Original Article

# Rheological characterization, In vitro release, and Ex vivo permeation of Nefopam Thermosensitive and mucoadhesive intranasal in situ gel

**Ammar A. Alabdly[1], Hanan J. Kassab[2]**

[1]Department of Pharmaceutics, College of Pharmacy, Anbar University, Anbar, Iraq

[2]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Email: amar.abdulmajeed1200a@copharm.uobaghdad.edu.iq

## Abstract

Nefopam nasal thermosensitive in situ gel based on poloxamer 407 and poloxamer 188 were formulated. The formulations that showed acceptable gelation temperature were selected for further evaluations; rheological, in vitro-release, and permeation behavior.

Rheological studies show significant increase in viscosity when solution formed gel at gelation temperature and follow pseudoplastic flow, and as viscosity increased, slower erosion and drug release occurred.

Drug release profiles were fitted to mathematical models (First-order, Zero order, Higuchi, Power law, and Peppas-Sahlin) using a DDSolver Excel Microsoft Add-in, dissolution profile comparison in addition to pairwise similarity factor (f2) was used for comparison between formulas dissolution.

The flux and effective permeability coefficient parameters at steady state were calculated for selected formulas and compared with permeation of 20mg/ml Nefopam aqueous solution as control.

**Keywords:** Nefopam Hydrochloride, Thermosensitive in situ gel, Poloxamer 407, Poloxamer 188, Rheological properties, In-vitro release, Ex-vivo permeation.

## Introduction

Drug release is crucial for the efficacy of the treatment and for the best patient compliance. The major objective of the pharmacokinetic modulation is to perform the lowest number of the least invasive administration, preserving the therapeutic effect. The fine design of thermosensitive nasal in situ gel, together with a knowledge of the physiological factors involved, can highly influence the time and the place of the drug release.(1)

Indeed, Nose-to-Brain in situ gel delivery system represent highly promising opportunity, as it opens the way of enhanced drug delivery to the brain, attributed to being the nose is one of the most permeable and highly vascularized sites for drug administration ensuring rapid absorption and onset of therapeutic action. It has been potentially explored as an alternative route for drugs passing harsh environment of oral route and increased brain penetration, as well as offer the possibility for controlled release of drugs.(2–4)

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.03.025

Different polymers are used in preparation of in situ gel including thermosensitive, ion responsive, and pH responsive polymers. Hence, the versatility of polymers, offering the possibility to deliver different molecules at different release rates.(5)

Unlike covalently crosslinked gels which may control the release of the drug over a period of months, Poloxamer 407 (PoloX 407) based gels are intended for shorter time periods.

**Address for correspondence: Ammar A. Alabdly,**
Department of Pharmaceutics, College of Pharmacy, Anbar University, Anbar, Iraq
Email: amar.abdulmajeed1200a@copharm.uobaghdad.edu.iq



How to cite this article: Ammar A. Alabdly, Hanan J. Kassab, Rheological characterization, In vitro release, and Ex vivo permeation of Nefopam Thermosensitive and mucoadhesive intranasal in situ gel, J PHARM NEGATIVE RESULTS 2022;13:155-164.

Their main advantage over covalently based gels is that neither special insertion instrument nor surgical removal of the gel is required since they dissolve without any toxic effect.

In vitro drug release from poloxamer gel into the medium is regulated by the diffusion of the drug and/or dissolution of the poloxamer gel, depending on the experimental conditions. For example, the diffusion of the drug from the gel into the media is the predominant mechanism of drug release if an experimental setup where the poloxamer gel is confined within a permeable membrane (with pores blocking the poloxamer molecules).(6)

A drug administered through the nasal cavity can permeate either passively by the paracellular pathway or both passively and actively via the transcellular pathway. This basically depends on the lipophilicity of the compound. Different factors can affect the permeability of drugs from nasal cavity, biological factors such as blood supply, exposed surface area, nasal secretions, and nasal clearance and formulation factors such as physicochemical properties of drug molecule, and physicochemical properties of formulation.(4)

Moreover, the flow rheological properties are of primary interest because they have been shown to largely govern the ease of application and dispersion of formulation at site of absorption. Also, prolonged retention at the site of application by using mucoadhesive polymers leading to increased therapeutic efficacy.

For nasal application, polymers should have optimum viscosity for easy instillation into the nose, which in turn should undergo rapid sol-gel transition at specific temperature of the nose (32-34 °C).(7)

Thus, evaluation of rheological properties of in situ gelling polymers is related to in vivo drug release and ex vivo permeation characteristics.

In the present investigation, blends of PoloX 407 and poloxamer 188 (PoloX 188) as thermosensitive in situ gel, hyaluronic acid (H.A), and methylcellulose as mucoadhesive agents are used for nasal delivery of nefopam hydrochloride.

## Materials and Methods

### Materials

Nefopam HCl (N.F), Hyaluronic acid (H.A) average molecular weight ≈ 33 kDa , purchased from (Baoji Guo Kang, Ltd–China), Methyl cellulose M.C average molecular weight ≈ 13 kDa, , Poloxamer PoloX 407, and PoloX 188 purchased from ( Eastman Chemical company – USA), all the chemicals and reagents, were of analytical grade.

### Methods

Preparation of polymers solution

In our previous work (not yet published), Nefopam nasal mucoadhesive thermosensitive in-situ gel were formulated using different concentrations of poloxamer and mucoadhesive polymers, the gelation temperature was measured for each formula; only compositions with a gelation temperature range of 32-34°C (7) were selected for further study (P.I No. 1,2,3,4,5,6,7,8,and 9) as shown in Table 1. The cold method was used to formulate different compositions of in situ gel, by mixing different concentrations of PoloX 407 (17-20%) with or without PoloX 188 (3-4%) in cold double distilled water immersed in ice with continuous stirring for 4-6 hours, then the obtained cloudy mixture was stored in the refrigerator at 4°C for 24 hours to get a clear solution(8). After that, different concentrations of the mucoadhesive polymers Hyaluronic acid (H.A), or Methylcellulose (M.C) were added slowly to a the poloxamer mixture with continuous stirring at 50 rpm. The slow stirring speed was applied to prevent foam formation that can affect the mucoadhesive polymer dispersion in the poloxamer mixture. Then N.F HCl and Benzalkonium chloride at a concentration (20 mg/ml) and (0.01 % w/v), respectively, were added slowly with continuous stirring. Finally, the mixture was stored for 24 hours at 4°C to eliminate any foam formed.

