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Formulation Variables Effect on Gelation Temperature of Nefopam Hydrochloride intranasal in Situ Gel (Conference Paper)

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Abstract

Nefopam (NF) HCl is a non-narcotic centrally-acting, non-opioid benzoxazocine analgesic used to relieve acute and chronic pain. It exhibits low bioavailability (about 36%) due to its first-pass degradation in the liver. Intranasal administration has been proposed as a new route for targeting active brain sites and enhancing the bioavailability of NF HCl by bypassing hepatic metabolism. In situ gel of NF HCl was prepared by the cold method using different concentrations of Poloxamer 407 (PoloX 407), Poloxamer 188 (PoloX 188), Hydroxypropylmethyl cellulose (HPMC K4M), Carbapol 934 (Car934), Methylcellulose (MC) and Hyaluronic acid(H.A) polymers. The results showed that identification tests including differential scanning calorimetry *(*DSC*)*, Fourier Transform Infra-Red Spectroscopy (FTIR), and spectrophotometric λ_{max} determination are superimposed with references, solubility study shows that NF HCl is suitable to be administered intranasally; Compatibility studies reveal incompatibility of N.F HCl with HPMC K4M and Car934; meanwhile, no interaction with MC and H.A.

In conclusion, the obtained results revealed the feasibility of the produced NF HCl intranasal in situ gel.

Keywords: Nefopam Hydrochloride, Thermosensitive in situ gel, Poloxamer 407, Poloxamer 188.

تأثير بعض متغيرات الصياغة على درجة حرارة تكون الهلام الموقعي داخل الانف لعلاج النيفوبام هايدروكلورايد (بحث مؤتمر)

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الخلاصة

ينتمي علاج النيفوبام الى فئة الادوية غير المخدرة من مسكنات الالآم للاستخدام القصير والطويل الأمد، فئتها الكيميائية البنزوكسازوسين، و يواجه مشكلة في توافره الحيوي فيبلغ (٣٦ %) لتكسره في الكبد. لذلك يرشح للتحضير كهلام موقعي للانف لتجاوز هذه المشاكل.

تم تحضيير الصيغة باستخدام الطريقة الباردة عن طريق مزج تراكيز مختلفة من بولوكزامر ٤٠٧ و بولوكزامر ١٨٨ و هيدروكسي بروبيل مثيل سليلوز ، حمض الهايلورونك ، كاربوبول ٩٣٤ ، و مثيل سليلوز. أظهرت النتائج تطابق المواد المستخدمة مع المصادر، و أظهرت دراسة الذوبانية قابلية استخدام النيفوبام على هذه الهيئة، اما التوافقية فأظهرت عدم توافق المزيج الذي يحتوي هايدروكسي بروبيل مثيل سليلوز و الكاربوبول بينما أظهرت توافق استخدام حمض الهايلورونك و المثيل سليلوز. يستنتج من ذلك ان النتائج المستحصلة كشفت عن القدرة لتصييغ النيفوبام كهلام موقعي داخل الانف لتحسين توافره الحيوي.

الكلمات المفتاحية : نيفوبام هايدروكلورايد ، هلام موقعي متحسس للحرارة ، بولوكزامر ٤٠٧ ، بولوكزامر ١٨٨ .

Introduction

A direct nose to brain delivery system is an excellent way to increase absorption to the central nervous system via the olfactory zone positioned at the top of the nasal cavities crossing the blood-brain barrier to reach the brain. Nose-to-brain delivery offers many advantages including the absence of stomach and pancreatic enzymes, mucus's pH neutrality in the nasal cavity, less dilution by the gastrointestinal tract contents, and the mucosa are more permeable to drugs than the gastrointestinal system. Additionally, It diminishes the therapeutic dosage and associated adverse effects[(1)]. This mode of administration may be advantageous for treating serious brain diseases like tumors or deteriorating neural illnesses such as Parkinson and Alzheimer [(2)] or as migraine therapy[(3)]. N.F HCl is a non-narcotic centrally-acting, non-opioid benzoxazocine analgesic used to relieve acute and chronic pain [(4)] and chemical name of 5- methyl -1 -phenyl -1,3,4,6-tetrahydro- 2,5-benzoxazocine; hydrochloride as seen in Figure 1 and molecular weight of 289.8 log P ~ 3.4[(5)].

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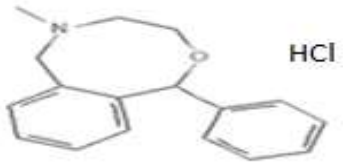

Figure 1. Chemical structure of Nefopam HCl

Orally administration is associated with low bioavailability (about 36%) due to its first-pass degradation in the [6] and shows several common side effects related to peripheral action of nefopam hydrochloride, such anticholinergic side effects include urinary retention, dry mouth, and nausea [7].

