

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

Ammar A. Alabdly\*<sup>1</sup>, Hanan J. Kassab<sup>2</sup>

<sup>1</sup> Department of Pharmaceutics, College of Pharmacy, Anbar University, Anbar, Iraq

<sup>2</sup> Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

E-mail: 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 ( $f_2$ ) 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)

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)

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

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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. 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  $\approx$  33 kDa , purchased from (Baoji Guo Kang, Ltd–China), Methyl cellulose M.C average molecular weight  $\approx$  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

<i>P.I. No.</i>	<i>NF mg</i>	<i>PoloX407(%)</i>	<i>PoloXI88(%)</i>	<i>H.A(%)</i>	<i>M.C(%)</i>
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.

$$C_t = C_0 - K_0 t \quad \text{Eq 1. (12)}$$

Where:

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

(  $C_0$  ) represents the initial concentration of N.F

(  $K_0$  ) 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.

$$\text{Log } C_t = \text{log } C_0 - \frac{K_1 t}{2.303} \quad \text{Eq 2. (12)}$$

Where:

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

(  $C_0$  ) represents the initial concentration of N.F

(  $K_1$  ) 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 ( $C_s$ ), in the matrix, into a surrounding fluid.

$$Q = KH t^{1/2} \quad \text{Eq 3. (13)}$$

Where:

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

(  $K$  ) is the release constant of Higuchi

(  $t^{1/2}$  ) 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.

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

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

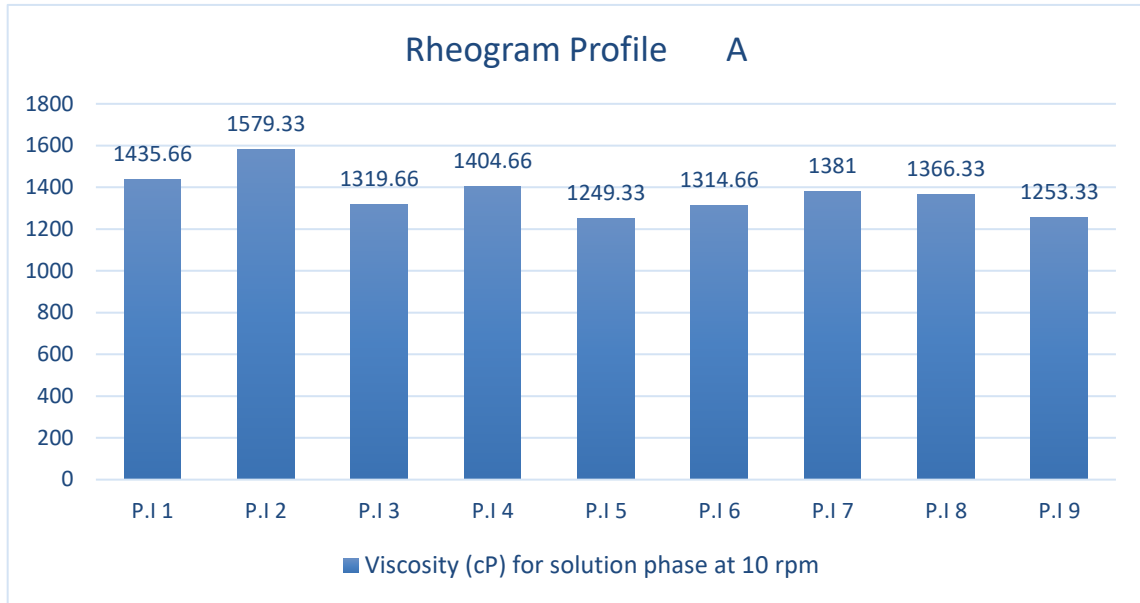
The results of the research were given as mean values  $\pm$  standard deviation (SD) and examined according to the one-way analysis of variance (ANOVA) at which significant results ( $p < 0.05$ ) and non-significant ( $p > 0.05$ ).

