer. About 3 ml of sample was withdrawn at a time interval of 1 hour and replaced with an equal volume of fresh diffusion medium. The aliquots were diluted with the diffusion medium and analysed at 363nm using UV spectrophotometer. [9]

***Ex-vivo* studies**

***In situ* gel formation in chick muscle**

The extensor digitorum muscle from *G. gallus domesticus* was procured fresh from a reputable hatchery and the tissue weighing 4.5 g was excised. The formulation (3 ml) was injected into the muscle using a 20gauge needle. Crystal violet dye was added previously to the formulation to increase the visibility of depot in the muscles. The tissue was tied to a tissue holder and immersed in the vessel of an organ bath containing 25 ml of phosphate buffer (pH 7.4) maintained at 37°C ± 2°C and aerated at constant rate of 10–12 bubbles/s. The formation of depot was determined by taking a section

of muscle after injection of formulation and observing the presence of any gelled mass. [10,11]

## RESULTS AND DISCUSSION

### Appearance and Clarity

All the gel formulations were clear and transparent. They were free from visible foreign particles and undissolved particles when determined against white or black background.

### pH

The pH of all the formulations were found to be in the range of 6.00-7.00 which is close to blood pH. Hence the formulations are non-irritant.

### Measurement of Gelation temperature and Gelation time

The data for determination of Gelation temperature and Gelation time of thermosensitive gel-forming solutions are shown in Table 2.

Formulations F3, F4 and F8 exhibited gelation temperature closed to 37℃. But upon addition of drug they liquified. Hence these formulations were not selected. Whereas F6, F7 and F9 exhibited gelation temperature closed to the body temperature even upon incorporation of drug. This can be attributed to the fact that the thermo-gelling behaviour of poloxamers arises out of the self-assembling of the molecules into micelles with a dehydrated polypropylene oxide (PPO) core surrounded by hydrated swollen polyethylene oxide (PEO) chains at the critical micellar concentration and temperature. At higher temperatures, micelles lose their water, become dehydrated, and form a gel. A gelation at temperatures of 33–37°C is considered optimum for development of thermosensitive *in situ* gelling formulation for the purpose of implantation since temperatures below 30°C would mean gel at room temperatures and problems in manufacture, handling, and administration. On the other hand, gelation temperatures exceeding 37°C would result in the formulation remaining in the liquid state after administration. Therefore, formulations **F6, F7 and F9** were considered for further study.

**Table 2:** Data for gelation temperature and Gelation time for all formulations.

| Formulation Code | Gelation Temperature (℃) | Gelation Time (min) |
|---|---|---|
| F1 | 29±1.5 | 3.5±0.4 |
| F2 | 27.5±0.7 | 4±1.5 |
| F3 | 37±0.6 | 3±0.8 |
| F4 | 37.5±0.9 | 3.5±0.5 |
| F5 | 40±0.4 | 4.9±0.7 |
| F6 | 33.5±0.5 | 7±0.4 |
| F7 | 35±0.2 | 6±0.2 |
| F8 | 37.5±0.8 | 4±0.3 |
| F9 | 34.5±0.5 | 5±1.3 |

After addition of drug to the formulations F6, F7 and F9 they did not show any change in appearance and clarity. They were also free from visible particles and were mobile solutions when stored under refrigeration at 4℃.

**Table 3:** Composition of final formulation of Felodipine in situ gels

| Formulation code | Poloxamer-407 (%) | Poloxamer-188 (%) | Felodipine (mg) | Benzalkonium chloride (%) | Distilled water (ml) |
|---|---|---|---|---|---|
| F6 | 20 | 3 | 35 | 0.02 | 10 |
| F7 | 20 | 5 | 35 | 0.02 | 10 |
| F9 | 20 | 17 | 35 | 0.02 | 10 |

**Table 4:** Data for gelation temperature, gelation time and pH of formulations

| Formulation code | Gelation temperature (℃) | Gelation time (min) | pH |
|---|---|---|---|
| F6 | 33.5-34 | 7±0.4 | 6.2-6.8 |
| F7 | 35 | 6±0.2 | 6.5-7.2 |
| F9 | 34.5-35 | 5±1.3 | 6.1-6.4 |

### Syringeability and Injectability

Syringeability plays a significant role in clinical application while administrating gels into the body. For evaluation 22 and 20gauge syringe is used. It was observed that the thermoreversible *in situ* gel formulations F6 and F7 showed good syringeability and F9 showed moderate syringeability as it was more viscous when compare to F6 and F7.

### Drug content

Drug content estimation was done for F6, F7 and F9 formulations and is indicated in Table 5.