N.F HCl has a pKa of 8.98, s0877t65dc vo it will be mainly in an ionized form in nasal mucosa pH (4.5-6.5) [8] mnhuitrewerjkgoi9 /and a molecular weight lower than 300,allow a better penetration via passive diffusion [9]. Also, the log P is higher than 3^5 therefore, penetration is predicted to be over 80% [10].

In situ gel is a solution that undergoes sol-gel transformation by a specific physiological trigger such as body temperature in the case of thermosensitive in situ gel. [3] Dalia M.N. Abouhussein et al. develop rivastigmine nose-to-brain targeted poloxamer based thermosinsitive mucoadhesive in situ gel[11].

Accordingly, the current study aims to explore the factors affecting on formulation and gelation temperature of polxamer 407 based intranasal in situ gel of nefopam.

Materials and Methods

Materials

Nefopam (NF) HCl, Hyaluronic acid (H.A), and HPMC K4M were acquired from (Baoji Guokang,Ltd–China),Car934,PoloX 407, and PoloX 188 were purchased from (Eastman Chemical company – USA), Benzalkonium chloride (BNZ) was obtained as a gift from (The State Company for Drug Industry and Medical Appliances, SDI – Iraq), Every other substance, including reagents, was of analytical grade.

Methods

Characterization of NF HCl

Identification tests to confirm the active pharmaceutical ingredients (API) and excipients' identities and purities.

Determination of the melting point and differential scanning calorimetry (*DSC*) *of NF HCl*

The melting point was determined using the capillary tube approach [12], and the thermal behavior was evaluated using a DSC-60 (Shimadzu 60 plus Japan). A small quantity of NF HCl. was sealed in ordinary aluminum pans and then heated at rate of 10°C every min. over a range of temperatures 50°C to 300°C.

Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR is effective method for identifying pure compounds and compatibility studies between the active pharmaceutical ingredient (API) and the excipient(s) [13,14]. FTIR was performed using Lambda 7600 Australia instrument for NF HCl, HPMC K4M, Car934, H.A, MC, and physical mixture.

The FTIR spectrum of N.F HCl intranasal gel of the selected formulas and the excipients used were recorded using FTIR spectrometer between spectral bands 4000 - 400 cm^{-1}. The sample was mixed in a mortar with potassium bromide (KBr), then compressed into a tiny disc using a hydraulic press.

Standard calibration curves of NF HCl

The Standard calibration curves of NF HCl in double distilled water (D.D.W), simulated nasal fluid (SNF, $CaCl_2.2\ H_2O$ 0.32 g/L , NaCl 7.45 g/L, and KCl 1.29 g/L.)[15], and aqueous phosphate buffer saline (PBS) at pH 7.4, were made by controlled dilutions of the stock solution (1mg/mL) at various concentrations (50–350 g/ml). Each solution's absorbance was measured at the N.F HCl λ_{max}. Plotting was done between the measured absorbance and the corresponding concentration.

Solubility study of NF HCl

The test tube method was used to measure the solubility of drugs in an aqueous buffer, simulated nasal fluid, and deionized water by adding an excess amount of NF HCl in 10 ml of solvents mentioned above in a glass tube, then stored at 34 ºC takes 72 hours to establish an equilibrium state in a shaker water bath. After that, a solution centrifuged for 15 minutes at 3000 rpm ; then, the solution is filtered using 0.45 µm filter paper and a final concentration of NF HCl measured after a suitable dilution to be read by UV-Vis [16]. this test is checked in triplicate manner.

Preparation of NF HCl intranasal in situ gel

The cold approach was applied to formulate different compositions of mucoadhesive thermosensitive in situ gel as detailed in Table 1. Different concentrations of Poloxamer 407 (PoloX 407) (17-20%) were blended with or without Poloxamer 188 (PoloX 188) (3-4%) in cold double distilled water (D.D.W) immersed in ice with continuous stirring for 4-6 hours.

The obtained cloudy mixture was stored in the refrigerator at 4 ºC for 24 hours to get a clear solution. The gelation temperature was determined in triplicate for each formula; only compositions with a gelation temperature in the range of 30-36 ºC [17] were selected for further addition to the final formula.

Table 1. Compositions of intranasal in situ gel polymer mixture (P).

P No.	PoloX 407 (%w/v)	PoloX 188 (%w/v)	D.D.W q.s ml
1	17		10
2	18		10
3	19		10
4	20		10
5	17	4	10
6	18	4	10
7	19	4	10
8	20	4	10
9	17	3	10
10	18	3	10
11	19	3	10
12	20	3	10

After that, different concentrations of different mucoadhesive polymers (HPMC K4M, Car934,H.A, and M.C) as shown in Table 2**.** were added slowly to a selected polymer mixture with continuous stirring for 30 min at 50 rpm. The slow stirring speed was applied to prevent foam formation that can affect the mucoadhesive polymer solubility in poloxamer solution.

Then N.F HCl and BNZ at a concentration (20 mg/ml) and (0.01 % w/v), respectively, were added slowly with continuous slow stirring for the same reason mentioned above, and complete the volume with double distilled water up to 10 ml.