**Table 1.** Nefopam nasal in-situ gel formula contents (all the formulas contain Benzalkonium chloride 0.01% w/v, and the volume completed to 10 mL with Double DW

| P.I. No. | NF mg | PoloX407(%) | PoloX188(%) | H.A(%) | M.C(%) |
|---|---|---|---|---|---|
| 1 | 200 | 17 | | 0.75 | |
| 2 | 200 | 19 | 3 | 0.75 | |
| 3 | 200 | 17 | | 0.5 | |
| 4 | 200 | 19 | 3 | 0.5 | |
| 5 | 200 | 17 | | 0.25 | |
| 6 | 200 | 19 | 3 | 0.25 | |
| 7 | 200 | 17 | | | 0.2 |
| 8 | 200 | 19 | 3 | | 0.2 |
| 9 | 200 | 17 | | | 0.1 |

Rheological studies

The rheological properties of all formulations (P.I 1-9) were evaluated by using a the Myr digital rheometer (Model VR 3000, Spain). The viscometer is initially calibrated.

The viscosities of the samples were measured at room temperature for the solution phase and at 34 °C for the gel phase in triplicates. Samples were sheared at 10 rpm with spindle R4 and R5 for formulas while cold and at 10,12, 20, 30,50,60,100,200 rpm with spindle number R7 after gelation (9).Each sample was allowed to be sheared for 2 min before the reading. The interpretation of rheological behavior was established using plots of shear stress (rpm) versus viscosity (cP).

Drug content

Accurately, 1 mL of the formulation (equivalent to 20 mg/mL N.F) from selected P.I formulas was suitably diluted with simulated nasal fluid SNF. Then the solution was filtered by

0.45 µm syringe filter. Finally, the absorbance was measured at the maximum absorption wavelength (266 nm) using UV-VIS spectrophotometer (Cary, Varian Australia).The samples were taken from three different regions of the gel from the upper, middle and bottom of the P.I formulas to ensure uniform distribution of N.F in formula, and the mean drug content was measured .(10)

In-vitro drug release study

Drug release studies were performed using modified Franz diffusion cell. Experiments were carried out at 34°C with a constant stirring of 50 rpm, using magnetic bead, for 2 hours. Simulated nasal fluid (SNF) was used as the receptor medium as it maintains sink condition. Dialysis membrane (Hi-Media Laboratories Pvt. Ltd. Mumbai, India), having a molecular weight cut-off between 12000-14000 Dalton and pore size 2.5 nanometers was immersed in SNF for 2 h before use. A sufficient quantity of SNF to achieve a sink condition was placed in the acceptor compartment, 1 mL of P.I formulas which contain 20 mg of N.F are taken in donor compartment and temperature increase to 34°C to allow formation of gel. 2 mL of receptor fluid was withdrawn at scheduled time interval (every 5 min.) and was replaced with fresh receptor fluid in order to maintain sink condition. The amount of drug diffused through dialysis membrane was determined spectrophotometrically at 266 nm using UV-Vis after diluting suitably with SNF.(11)

The control drug release was performed with the same procedure mentioned above, with the use of 20 mg N.F HCl in aqueous solution, to compare the drug release from selected formulas and free drug.

Analysis of release mechanism

The mechanism of N.F release from different P.I formulas was mathematically analyzed according to different mathematical release equations including:

Zero-order model

For zero-order kinetics, the release of an active agent is only a function of time and the process takes place at a constant rate independent of active agent concentration.

$Ct = C0 – K0\ t$ Eq 1. (12)

Where:

( Ct ) represents the concentration of N.F remaining at the time (t)

( C0 ) represents the initial concentration of N.F

( K0 ) represents the zero-order release constant

(t) Time

First-order model

For first-order kinetics, the amount of drug released is proportional to the amount of remaining drug. Thus, the amount of active released tends to decrease in function of time.

$$\text{Log } C_t = \log C_0 - \frac{K1\ t}{2.303} \quad \text{Eq 2. (12)}$$

Where:

( Ct ) represents the concentration of N.F remaining at the time (t)

( C0 ) represents the initial concentration of N.F

( K1 ) represents the first-order release constant

(t) Time

Higuchi model

For Higuchi model, the rate of release of a drug, from a matrix, usually a polymer, where the loading of solute(A), exceeds its solubility (Cs), in the matrix, into a surrounding fluid.

$Q = KH\ t^{½}$ Eq 3. (13)

Where:

( Q ) Represents the amount of N.F remaining at the time (t)

( K ) is the release constant of Higuchi

( $t^{½}$ ) Represents the square root of time

Ritger-Peppas and Korsmeyer-Peppas model (power law)

For power law model, the model establishing the exponential relationship between the release and the time.

This model was developed specifically for the release of a drug molecule from a polymeric matrix, such as a hydrogel. The power law model is useful for the study of drug release from polymeric systems when the release mechanism is not known or when more than one type of phenomenon of drug release is involved.

$$\log \frac{M\ t}{M} = K + n\ log\ t \quad \text{Eq 4. (12)}$$

Where:

( $\frac{M\ t}{M}$ ) Represents the fraction of N.F release at the time t

(K) The constant of incorporation of structural modifications and geometrical characteristics of the system

(n) Represents an indicatives of the release mechanism

(t) time

The (n) value $0.45 < n < 0.89$, and $1 < n$ means a non Fickian (anomalous) , and non Fickian solvent crazing release (Case II), respectively, and $n < 0.45$ mean Fickian diffusion (Case I).

Peppas-Sahlin model

This release kinetics model is a consequence of the indication that it is possible to calculate the approximate two contribution mechanisms (diffusional and relaxational) in an anomalous drug release process.

$F/R = K_1 t^m + K_2 t_2^{2m}$ Eq 5. (14)

Where:

(F/R) Represents the fraction of N.F release at the time (t) from diffusional and relaxational process

(K 1 , 2) are the kinetics constants

(m) The coefficient of the Fickian diffusion exponent for a system

(t) time

Trans-nasal drug permeation study

The trans nasal permeation study of N.F in situ nasal gel was carried out using sheep nasal epithelium membrane. Nasal cavity of the freshly sacrificed sheep was procured from the slaughter house. These nasal cavities were conscientiously dissected and nasal septum was taken out without any damage to it. Then the nasal epithelium membrane was removed carefully from the underlying bone, washed thoroughly and stored in cold saline buffer of pH 7.4.

The membrane of nasal epithelium was tied efficiently to one end of the hollow cylindrical tube (2 cm in diameter). The cylindrical tubes were then suspended into 10 ml of phosphate buffer saline (PBS) at 37 °C and adjusted to rotation at 50 rpm. N.F in situ nasal gel equivalent to 20 mg N.F (1 mL) was applied over the epithelium membrane.