## RESULTS AND DISCUSSIONS

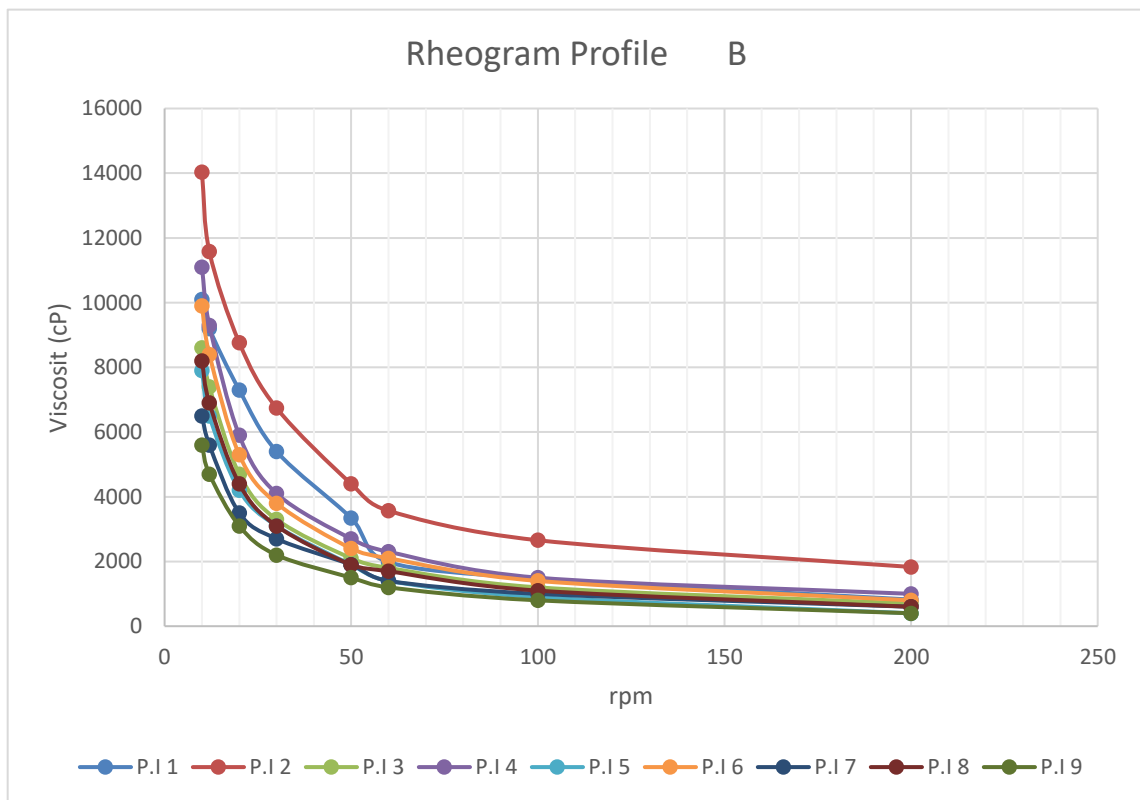
### Rheological studies

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  $24 \pm 2$  °C, at 10 rpm

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

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

P.I No.	Viscosity ( cP )								
	10rpm (Mean $\pm$ SD, (n=3))	12rpm (Mean $\pm$ SD, (n=3))	20rpm (Mean $\pm$ SD, (n=3))	30rpm (Mean $\pm$ SD, (n=3))	50rpm (Mean $\pm$ SD, (n=3))	60rpm (Mean $\pm$ SD, (n=3))	100rpm (Mean $\pm$ SD, (n=3))	200rpm (Mean $\pm$ SD, (n=3))	Shear rate
1	10118.33 $\pm$ 64.592	9179.33 $\pm$ 21.330	7300 $\pm$ 8.165	5394.67 $\pm$ 4.989	3354 $\pm$ 35.367	1981.667 $\pm$ 30.64	1475.66 $\pm$ 19.6	814 $\pm$ 7.789	
2	14034 $\pm$ 4.082	11576.67 $\pm$ 10.656	8765 $\pm$ 9.798	6748.33 $\pm$ 5.312	4392.33 $\pm$ 11.728	3575 $\pm$ 10.677	2651.67 $\pm$ 5.557	1831.67 $\pm$ 1.247	
3	8603.162 $\pm$ 9.052	7410.172 $\pm$ 10.434	4725.944 $\pm$ 18.971	3334.123 $\pm$ 24.369	2141.133 $\pm$ 29.168	1842.885 $\pm$ 10.384	1246.39 $\pm$ 22.828	749.3108 $\pm$ 34.877	
4	11088.56 $\pm$ 13.896	9299.073 $\pm$ 9.501	5918.934 $\pm$ 14.689	4129.449 $\pm$ 21.248	2737.628 $\pm$ 26.755	2339.964 $\pm$ 28.361	1544.638 $\pm$ 21.604	1047.558 $\pm$ 33.647	
5	7907.251 $\pm$ 9.556	6515.429 $\pm$ 12.777	4228.865 $\pm$ 20.864	3135.291 $\pm$ 25.161	1942.302 $\pm$ 9.978	1445.222 $\pm$ 12.012	948.1425 $\pm$ 4.056	451.0633 $\pm$ 26.110	
6	9895.568 $\pm$ 10.56	8404.33 $\pm$ 9.10	5322.439 $\pm$ 16.773	3831.202 $\pm$ 22.409	2439.38 $\pm$ 17.959	2141.133 $\pm$ 19.168	1445.222 $\pm$ 12.012	848.7267 $\pm$ 14.466	
7	6515.429 $\pm$ 12.777	5620.687 $\pm$ 15.714	3532.954 $\pm$ 23.581	2737.628 $\pm$ 26.755	1942.301 $\pm$ 19.978	1445.222 $\pm$ 2.012	1047.558 $\pm$ 23.647	649.895 $\pm$ 5.288	
8	8205.498 $\pm$ 9.226	6913.093 $\pm$ 11.639	4427.697 $\pm$ 10.101	3135.291 $\pm$ 15.161	1942.301 $\pm$ 9.978	1743.469 $\pm$ 10.790	1146.974 $\pm$ 13.237	649.895 $\pm$ 25.288	
9	5620.687 $\pm$ 15.714	4725.944 $\pm$ 18.971	3135.291 $\pm$ 15.161	2240.548 $\pm$ 8.764	1544.638 $\pm$ 11.604	1246.390 $\pm$ 22.828	848.727 $\pm$ 14.466	451.063 $\pm$ 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