Finally, the mixture was stored for 24 hours at 4 ºC to eliminate a formed foam.

Table 2. Compositions of the formulated intranasal in situ gel with mucoadhesive polymers (P.I.).

P.I. No.	**NF mg**	**PoloX 407(%)**	**PoloX 188(%)**	**HPMC K4M (%)**	**H.A (%)**	**Car934 (%)**	**M.C (%)**
1	200	17		1			
2	200	19	4	1			
3	200	18	3	1			
4	200	19	3	1			
5	200	17		0.75			
6	200	19	4	0.75			
7	200	18	3	0.75			
8	200	19	3	0.75			
9	200	17		0.5			
10	200	19	4	0.5			
11	200	18	3	0.5			
12	200	19	3	0.5			

Continued table 2.

P.I. No.	NF mg	PoloX 407(%)	PoloX 188(%)	HPMC K4M (%)	H.A (%)	Car934 (%)	M.C (%)
13	200	17			0.75		
14	200	19	4		0.75		
15	200	18	3		0.75		
16	200	19	3		0.75		
17	200	17			0.5		
18	200	19	4		0.5		
19	200	18	3		0.5		
20	200	19	3		0.5		
21	200	17			0.25		
22	200	19	4		0.25		
23	200	18	3		0.25		
24	200	19	3		0.25		
25	200	17				0.3	
26	200	19	4			0.3	
27	200	18	3			0.3	
28	200	19	3			0.3	
29	200	17				0.2	
30	200	19	4			0.2	
31	200	18	3			0.2	
32	200	19	3			0.2	
33	200	17				0.1	
34	200	19	4			0.1	
35	200	18	3			0.1	
36	200	19	3			0.1	
37	200	17					0.3
38	200	19	4				0.3
39	200	18	3				0.3
40	200	19	3				0.3
41	200	17					0.2
42	200	19	4				0.2
43	200	18	3				0.2
44	200	19	3				0.2
45	200	17					0.1
46	200	19	4				0.1
47	200	18	3				0.1
48	200	19	3				0.1

Gelation temperature

The gelation temperature is determined in triplicate by visual inspection of the glass tube when it is tilted at 90 degrees. Gelation temperature recorded when no movement of solution occurred as a result of conversion into a gel. Each glass tube that contained 2 ml of the formulation was kept in the water bath at 25 ºC; then, the temperature raised gradually at a rate of 1 ºC / min, and at the end of the minute, a glass tube titled at 90^0 in a horizontal position to show if the solution is converted into a gel [18].

Appearance

Visual inspection of selected formulas against white and black paper in triplicate were conducted to examine the occurrence of any incompatibility due to an interaction between API with excipients or among excipients themselves. A change in color or precipitation was considered as a sign of incompatibility [19].

Compatibility study

FTIR was carried out on several separated formulas observed by appearance test to compare its FTIR spectra with the original spectra of drug and polymers.

FTIR was performed with the same method mentioned previously in FTIR section.

Gelling capacity

By adding 1 ml of the system to a vial containing 2 ml of freshly prepared and equilibrated at 34° C simulated nasal fluid, and visually observing the gel formation and noting the time for gelation every 5 sec. and the time taken for the gel formed to dissolve, noting desolvation every 15 min., the gelling capacity was determined in triplicate[20].

pH of NF HCl intranasal in situ gel

The pH of the selected P.I formulas was measured by using HI98107 pH meter, Hanna, Italy, in triplicate at room temperature using pH meter. pH 4 and pH 7 standard buffer solutions were utilized to calibrate the equipment [21].

The osmolarity of NF HCl intranasal in situ gel

Osmotic pressure was measured by using a OSMOMAT 030 cryoscopic osmometer. The osmometer was zeroed by purified water and then 300 mOsmol NaCl (1%NaCl) standard solution for calibration. Then, 50 µL of the P.I formulas were used in a clean, dry microtube. after checking for bubbles of air , the measurement performed. [22], and the results were recorded in triplicate.

Mucoadhesive strength

The weight needed to separate a preparation from nasal mucosa of sheep using a modified pan balance for P.I formulae yielded the mucoadhesive detachment force. Within 30 minutes of the sheep's sacrifice, we were able to retrieve a sheep nose from the slaughterhouse. The sheep's nasal mucosa was preserved in 0.9% NaCl. It was prepared for usage after blood and bony cartilage were taken out of the mucosal membrane. A glass vial was used to hold the mucosal side. Before they were connected to the equipment, these vials were held at 34 °C for 5 minutes. One side included a modified syringe vial that was inverted and held 1 mL of formula, and the other side contained a small plastic beaker that had previously been weighted. The syringe held specific amounts of each formulation (1 mL). By permitting two minutes of contact time, it was possible to confirm that tissue glued to a petri dish underneath the syringe was in intimate contact with the formula. The weight increased in gradually in the plastic pan on the other side until the formula separated from the tissue. The bioadhesive force, which represents the minimal weight necessary for tissues to detach from the surface for any formulation, is determined using the equation;

Mucoadhesive force (dynes/cm^2) = MG / A

where M is the smallest weight in grams needed to separate two vials, G is the acceleration gravity which is (980 cm/s^2), and A is the exposed area of the tissue. For each measurement, the newly formed nasal mucosa was employed [21].