At time interval every 30 min, 1 mL aliquots of receptor fluid were withdrawn over 6 h and were replaced with an equal volume of fresh PBS maintained at 37 °C. Aliquots of withdrawn samples were filtered and diluted suitably with PBS and analyzed for drug concentration spectrophotometrically double beam UV-Vis spectrophotometer at 266 nm.(11)Absorbance data were converted to concentration and by multiplying it by dilution factor (10 mL) an amount permeated per unit time will be obtained.

### Statistical analysis

The results of the research were given as mean values ± standard deviation (SD) and examined according to the one-way analysis of variance (ANOVA) at which significant results ($p<0.05$) and non-significant ($p>0.05$).

## Results and Discussions

### Rheological studies

The viscosity data of selected P.I formulas (1-9) at gel phase under different shear rate (rpm) are shown in Table 2a and 2b and Fig. 1a and 1b, all formulas form solution with low viscosity at room temperature, while a significant increase in viscosity at gelation temperature. In situ gel dissent the Arrhenius equation relates the viscosity and temperature due to the presence of thermosensitive polymers that form gel and increase viscosity when temperature increase.(15)

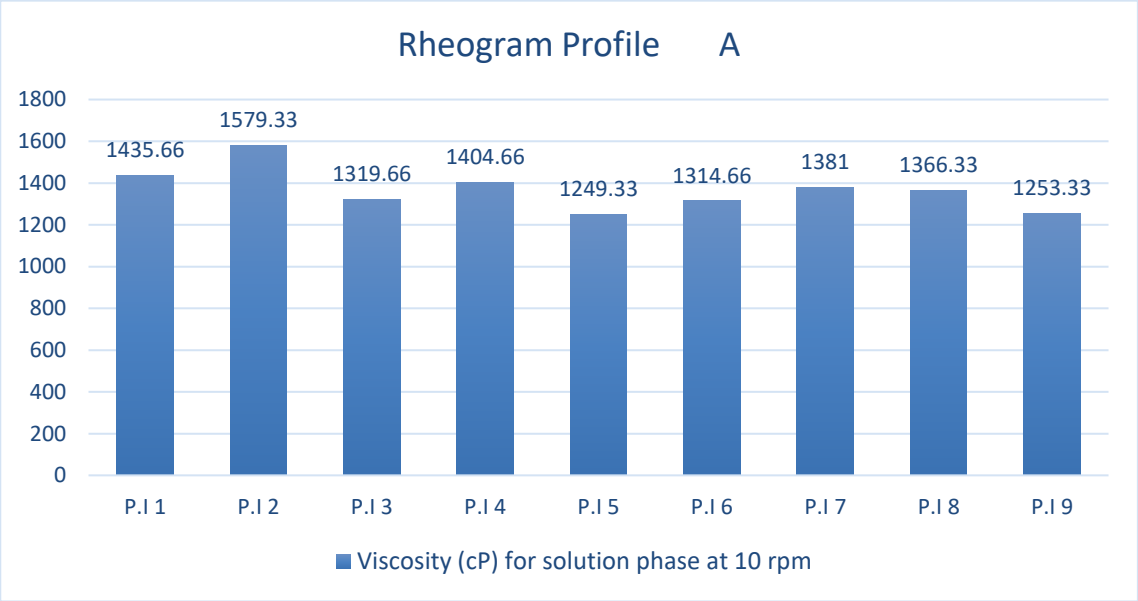



**Fig. 1a.** Rheogram profiles for P.I formulas at 24 oC using spindle No. R4 and 5

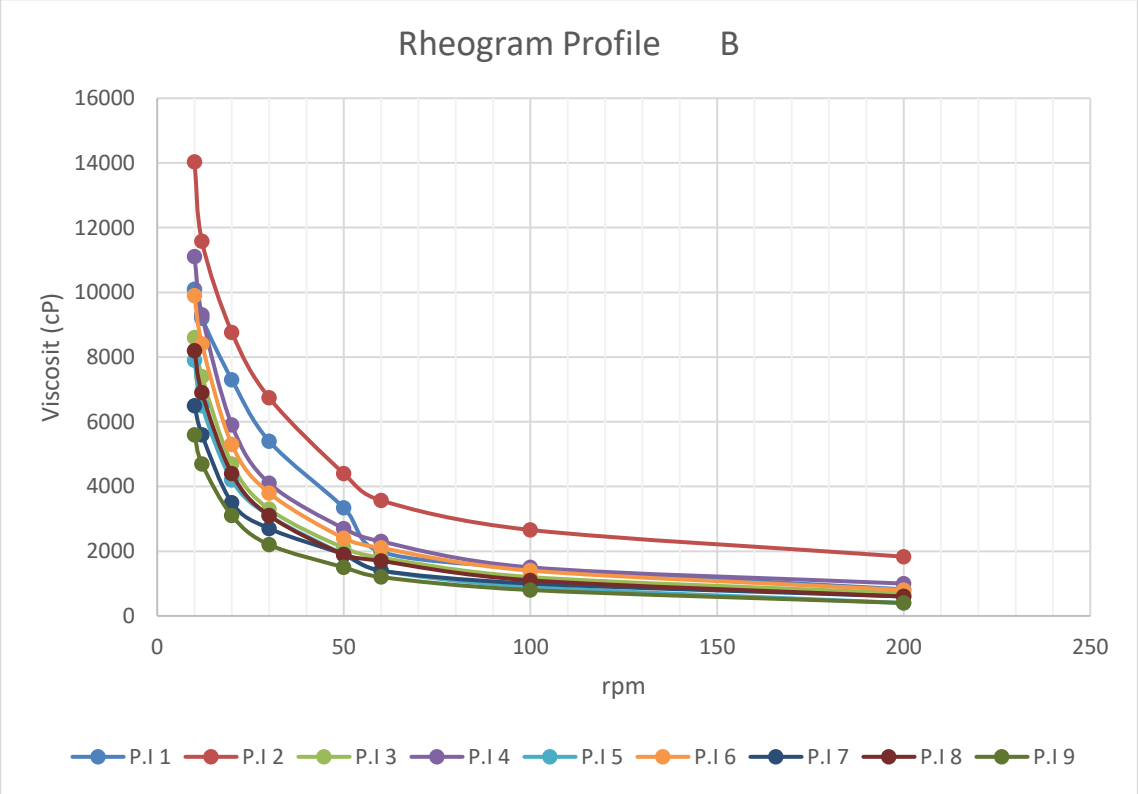



**Fig. 1b.** Rheogram profiles for P.I formulas at 34 oC using spindle No. R7

**Table 2a.** Viscosity of P.I formulas at room temperature 24 ± 2 °C, at 10 rpm

| P.I Formula No. | Spindle No. | Viscosity ( cP ) (Mean ±SD, (n=3)) |
|---|---|---|
| **1** | R5 | 1435.66 ± 10.530 |
| **2** | R5 | 1579.33 ± 11.440 |
| **3** | R5 | 1319.66 ± 13.573 |
| **4** | R5 | 1404.66 ± 4.189 |
| **5** | R5 | 1249.33 ± 4.988 |
| **6** | R5 | 1314.66 ± 3.680 |
| **7** | R4 | 1381 ± 6.164 |
| **8** | R4 | 1366.33 ± 8.993 |
| **9** | R4 | 1253.33 ± 3.399 |