<i>P.I Formula No.</i>	<i>Drug content % w/v (Mean ±SD, n=3).</i>
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 ( $R_{sq}$ ), 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  $f_2$ ) were used to compare formulas dissolution with 20mg/ml nefopam aqueous solution release profile as a control.

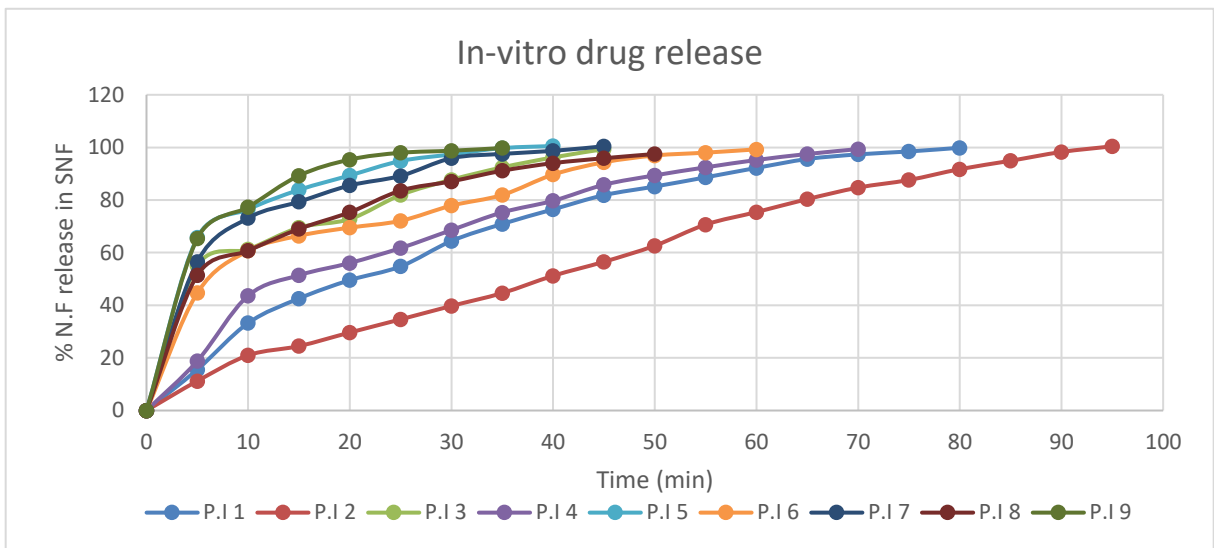


Fig. 2: Graphical representation of In-vitro drug release study of N.F HCl from in situ nasal gel formulation P.I (1-9) in SNF at 34°C.

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

Model (R2)	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 (R<sup>2</sup>)(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 f<sub>2</sub> show that P.I No. (1,2,3,4,6,7,8, and 9) have  $f_2 < 50$ , that mean these formulas have a different release profile from the control (Nefopam powder alone), meanwhile P.I No. (5 and 9) has  $f_2 > 50$ , 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.