Results and Discussion

Melting point determination

The temperature at which NF HCl melted by the method mentioned above was found to be 260 °C, which coincides with the reported melting point [23].

Differential Scanning Calorimetry (DSC)

The DSC of NF HCl, shown in Figure 2 , agrees with the reference that it exhibits an endothermic peak at 259.8 ºC [24] both melting point and DSC give good evidence for the purity of crystalline drug powder.

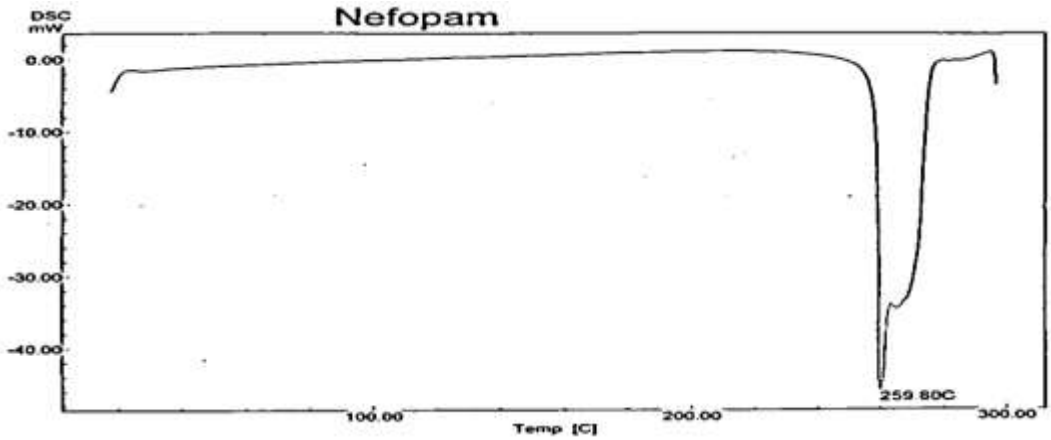

Figure 2. DSC thermogram of N.F HCl

Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR was carried out on drug and polymers powder, as shown in the Figures 3 and 4,

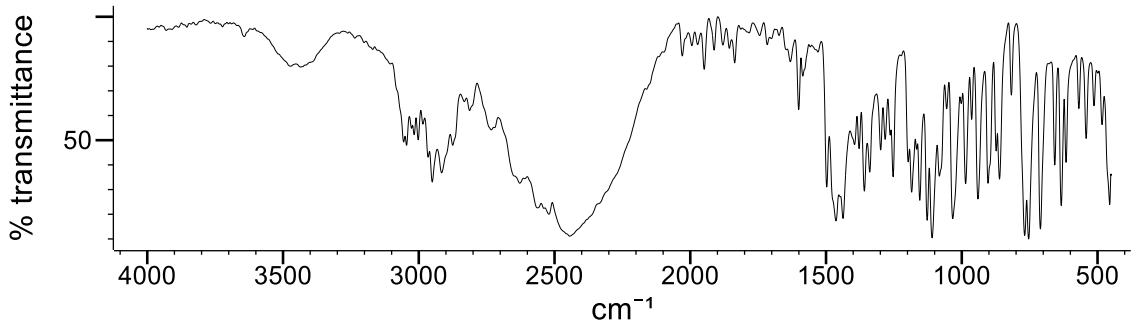

Figure 3. FTIR of NF HCl

Spectra is compatible with the characteristics absorption peaks of N.F HCl FTIR spectra, at 3440 cm^{-1} ,3049 cm^{-1} , 2947 cm^{-1} , 2440 cm^{-1} , 1464 cm^{-1} , 1437 cm^{-1} , 1357 cm^{-1}, 1107 cm^{-1} , 1028 cm^{-1} , and 760 cm^{-1} ,correlates to C-O-C of ring, substituted aromatic ring, C-H of alkane(s), resonance of amine salt NH^{+} , C=C of substituted aromatic ring, C_3-N, C-O stretch of cyclic ether, o-disubstituted aromatic ring, and mono-substituted and o-disubstituted aromatic rings, respectively. These findings are superimposed with reference [25].