**Table 2b.** Viscosity of P.I formulas at 34°C, at different shear rate (rpm)

| Shear rate / P.I No. | Viscosity ( cP ) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **10rpm (Mean ±SD, (n=3))** | **12rpm (Mean ±SD, (n=3))** | **20rpm (Mean ±SD, (n=3))** | **30rpm (Mean ±SD, (n=3))** | **50rpm (Mean ±SD, (n=3))** | **60rpm (Mean ±SD, (n=3))** | **100rpm (Mean ±SD, (n=3))** | **200rpm (Mean ±SD, (n=3))** |
| **1** | 10118.33 ± 64.592 | 9179.33 ± 21.330 | 7300 ± 8.165 | 5394.67 ± 4.989 | 3354 ± 35.367 | 1981.667 ± 30.64 | 1475.66 ± 19.6 | 814 ± 7.789 |
| **2** | 14034 ± 4.082 | 11576.67 ± 10.656 | 8765 ± 9.798 | 6748.33 ± 5.312 | 4392.33 ± 11.728 | 3575 ± 10.677 | 2651.67 ± 5.557 | 1831.67 ± 1.247 |
| **3** | 8603.162 ± 9.052 | 7410.172 ± 10.434 | 4725.944 ± 18.971 | 3334.123 ± 24.369 | 2141.133 ± 29.168 | 1842.885 ± 10.384 | 1246.39 ± 22.828 | 749.3108 ± 34.877 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **4** | 11088.56 ± 13.896 | 9299.073 ± 9.501 | 5918.934 ± 14.689 | 4129.449 ± 21.248 | 2737.628 ± 26.755 | 2339.964 ± 28.361 | 1544.638 ± 21.604 | 1047.558 ± 33.647 |
| **5** | 7907.251 ± 9.556 | 6515.429 ± 12.777 | 4228.865 ± 20.864 | 3135.291 ± 25.161 | 1942.302 ± 9.978 | 1445.222 ± 12.012 | 948.1425 ± 4.056 | 451.0633 ± 26.110 |
| **6** | 9895.568 ± 10.56 | 8404.33 ± 9.10 | 5322.439 ± 16.773 | 3831.202 ± 22.409 | 2439.38 ± 17.959 | 2141.133 ± 19.168 | 1445.222 ± 12.012 | 848.7267 ± 14.466 |
| **7** | 6515.429 ± 12.777 | 5620.687 ± 15.714 | 3532.954 ± 23.581 | 2737.628 ± 26.755 | 1942.301 ± 19.978 | 1445.222 ± 2.012 | 1047.558 ± 23.647 | 649.895 ± 5.288 |
| **8** | 8205.498 ± 9.226 | 6913.093 ± 11.639 | 4427.697 ± 10.101 | 3135.291 ± 15.161 | 1942.301 ± 9.978 | 1743.469 ± 10.790 | 1146.974 ± 13.237 | 649.895 ± 25.288 |
| **9** | 5620.687 ± 15.714 | 4725.944 ± 18.971 | 3135.291 ± 15.161 | 2240.548 ± 8.764 | 1544.638 ± 11.604 | 1246.390 ± 22.828 | 848.727 ± 14.466 | 451.063 ± 6.110 |

The rheogram profiles of different polymers used in this study showed that as the rotation speed increased (shear rate) (Fig. 1) the viscosity decreased indicating the pseudoplastic (shear thinning liquids) flow of the preparation.(16) As expected , P.I formulas No, (1-6) containing H.A show higher viscosity in concentration dependent manner at both room temperature and 34°C (17) than formulas ( P.I 7-9 ) , also poloxamers show impact on viscosity in proportion to its concentration as a result of more physical entanglement when concentration increase (18). P.I 16 show higher viscosity due to higher concentration of H.A (0.75%) , PoloX 407 ( 19%) and PoloX 188 (3%).

## Drug content

The drug content was found to be (97.66-107.76%) that is in the acceptable range according to USP (19) of all the formulations. This indicates that the process employed in this study was capable of producing gels with uniform drug content and minimal variability, as seen in Table 3. Also, nonsignificant differences ($p<0.05$) shown among upper, middle, and lower samples, that indicate to a good uniform distribution of drug and preparation efficiency.

**Table 3.** N.F intranasal gel drug content of the formulas

| P.I Formula No. | Drug content % w/v (Mean ±SD, n=3). |
|---|---|
| **1** | 100.44 ± 0.901 |
| **2** | 99.62 ± 0.460 |
| **3** | 101.54 ± 1.03 |
| **4** | 102.623 ± 0.460 |
| **5** | 102.98 ± 0.582 |
| **6** | 98.95 ± 1.138 |
| **7** | 101.607 ± 0.575 |
| **8** | 102.28 ± 0.789 |
| **9** | 99.19 ± 0.347 |

## Drug release kinetics

Release could be directly related to permeation and subsequently to bioavailability of a drug , Fig. 2 show release profile for P.I formulas (1-9) , P.I No. 9 with low concentration of M.C (0.1%) , and PoloX (17%) show rapidest and higher release percent after 15 min. , meanwhile P.I No. 2 composed of H.A (0.75%) and PoloX 407,188 (19,3 w/v% respectively) show lowest and slow release to reach 80% after 65 min. Other formulas with different concentration of mucoadhesive and poloxamer(s) show release in concentration dependent pattern. Drug release from poloxamer based in-situ gels is controlled by both diffusion and gel erosion, However, the rate-determining step for release varies according to the composition of the gel matrix and the physicochemical properties of the loaded drug, in case of small hydrophilic molecule such as nefopam hydrochloride drug diffusion from poloxamer based gels plays a dominant role due to the presence of a continuous aqueous channel within the gel microstructure and thus diffuse rapidly to the surrounding environment

Increment of mechanical and rheological properties causes a slower erosion and drug diffusion and attributes for sustaining the release from poloxamer based in-situ gels due to trapped drug inside polymer micelle that form at higher concentration leading to slower drug release.