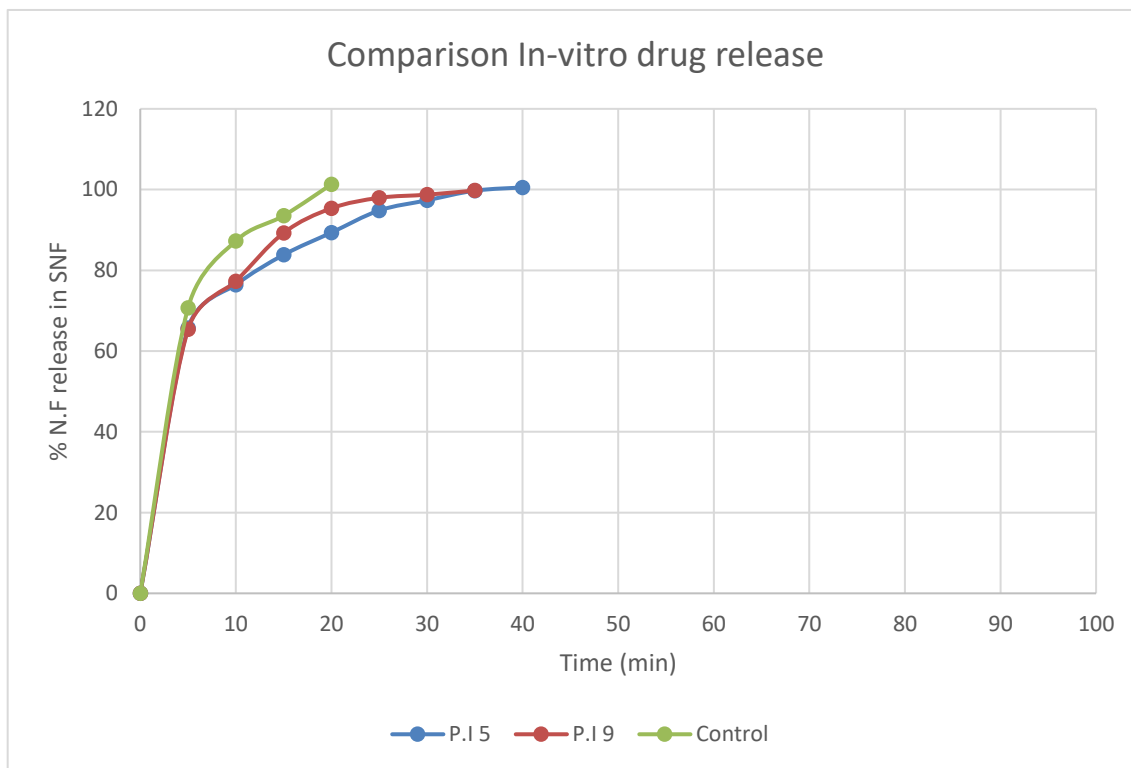


Fig. 3. Graphical representation of In-vitro drug release study of N.F HCl from in situ nasal gel formulation P.I (5 and 9) and as Nefopam aqueous solution (control ) release in SNF at 34 °C.

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 comparison 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 portion of curve) were calculated from the slope according to Eq 7, and Eq 8, respectively.

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

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

Where

J: Flux at steady state (mg/cm<sup>2</sup> min)