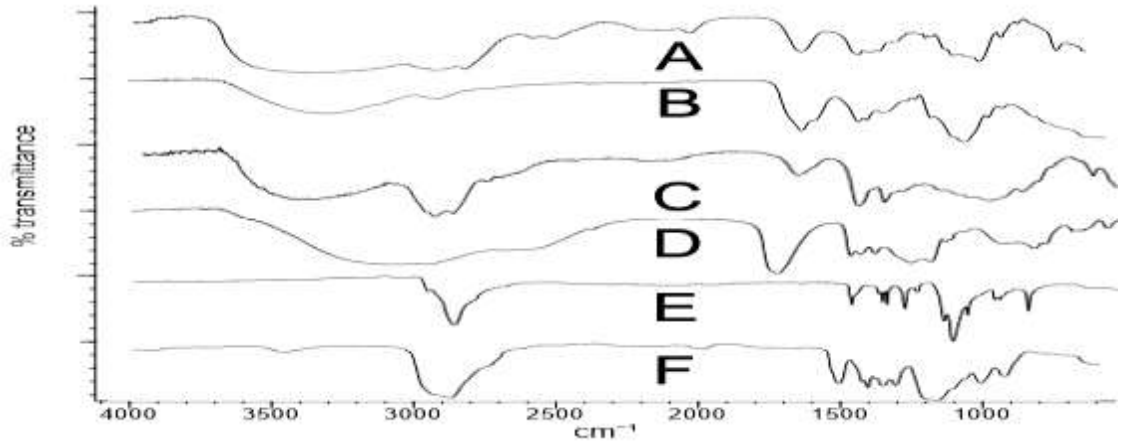

Figure 4. FTIR for HPMC K4M (A), H.A (B), MC (C), Car934 (D), PoloX 407 (E), and PoloX 188 (F).

Spectra show characteristics peaks of HPMC at 3650-2800 cm^{-1} broad peak, 1653 cm^{-1}, 1450 cm^{-1}, and 1026 cm^{-1}, that are corresponded to a broad range of hydroxyl H-bond that overlap and cover sp^3 C-H stretching of alkane, cellulose nitrate ester group that converted from cellulose hydroxyl groups, spectra of poly-(vinyl alcohol) O-H deformation spectra of a secondary alcohol, and C-O-C spectra, respectively. Spectra coincide with reference [26].

H.A peaks at 3500-3000 cm^{-1},2891 cm^{-1}, 1603 cm^{-1}, and 1030 cm^{-1} are indicated for broad spectra of H-bond of hydroxyl of acidic, alcoholic, and amine groups of hyaluronate, C-H stretching of ring moieties, COOH group of an acidic group, and C-O-C ether group. That agrees with reference [27].

MC peaks at 3600-3050 cm^{-1},2926 cm^{-1}, 1640 cm^{-1}, 1456 cm^{-1}, and 1046 $cm^{-1;}$ these are spectra for broad H-bond spectra of acidic and alcoholic -O.H. group that overlap weak C-H stretching peak at 3000-3100 cm^{-1}, C-H stretching of cyclic alkane, cellulose nitrate ester group that converted from cellulose hydroxyl groups, poly- (vinyl alcohol), and C-O-C peak, respectively. These peaks are compatible with the reference peaks[28].

Car934 peaks at 3500-2500 cm^{-1}, 1711 cm^{-1}, and 1165 cm^{-1}, correlate to carboxylic dimer H-bond COOH that hide sp^3 C-H stretching peak, C=O, and C-O stretching mode, respectively. That is match the reference[29].
PoloX 407 , and PoloX 188 has two characteristic peaks appear at 2868 cm^{-1} and 1100 cm^{-1}, representing the reading of R-CH_3 and C-O-C peaks, respectively. These are agreed with the reference[30,31].

Standard calibration curves

NF HCl shows a maximum absorption peak at 266 nm. That is compatible with the results of references [32,33]. Accordingly, standard calibration (STD) curves Figure 5 were used to calculate saturated solubility of NF in different media.

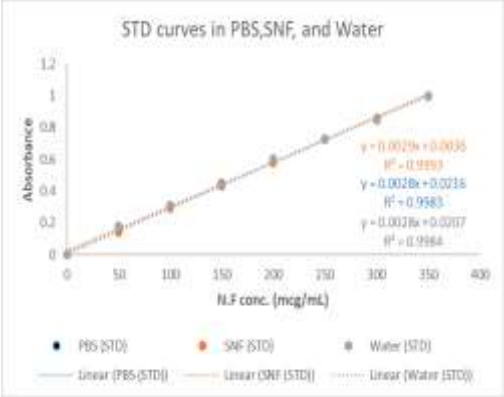

Figure 5. Standard calibration curves of NF HCl in different solvents

Solubility

The solubility of NF HCl in aqueous buffer saline at pH 7.4, simulated nasal fluid pH 6.4, and deionized water pH 7, shown in Table 3.

Table 3. Saturated solubility of NF HCl in different solvents.

Solvent	Saturated solubility (mg/ml) (Mean±SD, n=3)
Aqueous buffer	21.957 ± 0.05
SNF	13.2 ± 0.11
Deionized water	21.679 ± 0.07

The fact that only (100 – 250 µl) can be instilled in a human nose [34] makes a potent drug with high solubility essential for intranasal formulations. Drugs with poor water solubility at high strengths are challenging to synthesize without employing significant amounts of solubilizing agents, which might possibly harmful excipients. [35].