In general, poloxamer based in-situ gels are highly porous structures with interpenetrating aqueous channels contributing to fast erosion of the gel matrix. Creating a tightly packed micellar structure leading to reduce number and size of aqueous channels , so it will reduce drug diffusion as well as erosion. (20)

## Analysis of release mechanism

Release data were fitted to mathematical models (First-order, Zero order, Higuchi, Power law, and Peppas-Sahlin) using a DDSolver Excel Microsoft Add-in program(21) to evaluate drug release mechanism according to corresponding higher (Rsqr), lower Akaike information criterion (AIC), and coefficient exponent value (n,m) for each formula, as shown in Table 4 , dissolution profile comparison ( pairwise similarity factor f2 )were used to compare formulas dissolution with 20mg/ml nefopam aqueous solution release profile as a control.

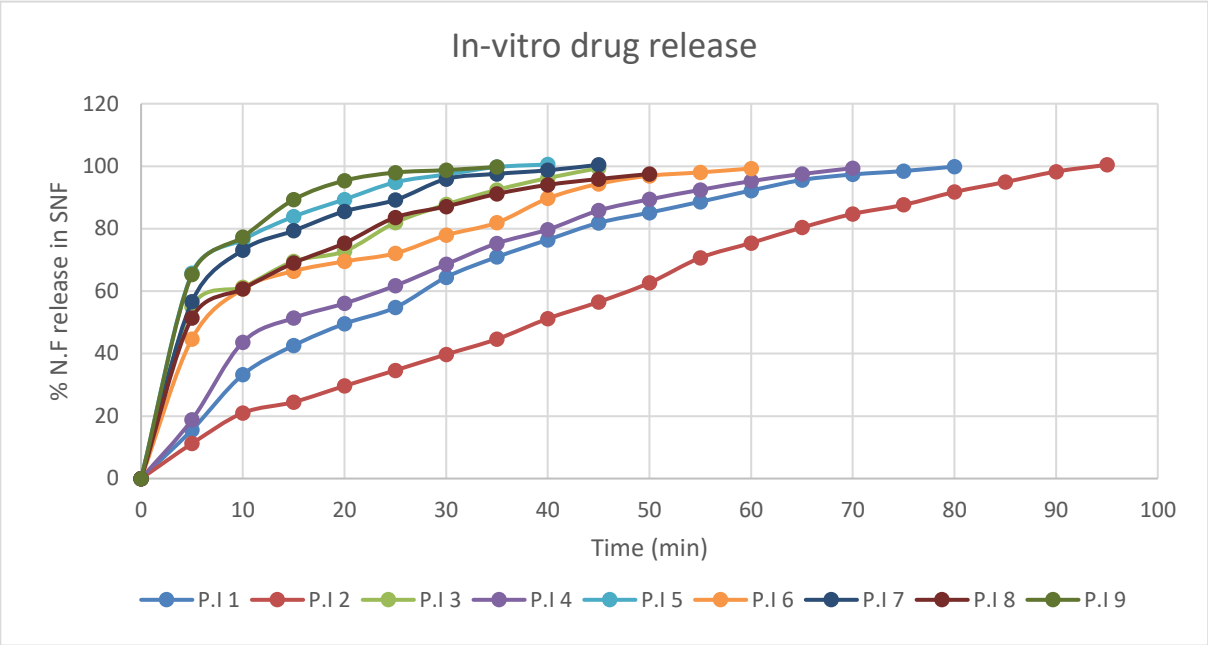



**Fig. 2.** Graphical representation of In-vitro drug release study of N.F HCl from in situ nasal gel formulation P.I (1-9) in SNF at 34°C.

**Table 4.** Release mechanism analysis by different models for P.I formulas

| Model ($R^2$) | P.I No. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| **Zero-order** | 0.7702 | 0.9679 | 0.3553 | 0.6979 | 0.0758 | 0.2808 | 0.1255 | 0.2544 | 0.1906 |
| **AIC** | 140.35 | 129.66 | 87.089 | 125.24 | 82.214 | 116.17 | 90.998 | 97.866 | 72.182 |
| **First-order** | 0.9891 | 0.9473 | 0.9240 | 0.9853 | 0.9710 | 0.9252 | 0.9738 | 0.9492 | 0.9882 |
| **AIC** | 88.454 | 139.555 | 65.704 | 79.912 | 51.06 | 86.751 | 55.918 | 68.319 | 38.373 |
| **Higuchi** | 0.9854 | 0.9247 | 0.9171 | 0.9875 | 0.8142 | 0.9151 | 0.8486 | 0.9016 | 0.8419 |
| **AIC** | 93.496 | 146.70 | 66.572 | 77.432 | 67.775 | 88.394 | 73.463 | 75.590 | 59.120 |
| **Power law** | 0.9876 | 0.9956 | 0.9942 | 0.9878 | 0.9988 | 0.9927 | 0.9960 | 0.9977 | 0.9936 |
| **n value** | 0.538 | 0.785 | 0.301 | 0.486 | 0.209 | 0.318 | 0.246 | 0.292 | 0.217 |
| **AIC** | 92.752 | 91.716 | 41.906 | 79.062 | 24.042 | 58.469 | 39.135 | 36.144 | 35.48 |
| **Peppas-Sahlin** | 0.9982 | 0.9956 | 0.9949 | 0.9928 | 0.9995 | 0.9930 | 0.9988 | 0.9980 | 0.9987 |
| **m value** | 0.807 | 0.397 | 0.225 | 0.677 | 0.325 | 0.242 | 0.415 | 0.367 | 0.460 |
| **AIC** | 62.103 | 93.708 | 42.598 | 73.2018 | 18.7381 | 59.9901 | 29.4134 | 36.7007 | 24.9664 |
| **Similarity factor, f2** | 16.20 | 11.18 | 33.33 | 19.33 | 52.84 | 30.06 | 44.17 | 33.15 | 60.66 |

Based on the statistically higher (R2)(22) and lower AIC, it is possible to get the mechanism by which the drug release is governed. Table 5 show that all P.I formulas are best fitted on Peppas-Sahlin model, in other words the release mechanism is a contribution of two mechanisms (diffusional and relaxational) release process, Eq 5. P.I No. (2,3,5,6,7,8, and 9) have $m < 0.45$, so it shows Fickian release process, Fickian diffusional release occurs by the usual molecular diffusion of the drug due to a chemical potential gradient.