**Table 5:** Data for Syringeability, Viscosity and Drug content.

| Formulation code | Syringeability | Viscosity at 25℃ (cps) | Drug content (%) |
|---|---|---|---|
| F6 | +++ | 216-217 | 97.6 |
| F7 | +++ | 199-200 | 97.75 |
| F9 | ++ | 155-156 | 97.32 |

**In vitro drug release studies**

The release profiles of Felodipine from the formulations in phosphate buffer pH 7.4 using the membranous diffusion method for 120hrs are graphically represented. Significant drug release was not observed beyond 120 hr from any of the formulations.

All the formulations showed an initial burst effect as a result of the immediate drug release from the sol form of the preparation before conversion to gel as seen in the initial phase of drug release profiles in Fig. 1, after which the formulation undergoes thermoconversion to form a gel and results in sustained release of the drug. In F6, F7 and F9 there was a burst release of 41.36±0.39, 43.81±0.12 and 40.81±0.28 respectively in the initial 2 hours. Such a high burst release with more than 40% drug released within two hours showed the limitation of poloxamer system to retard the burst release. It was observed that formulation F7 exhibited a complete drug release of 98.01 ±0.23 % at 120 hrs. In case of F6 and F9 showed a complete drug release of 95.85±0.31 % and 93.21±0.2% respectively at 120 hrs. It reveals that Poloxamer formulations could sustain the drug release only up to 120 hrs. The drug release was dependent on poloxamer concentration. As the poloxamer 188 concentration increased from 3 to 5%w/v, drug release was sustained for 120 hrs. But further increase in poloxamer concentration to 17% w/v could not further sustain the drug release.

**Table 6:** Data for in vitro drug release studies

| Time (hr) | Cumulative % Drug release | | |
|---|---|---|---|
| | F6 | F7 | F9 |
| 0 | 0 | 0 | 0 |
| 1 | 13.41±0.14 | 18.27±0.2 | 15.61±0.19 |
| 2 | 41.36±0.39 | 43.81±0.12 | 40.81±0.28 |
| 3 | 51.65±0.26 | 54.01±0.3 | 46.52±0.24 |
| 4 | 59.05±0.41 | 61.06±0.23 | 56.64±0.24 |
| 8 | 67.05±0.35 | 69.81±0.15 | 63.81±0.11 |
| 12 | 71.28±0.23 | 75.01±0.13 | 69.03±0.32 |
| 24 | 75.66±0.16 | 79.04±0.14 | 72.52±0.49 |
| 48 | 79.42±0.13 | 85.64±0.16 | 78.20±0.22 |
| 72 | 84.31±0.12 | 91.03±0.34 | 82.12±0.45 |
| 96 | 89.16±0.42 | 93.65±0.21 | 87.16±0.13 |
| 120 | 95.85±0.31 | 98.01±0.25 | 93.21±0.2 |

It was observed that the concentration of polymers affected the drug release from the formulations. There was a retardation of drug release with increase in the concentration of poloxamers.

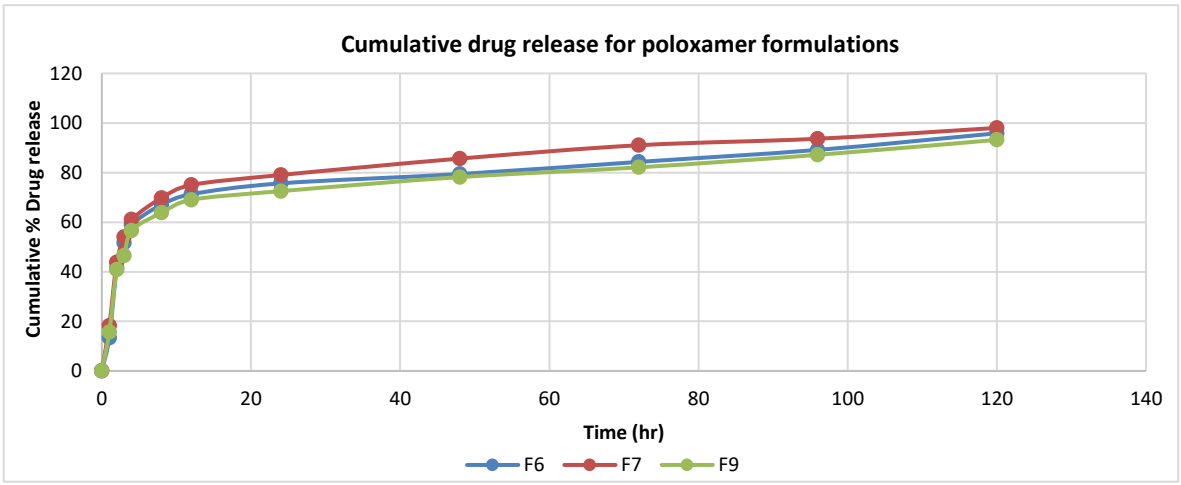


**Figure 1:** In vitro release of felodipine from F6, F7 and F8 in situ gel formulations.

**Mechanism of drug release**

The *in vitro* drug release data were subjected to kinetic analysis and fitted to zero-order, first-order Higuchi and korsmeyer-peppas models, as shown in fig.2. The drug release mechanism was determined using the Korsemeyer's–Peppas equation, and the release exponent (n) was calculated by regression analysis.