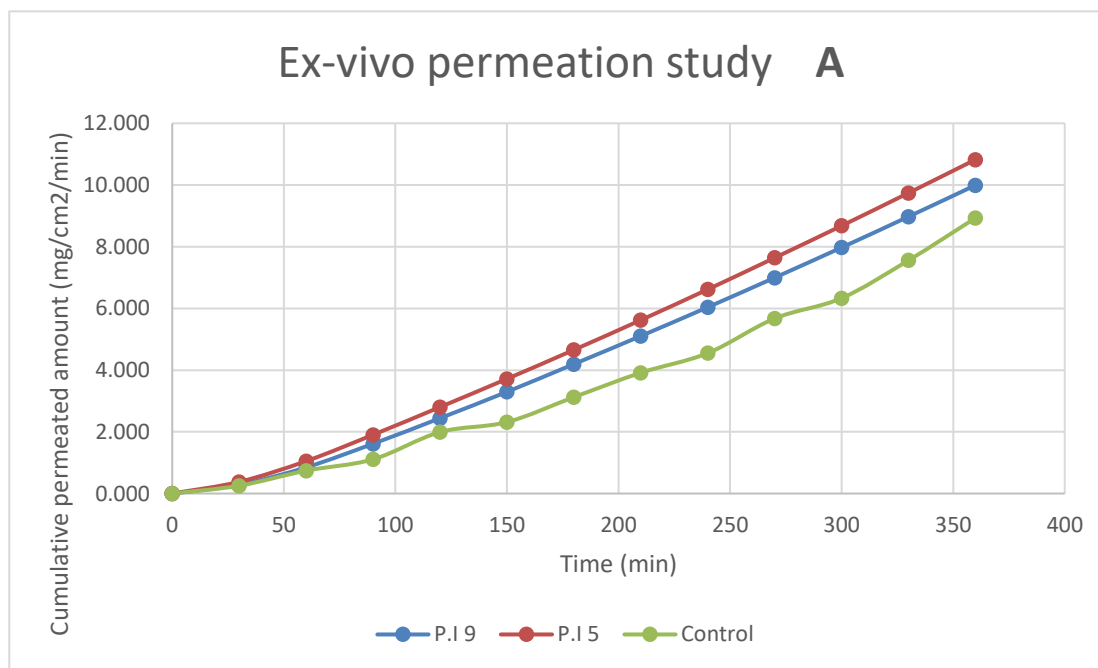
$\left( \frac{\Delta y}{\Delta x} \right)$  Slope of linear portion for Cumulative permeated amount (mcg/cm<sup>2</sup>/min) versus time (min)

( C<sub>0</sub> ) represents the initial concentration of the drug in the

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 <sup>2</sup> 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



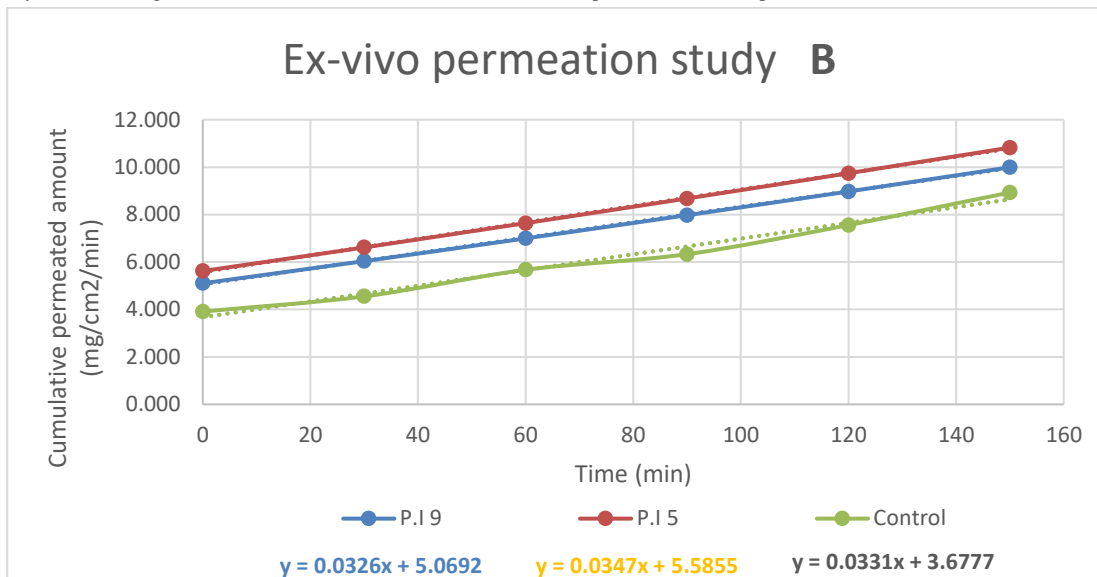


Fig. 4. Cumulative permeation of N.F from P.I 5 and 9 through nasal mucosa (A) , Linear portion of permeation curve (steady state) (B).

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.	<i>Jss</i> (mg/cm <sup>2</sup> min)	<i>Peff</i> (cm/min)
Control	0.0331	1.655 * 10 <sup>-3</sup>
P.I 5	0.0347	1.735 * 10 <sup>-3</sup>
P.I 9	0.0326	1.63 * 10 <sup>-3</sup>

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).

concentrations on rheological, release, and permeation properties of intranasal in 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 comparison 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.

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

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