P.I No. (1, and 4) have $0.45 < m < 0.89$ , so it show Case II non-Fickian relaxational release process its mechanism associated with stresses and state-transition in hydrophilic polymers which swell in SNF, attributed to the erosion of the hydrophilic polymer which takes place after complete hydration of the outer layer, and then the gel layer start to disperse due to an attrition process.(21)

Similarity factor f2 show that P.I No. (1,2,3,4,6,7,8, and 9) have $f2 < 50$, that mean these formulas have a different release profile from the control (Nefopam powder alone), meanwhile P.I No. (5 and 9) has $f2 < 50$, so it has similar release profile with the control.

As a formulation intended to be used for pain killer with rapid release not sustained, so only the formulas that show similar release profile with the control will be chosen for further studies.

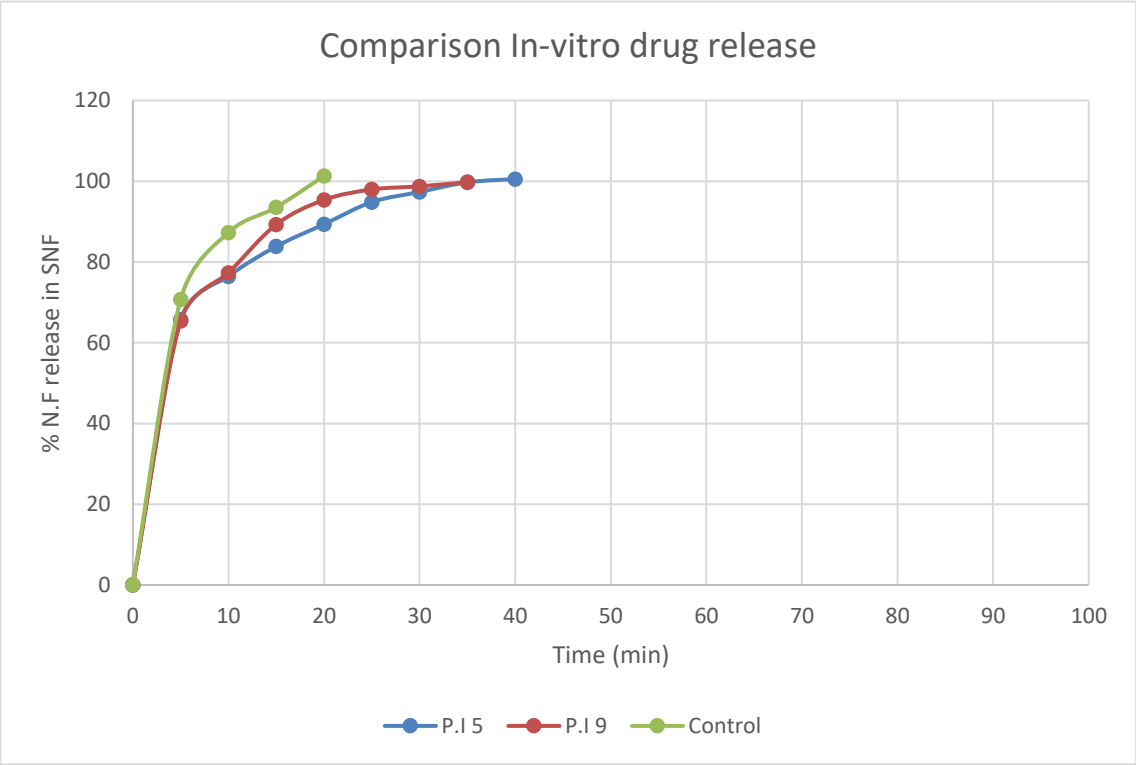



**Fig. 3.** Graphical representation of In-vitro drug release study of N.F HCl from in situ nasal gel formulation P.I (5 and 9) and as Nefopam aqueous solution (control ) release in SNF at 34 °C.

The percentage of N.F HCl released from control (aqueous N.F HCl solution alone) was compared with that from intranasal P.I using the dialysis membrane. A faster release from the control was obtained with a significant similarity in comparism with that from the selected P.I formulas (5,and 9). The percent of drug release from the control was 101.32% at 20 min in comparison with the percent of drug release from P.I 9 which was 99.78% at 35 min and with P.I 5 100.53% at 40 min as shown in Fig. 3 , due to viscosity effect as explained above.

## Trans-nasal drug permeation study

The permeation from nasal P.I preparations was investigated. The cumulative amount over time profiles that presented in Table 5 were plotted as shown in Fig. 4. A linear profile (steady state) was observed during 6-hour period and the slope of the linear portion of the curve was determined by linear regression. The effective permeability coefficients and flux values at steady state (linear potion of curve) were calculated from the slope according to Eq 7, and Eq 8, respectively.

$$J_{ss} = \left(\frac{\Delta y}{\Delta x}\right) \qquad \text{Eq 7. (23)}$$

$$(P_{eff}) = J_{ss} / C_0 \qquad \text{Eq 8. (23)}$$

Where

J: Flux at steady state (mg/cm2 min)

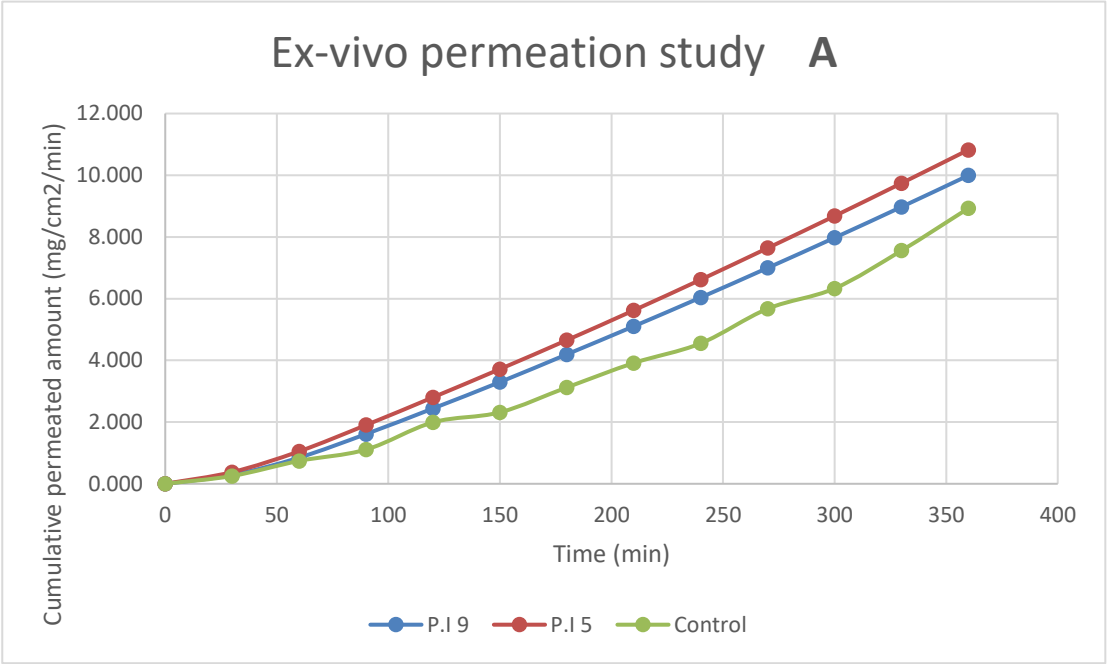
( (Δ y)/(Δ x) ) Slope of linear portion for Cumulative permeated amount (mcg/cm2/min) versus time (min)