**Korsmeyer-peppas equation is**:

$$F = (M_t/M) = K_M t^n$$

F = Fraction of drug released at time 't'

$M_t$ = Amount of drug released at time 't'

M = Total amount of drug in dosage form

$K_m$ = Kinetic constant

n = Diffusion or release exponent

t = Time in hours

If n value is n≤4.5 it indicates fickian diffusion and n≥1 indicates non-fickian diffusion.

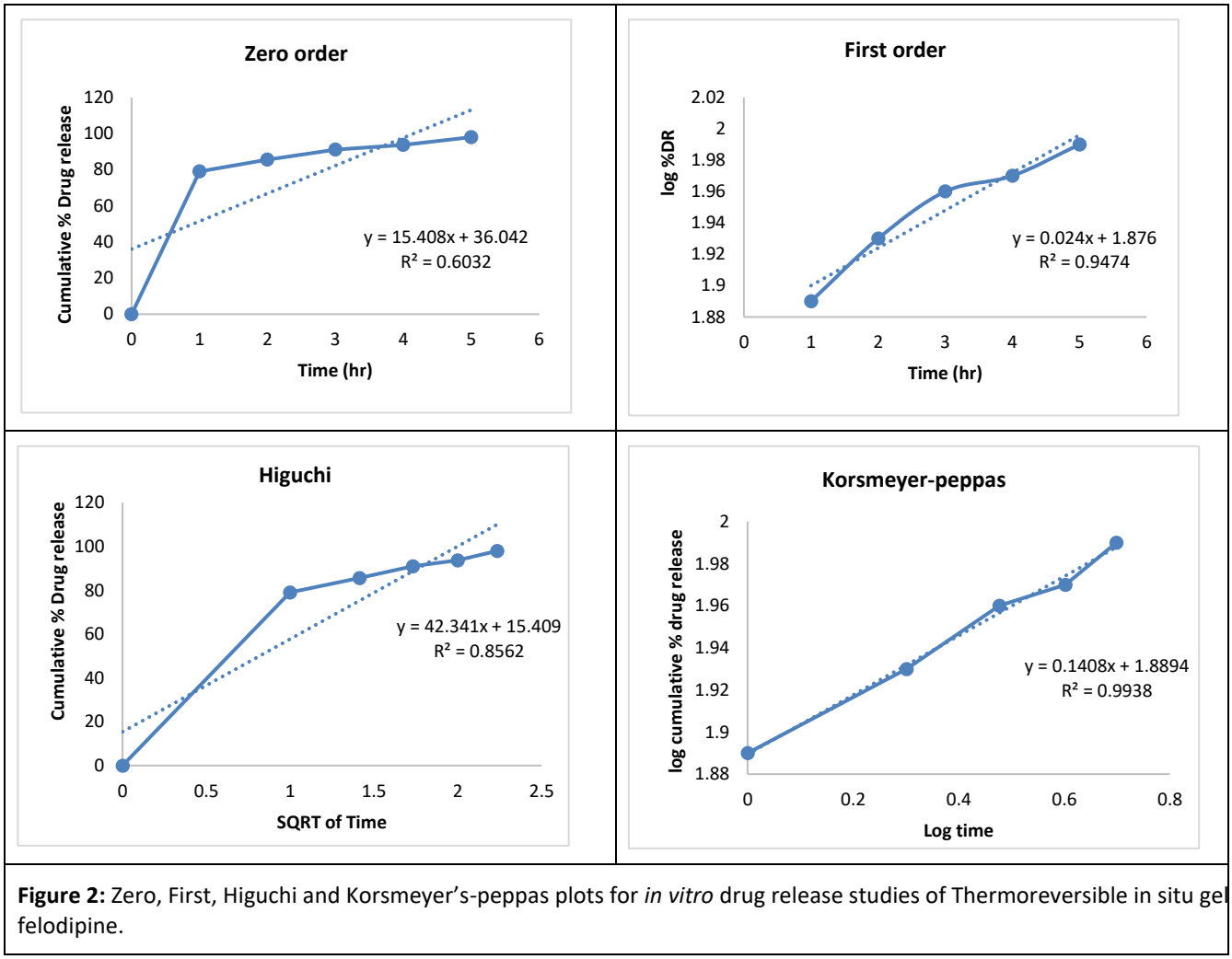


**Figure 2:** Zero, First, Higuchi and Korsmeyer's-peppas plots for *in vitro* drug release studies of Thermoreversible in situ gel felodipine.