Gelation temperature

Initially sol-gel temperature was measured for polymer mixtures that composed of only poloxamer 407 and poloxamer 188 as shown in Table 4.

Table 4. Temperature of gelation for intranasal in situ gel polymer mixture (P).

P No.	PoloX 407 (%w/v)	PoloX 188 (%w/v)	D.D.W q.s ml	Sol-Gel Temp. (Mean±SD, n=3)
1	17		10	32.1 ± 0.653
2	18		10	29.5 ± 0.430
3	19		10	27.3 ± 0.120
4	20		10	23.8 ± 0.115
5	17	4	10	42.4 ± 0.346
6	18	4	10	38.5 ± 0.152
7	19	4	10	35.1 ± 0.247
8	20	4	10	28.4 ± 0.432
9	17	3	10	40.6 ± 0.461
10	18	3	10	36.0 ± 0.154
11	19	3	10	32.3 ± 0.280
12	20	3	10	27.8 ± 0.316

Only P formula with gelation temperature 30-36 ºC [15] were selected for further addition of mucoadhesive polymers and N.F HCl.

Formulations inclusion criteria and exclusion criteria

Gelation occurs at room temperature if the temperature is lower than 32ºC, which makes it difficult to manufacture, handle, and administer. The formulation will remain liquid at body temperature if the gelation temperature is higher than 34ºC, resulting in nasal drainage. Drug and additives may increase or decrease gelation temperature. As a result, the formulation's gelation temperature must fall between the following range: 30-36ºC for P formula to allow study the effect of drug and additives on gelation temperature. And the P.I formulations of Table 5 must have gelation temperature 32-34 ºC.

For the same reasons mentioned above, any formula above 36 ºC or below 30 ºC for P formula , and any P.I formula above 34 ºC or below 32 ºC are excluded from further studies.

Results from Table 4 indicated that PoloX 407 or PoloX 188 alone could not provide a suitable gelation temperature. Only formula (P No. 1) with (17% PoloX 407) show gelation temperature at suitable range. In cases of mixtures of PoloX 407 and PoloX188, several formulations (P No. 7,10,and 11) are gelled at the included temperature.Table 5 show that (P.I No. 1, 4, 5, 8, 9, 12 ,17 ,20, 21, 24, 28, 29, 32,33,36,41,44,and 45) are gelled within included temperature.

Table 4 and 5 show that as the concentration of PoloX 407 increase, the gelation temperature decreases due to aggregation and formation of critical gel concentration (cgc),which become more easily as the concentration increases, and as the concentration of PoloX188 , HPMC K4M, H.A, M.C, and Car934 increases, the Sol-Gel temperature increase because these polymers will build up inside a gel texture of PoloX407 and inhibit gel formation so that the critical gel temperature will be higher[36].

Table 5. Gelation temperature(S-G) of the P.I formula.

P.I	1	2	3	4	5	6	7	8
S-G Temp (Mean±SD, n=3)	33.9 ± 0.113	40.1 ± 0.425	38.2 ± 0.236	34 ± 0.364	33.2 ± 0.168	39.4 ± 0.536	40.2 ± 0.325	33.1 ± 0.364
P.I	9	10	11	12	13	14	15	16
S-G Temp (Mean±SD, n=3)	33.2 ± 0.424	40.2 ± 0.244	41.3 ± 0.461	32.2 ± 0.145	34.1 ± 0.295	41.2 ± 0.196	42.1 ± 0.325	34.2 ± 0.212
P.I	17	18	19	20	21	22	23	24
S-G Temp (Mean±SD, n=3)	33.2 ± 0.315	40.4 ± 0.213	41.5 ± 0.116	34 ± 0.187	34 ± 0.285	41.9 ± 0.314	39.8 ± 0.412	33.9 ± 0.440
P.I	25	26	27	28	29	30	31	32
S-G Temp (Mean±SD, n=3)	34.8 ± 0.173	41.7 ± 0.238	42 ± 0.315	34 ± 0.164	34 ± 0.253	42.1 ± 0.426	40.2 ± 0.154	33.1 ± 0.332
P.I	33	34	35	36	37	38	39	40
S-G Temp (Mean±SD, n=3)	33.9 ± 0.227	37.8 ± 0.463	38.9 ± 0.352	33.1 ± 0.109	35.9 ± 0.267	39 ± 0.315	40.7 ± 0.573	38.8 ± 0.012
P.I	41	42	43	44	45	46	47	48
S-G Temp (Mean±SD, n=3)	34 ± 0.173	38.5 ± 0.532	38.9 ± 0.425	34 ± 0.316	33.1 ± 0.305	35.7 ± 0.311	36.9 ± 0.130	35.8 ± 0.189

Appearance

Appearance and clarity test reveal formation of cloudy and precipitate at the bottom of container for formulas that contain HPMC K4M and Car 934 in all concentrations.