( C0 ) represents the initial concentration of the drug in the apical side (mg/mL)

Peff: Effective permeability coefficient (cm/min)

**Table 5.** The results of Ex-vivo Permeation Studies for P.I of N.F From In-situ Intranasal Gel

| Time (min) | Cumulative permeated amount ( mg / cm$^2$ min) | | |
|---|---|---|---|
| | Control | P.I 5 | P.I 9 |
| **0** | 0 | 0.000 | 0.000 |
| **30** | 0.254 | 0.032 | 0.080 |
| **60** | 0.742 | 0.139 | 0.294 |
| **90** | 1.11 | 0.312 | 0.575 |
| **120** | 1.992 | 0.589 | 0.920 |
| **150** | 2.316 | 0.934 | 1.295 |
| **180** | 3.124 | 1.378 | 1.750 |
| **210** | 3.915 | 1.912 | 2.290 |
| **240** | 4.552 | 2.542 | 2.896 |
| **270** | 5.674 | 3.270 | 3.566 |
| **300** | 6.332 | 4.095 | 4.306 |
| **330** | 7.559 | 4.973 | 5.116 |
| **360** | 8.931 | 5.989 | 6.031 |



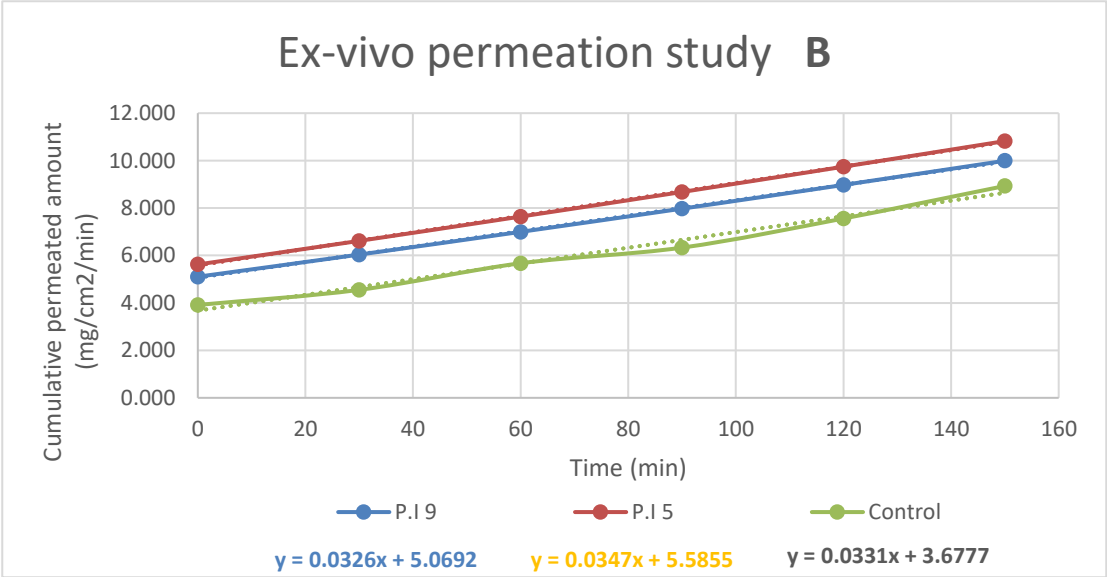




**Fig. 4.** Cumulative permeation of N.F from P.I 5 and 9 through nasal mucosa (A) , Linear portion of permeation curve (steady state) (B).

The slope of the linear portion of the curve was determined by linear regression Table 6 . The effective permeability coefficients and flux values at steady state were calculated from the slope according to the equations Eq 7, and Eq 8, respectively.

**Table 6.** In-vivo permeation parameters for selected P.I formulas

| P.I No. | $J_{ss}$ (mg/cm$^2$min) | $P_{eff}$ (cm/min) |
|---|---|---|
| **Control** | 0.0331 | 1.655 * 10 $^{-3}$ |
| **P.I 5** | 0.0347 | 1.735 * 10 $^{-3}$ |
| **P.I 9** | 0.0326 | 1.63 * 10 $^{-3}$ |

Permeation results revealed that P.I No. 5 ,and 9 as well as the control shows the initial high flux value followed by a decrease in value after about 90 min , as shown in Fig. 4, and Table 6.

The contact of fresh surface of drug present in P.I formula to the sheep mucosa cause initial rapid permeation and sustained drug permeation after 90 min due to the thermosensitive polymer like PoloX 407 (24) and mucoadhesive polymers included in the formula, the poloxamer slightly decreases the rate of drug release due to enhanced micellar core and gel network with mucoadhesive polymer which entrap the drug and sustain the release (25).

Permeation results also showed that P.I formulas have higher Jss , and Peff than the control , that can be attributed to the

permeation enhancing effect or due to increasing retention time by mucoadhesive effect of H.A (26) and MC (27) as well as for PoloX 407,and 188(28).

## Conclusion

Aqueous solutions of different compositions containing blend polymer solutions have been studied to seek their suitability as thermosensitive in situ forming gel, these studies highlighted the impacts of polymer type and concentrations on rheological, release, and permeation properties of intranasal is situ gel.

Results obtained for poloxamer based in situ gel with mucoadhesive polymers shows that drug release from P.I No. 5, and 9 have a significant similarity in comparism with that from the control and an increase in gel viscosity causes a slower erosion and drug release due to trapped drug inside polymer micelle that form at higher concentration leading to slower drug release.

Drug release analysis from P.I No. 5 and 9 fit to Peppas-Sahlin model and have m value < 0.45, so it shows Fickian release process, Fickian diffusional release occurs by a chemical potential gradient.

Permeation study show enhancement of permeation parameters for selected P.I No. 5 and 9 (Jss, and Peff) in comparing with control, due to permeation enhancing effect of H.A and M.C.