The *in vitro* release profiles of the drug from all the formulations appeared to follow korsmeyer-peppas model. The drug was dissolved from in situ gels by Fickian diffusion through the extramicellar aqueous channels of the gel matrix. The 'n' value was found to be 0.14 and regression co-efficient was 0.9938.

***Ex-vivo* studies**

***In situ* gel formation in chick muscle**

The optimized formulation was injected into the chick muscle tissue, from where they should release the drug in a prolonged or controlled fashion. Therefore, this study was carried out to confirm the formation of a depot after injection of the solution into the excised chick muscle or extensor digitorum. To increase the visibility of the depot in the muscles, crystal violet dye was added to the formulation. The formation of depot was confirmed by taking a section of muscle after injecting the formulation. A semisolid violet-colored depot was observed in the muscle which confirmed the formation of depot in the muscle. Depot formation in the muscle after thermoconversion of the *in-situ* gelling solution for the optimized formulation is shown in Fig. 3.

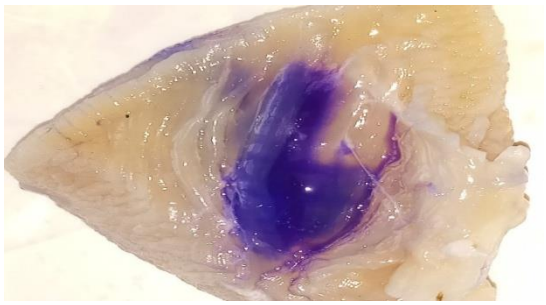

**Figure 3:** Formation of depot inside extensor digitorum muscle (Formulation F7).

## CONCLUSION

Thermoreversible in situ gels of felodipine were formulated using poloxamer-407 (20%), poloxamer-188 (5%), Felodipine (35mg), Benzalkonium chloride (0.02%) and DMSO (0.5ml). These gels could sustain the drug release for 120hrs. *In situ* gels are a promising approach to enhance the bioavailability and sustain the drug release.

## REFERENCES


1. X.-Q. Wang and Q. Zhang, PH-sensitive polymeric nanoparticles to improve oral bioavailability of peptide/protein drugs and poorly water-soluble drugs, European Journal of Pharmaceutics and Biopharmaceutics, vol. 82, no. 2, 2012, pp. 219–229.
2. H. Li, X. Zhao, Y. Ma, G. Zhai, L. Li, and H. Lou, Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles,Journal of Controlled Release, vol. 133, no. 3, 2009, pp. 238–244,.
3. P. Khadka, J. Ro, H. Kim et al., Pharmaceutical particle technologies: an approach to improve drug solubility, dissolution and bioavailability, Asian Journal of Pharmaceutical Sciences, vol. 9, no. 6, 2014, pp. 304–316.
4. Choi, H.G., Jung, J.H., Ryu, J.M., Yoon, S.J., Sung, Oh, Y.K., Kim, C.K., Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int. J. Pharm. 165, 1998a, 33–44.
5. Yong, C.S., Yang, C.H., Rhee, J.D., Lee, B.J., Kim, D.C., Kim, D.D., Kim, C.K., Choi, J.S., Choi, H.G., Enhanced rectal bioavailability of ibuprofen in rats by poloxamer 188 and menthol. Int. J. Pharm. 269, 2004, 169–176.v8
6. Baloglu E, Karavana, Senyigit YS, Hilmioglu-Polat A, Metin S, Jones DS. In-situ gel formulations of econazole nitrate preparation and in-vitro and in-vivo evaluation. J Pharm Pharmacol. 63(10), 2011, 1274-82.
7. Lalloo A, Chao P, Hu PS. Pharmacodynamic evaluation of a novel insitu poly (ethylene glycol)based hydrogel for controlled delivery of camptothecins. Journal of controlled release, 112(3), 2006, 333-42
8. Ricci EJ, Bentley MV, Farah M, Bretas RE, Marchetti JM. Rheological characterization of poloxamer 407 lidocaine hydrochloride gels. Eur J Pharm Sci, 17, 2002, 161-7
9. Venkatesh MP, Anis S, Kumar TP. Design and development of an injectable in situ forming drug delivery system of methotrexate for the treatment of rheumatoid arthritis. Journal of Drug Delivery Science and Technology. 23(5), 2013 Jan 1, 445-53.
10. Dawre S, Devarajan PV. Novel *ex vivo* Method for Intramuscular *in situ* Depot Formulation. International symposium on Disso Asia; 2014, p. 43.
11. Avachat AM, Kapure SS. Asenapine maleate *in situ* forming biodegradable implant: An approach to enhance bioavailability. Int J Pharm, 477, 2014, 64-72.


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