Table 6. shows that P.I formula No. (1-12) and (25-36) are cloudy with precipitate, and one selected precipitated cloudy formula for HPMC K4M (P.I No.4) and Car934 (P.I No.26) are subjected to FTIR scan to check its compatibility and reason of separation.

Table 6. Appearance (P.A) results (Cloudy -, Very Cloudy --, Clear +, Glassy ++, Precipitate (ppt.))

P.I	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
P.A	- ppt.	- ppt.	- ppt.	-- ppt.	- ppt.	- ppt.	- ppt.	- ppt.
P.I	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
P.A	- ppt.	- ppt.	- ppt.	- ppt.	++	+	+	+
P.I	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
P.A	++	+	++	+	++	++	++	++
P.I	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
P.A	- ppt.	-- ppt.	-- ppt.	-- ppt.	- ppt.	-- ppt.	- ppt.	- ppt.
P.I	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
P.A	- ppt.	-- ppt.	- ppt.	- ppt.	+	+	+	+
P.I	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
P.A	+	+	+	+	++	+	+	+

Compatibility study

Physicochemical compatibility between NF HCl and different excipients was studied using FTIR to examine any interactions between the NF HCl and the excipients used in the P.I formulation. The spectrums were compared to the spectrum of NF HCl alone. FTIR spectra of P.I No. 4 and 26. are shown in Figure 6.

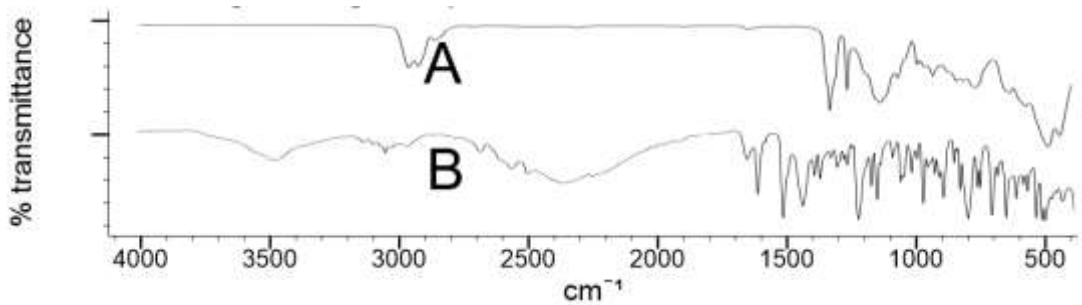

Figure 6. FTIR of P.I No. 4 (A) and P.I No. 26 (B).

In comparing with FTIR of pure drug Figure 3., we find that a peak has lower intensity with the absence of peaks at 2800 cm^{-1} – 1460 cm^{-1} region and absence of a characteristic peak at 1000 cm^{-1} – 1100 cm^{-1} of cyclic C-O-C; these might be resulted from the interaction of N.F HCl with HPMC K4M and Car934.

Despite the critical need to verify API-excipient compatibility, there is currently no globally acknowledged standard for evaluating such interactions; FTIR and appearance are straightforward methods often used in analytical labs [13].

Incompatibility is characterized as a change that occurs and an unfavorable product is produced, which may influence the pharmaceutical product's safety, effectiveness, stability, and appearance [19].

Gelling capacity

Gelation time is an important feature as gelation temperature, because the formula should transform into gel upon instillation but also remain as gel for the period of time sufficient for the drug release. Only clear and un precipitated formulas are checked. Gelation time is described in Table 7.

Table 7. Gelation time and gelling capacity of PI formulas

P.I Formula No.	Gelation time (Sec.) (Mean±SD, n=3)	Gelling capacity (hr:min) (Mean±SD, n=3)
13	56.66 ± 2.886	6:15 ± 0.00
16	60 ± 0.00	6.1 ± 0.086
17	46.66 ± 2.886	5.2 ± 0.173
20	48.33 ± 2.886	4:15 ± 0.00
21	46.66 ± 2.886	5.1 ± 0.086
24	40 ± 0.00	3.63 ± 0.317
41	50 ± 0.00	5.1 ± 0.086
44	46.66 ± 2.886	3.816 ± 0.317
45	50 ± 0.00	5.1 ± 0.173

The gelation study was conducted in SNF (pH 6.5). All the formulations on contact with the gelation medium had undergone sol-to-gel transition due to the presence of gel-forming polymers such as poloxamer. Gelation characteristics of the formulations showed that increasing the concentration of poloxamer 407 increased the gelation time, while the combination with poloxamer 188 showed less gelling capacity as seen in Table 7. Also the rise in the concentration of HA and M.C increased the time of gelation for the formulas [3] , meanwhile different concentrations of different polymers show non-significant effect on gelation time.

pH of selected NF HCl intranasal in situ gel

The degree of ionization of a drug molecule intended to be administered intranasally depends on two factors, the dissociation constant of the drug and the pH value [37] of nasal fluid (4.5-6.5) [38].