## References


1. Carradori D, Gaudin A, Brambilla D, Andrieux K. Application of Nanomedicine to the CNS Diseases. Vol. 130, International Review of Neurobiology. 2016. p. 73–113.
2. Erdő F, Bors LA, Farkas D, Bajza Á, Gizurarson S. Evaluation of intranasal delivery route of drug administration for brain targeting. Brain Research Bulletin Oct 1, 2018 p. 155–70.
3. Katona G, Sabir F, Sipos B, Naveed M, Schelz Z, Zupkó I, et al. Development of Lomustine and n-Propyl Gallate Co-Encapsulated Liposomes for Targeting Glioblastoma Multiforme via Intranasal Administration. Pharmaceutics. 2022 Mar 1;14(3).
4. Arora P, Sharma S, Garg S. Permeability issues in nasal drug delivery. Vol. 7, Drug Discovery Today. 2002. p. 967–75.
5. Teotia AK, Sami H, Kumar A. Thermo-responsive polymers: Structure and design of smart materials. In: Switchable and Responsive Surfaces and Materials for Biomedical Applications. Elsevier Inc.; 2015. p. 3–43.
6. Ur-rehman T. Controlled Release Gel Formulations and Preclinical Screening of Drug Candidates. 2011.
7. Yu S, Sun X, Liu Y. Numerical Analysis of the Relationship between Nasal Structure and Its Function. Schloss M, Sugimoto N, Maune S, editors. Sci World J [Internet]. 2014;2014:581975. Available from: https://doi.org/10.1155/2014/581975
8. Chaudhary B, Verma S. Preparation and evaluation of novel in situ gels containing acyclovir for the treatment of oral herpes simplex virus infections. Sci World J. 2014;2014.
9. Verekar RR, Gurav SS, Bolmal U. Thermosensitive mucoadhesive in situ gel for intranasal delivery of Almotriptan malate: Formulation, characterization, and evaluation. J Drug Deliv Sci Technol. 2020 Aug 1;58.
10. Karpagavalli L, Narayanan N, Maheswaran A, Raj A, Priya J, Kasiramar G. FORMULATION AND EVALUATION OF ZOLPIDEM NASAL IN SITU GEL. World J Pharm Res. 2019 May 1;6:940–51.
11. Agrawal A, Maheshwari RK. Formulation development and evaluation of in situ nasal gel of poorly water soluble drug using mixed solvency concept. Asian J Pharm. 2011 Jul;5(3):131–40.
12. mathematical-models-of-drug-release-2015.pdf [Internet]. 2015. Available from: https://www.sciencedirect.com/science/article/pii/B9780081000922000059
13. Paul DR. Elaborations on the Higuchi model for drug delivery. Vol. 418, International Journal of Pharmaceutics. 2011. p. 13–7.
14. Zhan S, Wang J, Wang W, Cui L, Zhao Q. Preparation and in vitro release kinetics of nitrendipine-loaded PLLA–PEG–PLLA microparticles by supercritical solution impregnation process. RSC Adv [Internet]. 2019;9(28):16167–75. Available from: http://dx.doi.org/10.1039/C9RA01068H
15. Peleg M. Temperature–viscosity models reassessed. Crit Rev Food Sci Nutr [Internet]. 2018 Oct 13;58(15):2663–72. Available from: https://doi.org/10.1080/10408398.2017.1325836
16. Shau PA, Dangre P, Potnis V V. Formulation of Thermosensitive in situ Otic Gel for Topical Management of Otitis Media. Indian J Pharm Sci. 2016 Jan 28;77:764–70.
17. Kim J, Chang J-Y, Kim Y-Y, Kim M-J, Kho H-S. Effects of molecular weight of hyaluronic acid on its viscosity and enzymatic activities of lysozyme and peroxidase. Arch Oral Biol [Internet]. 2018;89:55–64. Available from: https://www.sciencedirect.com/science/article/pii/S0003996918300414
18. Fakhari A, Corcoran M, Schwarz A, Fakhari A. Thermogelling properties of purified poloxamer 407. Heliyon [Internet]. 2017;3:390. Available from: http://dx.doi.org/10.1016/j.heliyon.2017.e00390240 5-8440/
19. USP-NF ⟨853⟩ Fluorescence Spectroscopy [Internet]. [cited 2022 Jul 12]. Available from: https://online.uspnf.com/uspnf/document/1_GUID-1996D278-BEC4-40A0-AC53-C8E3B31008D8_1_en-US?source=Search Results&highlight=content uniformity
20. Abdeltawab H, Svirskis D, Sharma M. Formulation strategies to modulate drug release from poloxamer based in situ gelling systems. Expert Opin Drug Deliv [Internet]. 2020 Apr 2;17(4):495–509. Available from: https://doi.org/10.1080/17425247.2020.1731469
21. Release Kinetics – Concepts and Applications. Vol. 8, International Journal of Pharmacy Research & Technology. 2019.
22. Abdul BI, Rajab NA. Preparation and in-vitro evaluation of mucoadhesive clotrimazole vaginal hydrogel. Iraqi J Pharm Sci (P-ISSN 1683-3597, E-ISSN 2521-3512). 2014;23(1):19–25.
23. Inoue D, Furubayashi T, Tanaka A, Sakane T, Sugano K. Quantitative estimation of drug permeation through nasal mucosa using in vitro membrane permeability across Calu-3 cell layers for predicting in vivo bioavailability after intranasal administration to rats. Eur J Pharm Biopharm. 2020 Apr 1;149:145–53.
24. Ricci EJ, Lunardi LO, Nanclares DMA, Marchetti JM. Sustained release of lidocaine from Poloxamer 407 gels. Int J Pharm [Internet]. 2005;288(2):235–44. Available from: https://www.sciencedirect.com/science/article/pii/S0378517304005927
25. Zhang L, Parsons DL, Navarre C, Kompella UB. Development and in-vitro evaluation of sustained release Poloxamer 407 (P407) gel formulations of ceftiofur. Vol. 85, Journal of Controlled Release. 2002. p. 73–81.
26. Son SU, Lim J, Kang T, Jung J, Lim E-K. Hyaluronan-Based Nanohydrogels as Effective Carriers for Transdermal Delivery of Lipophilic Agents: Towards Transdermal Drug Administration in Neurological Disorders. Vol. 7, Nanomaterials . 2017.
27. Franz-Montan M, de Araújo DR, de Morais Ribeiro LN, de Melo NFS, de Paula E. Nanostructured systems for transbuccal drug delivery. Nanostructures for Oral Medicine. 2017. p. 87–121.
28. Li Y, Li J, Zhang X, Ding J, Mao S. Non-ionic surfactants as novel intranasal absorption enhancers: in vitro and in vivo characterization. Drug Deliv. 2016 Sep 1;23(7):2272–9.