The pH value of the formula plays a significant role in the ionization process of the molecule so that it will affect its solubility and penetration capacity; the intranasal formulation should have a controlled pH range (4.5-6.5) [38] to be tolerable. Increased pH increases the likelihood of nasal cavity infections by inhibiting the lysozymes responsible for eliminating them, while a decrease in its value will irritate the tissue [2].

The pH range of selected formulas as shown in Table 8 were 5.7-6.1 , which are accepted to be used intranasally and compatible with the nasal pH range 4.5 – 6.5 [38] at this pH range, the drug NF HCl will be mainly in ionized form to be completely soluble in the formula.

Table 8. The pH value of selected IP formulas.

P.I	13	16	17	20	21	24	41	44	45
pH (Mean±SD, n=3)	5.7 ± 0.243	5.8 ± 0.152	5.9 ± 0.216	5.9 ± 0.022	6.1 ± 0.171	6.1 ± 0.142	6 ± 0.415	6 ± 0.104	6.1 ± 0.028

Osmolarity of NF HCl intranasal in situ gel

Osmotic pressure is required for all biological activities that involve solute diffusion or fluid transfer across membranes. While hypotonic formulation induces epithelial cells to expand and promote water intake and drug absorption, hypertonic solutions increase cell shrinking and decrease the likelihood of release. The formulation should, however, range from 200 to 600 mOsm/l. to protect the nasal mucosa's since these changes might have a harmful impact during chronic treatment. Finally, hypertonic formulations stimulate mucociliary clearance,

increasing medication excretion [2]. Table 9 shows the osmolarity range 590 – 311 was within an acceptable range; osmolarity is directly proportional to the concentration of thermosensitive and mucoadhesive polymers, with H.A It shows the significant impact on osmolarity.

Table 9. Osmolarity value of selected P.I formula

P.I	13	16	17	20	21	24	41	44	45
Osmolarity (Mean±SD, n=3)	543 ± 0.081	590 ± 0.102	484 ± 0.119	552 ± 0.258	403 ± 0.317	461 ± 0.163	358 ± 0.401	396 ± 0.259	311 ± 0.066

Mucoadhesive strength

The residence time of the P.I formulas in the nose can be prolonged by the mucoadhesive force of the gel. The mucoadhesive force of the P.I formulas should be enough to supply good opposition to the gel mucocilliary clearance. The oligosaccharide sequence of the mucin glycoprotein in the mucus membrane forms a hydrogen bond with the polymer in the formula, producing the mucoadhesive force. Evaluation of the mucoadhesive strength in terms of detachment stress demonstrated (Table 10) that formulations with higher concentrations had improved mucoadhesive qualities.

Table 10. shows the mucoadhesive force of the selected P.I formulations

P.I Formula No.	Detachment weight (g) (Mean, n=3).	Mucoadhesive force (dyne/cm^2)
13	7.03 ± 0.055	8776.31
16	7.52 ± 0.170	9388.03
17	6.48 ± 0.271	8089.68
20	7.31 ± 0.320	9125.86
21	6.01 ± 0.076	7502.93
24	6.73 ± 0.176	8401.78
41	2.77 ± 0.116	3458.09
44	3.55 ± 0.273	4431.85
45	2.63 ± 0.079	3283.31

Mucoadhesive strength for formulations containing H.A (P.I No. 13,16,17,20,21,and 24) show two-three folds higher mucoadhesive detachment force than that of formulations that containing M.C (P.I No. 41,44,and 45) , due to the HA capacity to include hydrogen bonding , and large molecular weight [39]. Also, poloxamers have shown mucoadhesive effect.

The faster fluid uptake from the mucus layer that enables the polymer chain to penetrate the mucin network and establish adhesive contacts is thought to be the cause of the higher mucoadhesive strength of the delivery system, which may lead to enhance retention and improved absorption.

Strong mucoadhesive force of the P.I can avoid the drainage of the drug from the nose, causing greater absorption through mucosal tissues and extended retention. The nasal mucosal membrane, however, can be harmed by excessive mucoadhesive force (i.e., more than 10,000 dyne/cm^2 gel) [40].All formulations does not topped the highest limit and hence appeared as formulations with optimal mucoadhesion properties.

Conclusion

From these research findings, it was determined that N.F HCl intranasal in situ gel that is prepared by the cold method could be formulated by Poloxamer 407 as a gel-forming polymer with Poloxamer 188 to modify its gelation temperature, along with Hyaluronic acid and Methylcellulose as mucoadhesive polymers, these studies highlighted the impacts of polymer type and concentrations on gelation temperature.

It was concluded that N.F HCl P.I formulas (13,16,17,20,21,24,41,44 and 45) could be used to formulate NF HCl intranasal mucoadhesive in situ gel.

Further studies on the compatibility, drug release, and permeation are recommended.

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