

Effect of different mucoadhesive polymers on release of ondansetron HCl from intranasal mucoadhesive in situ gel

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Abstract:

The aim of this work was to promote an intranasal mucoadhesive drug allocation system to simplify the systemic delivery of ondansetron HCL (OND-HCl) for the immediate and sustain prevention of repeated nausea and vomiting caused by the initial and repeated courses of anticancer therapy. An in situ gel dosage form was used to raise the permanence time and hence the absorption of OND-HCl from nasal mucosa. Temperature stimulated in situ gel formulations were intended by cold method using polymers like kolliphor 407, chitosan and HPMC E15. A mixture of polysorbate 20 and ethanol (1: 2 ratio) was used as solvent to dissolve the drug. The pH of in situ gel solution was measured to pH range (4.5-6.5). The six in situ gel formulas were characterized for gelation temperature, pH, viscosity, drug content, mucoadhesion and dissolution release. The temperatures of conversion of all the formulas solution to gel were within the range of 30–43°C. The drug content of all six in situ gel formulas showed drug content uniformity (99.15–99.76%). Dissolution release of the drug from in situ gel formulas showed immediate and sustained release features with Higuchi model and zero order model mechanisms.

Key words: in situ gel, OND-HCl, kolliphore 407, chitosan, HPMC E15.

تأثير البوليمرات اللاصقة المخاطية المختلفة على تحرر دواء الأوندانسترون هايدروكلورايد من جل لاصق مخاطي أنفي

الخلاصة:

أن الهدف من هذا العمل هو تطوير نظام تسليم الدواء أنفي لاصق مخاطي لتسهيل إيصال أوندانسترون هايدروكلورايد داخل الجسم للوقاية الفورية والمستديمة من الغثيان والقيء المتكررة الناجمة عن الدورات الأولية والمتكررة من العلاج المضادة للسرطان. تم استخدام نظام التبلور في الموقع لزيادة وقت الإقامة وبالتالي الامتصاص للأوندانسترون هايدروكلورايد بزيادة في الغشاء المخاطي للأنف. تم إعداد السائل المتحول حرارياً إلى جل باستخدام الطريقة الباردة باستخدام البوليمرات مثل البولكسامير 407، كيتوسان والهايدروكسي بروبيل ميثيل سيليلوز، تم استخدام خليط من توين 20 والإيثانول (1: 2 نسبة) كمذيبات للدواء، قياس الأس الهيدروجيني لدرجة الحموضة لمحلول بولوكسامير (4.5-6.5)، تم تقييم الصيغ الدوائية من ناحية، درجة حرارة التحول المحلول إلى جل، قابلية التبلور، مقياس الحموضة، اللزوجة، المحتوى الدوائي، قوة التلاصق المخاطي وقابلية التحرر الدوائي في المختبر. إن درجة حرارة التحول من سائل إلى جل لجميع الصيغ كانت ضمن النطاق (30-43 درجة مئوية)، وكل الصيغ الدوائية تؤثر إلى محتوى دوائي عالي (99.15-99.76%). أظهرت دراسة التحرر للدواء من جميع الصيغ الدوائية خصائص الافراج المستمر. وجاء الافراج عن الدواء من خلال هذه التركيبات السائل المتحول إلى جل نموذج يتبع أليات هيكوشي والمرتبة الصفرية.

Introduction:

Oral route is the desirable and convenient method of drug administration because of easy manufacturing and administration. Low absorption for many drugs through the gastrointestinal tract led to research on alternate routes of drug delivery (1). Researchers have selected nasal mucosa as an alternate route to achieve faster and higher drug absorption. Revulsion of the nasal mucosa as a therapeutically alternate

route came in recent years as a convenient and reliable route, not only for local but also for the systemic administration of drugs (2).

Ondansetron hydrochloride (OND-HCl) is selective serotonin 5-HT₃ receptor antagonists used to prevent nausea and vomiting associated with initial and repeated courses of cancer chemotherapy, radiotherapy, anesthesia and surgery (3). Mean bioavailability in healthy

subjects, following administration of a single 8-mg tablet, is approximately 56% due to extensive metabolism in the liver. Finally the OND-HCl exhibits nonlinear pharmacokinetics, possibly due to saturation of hepatic metabolism. Mean elimination half-life is 5.5 hr (4, 5).

In-situ gel is a novel drug delivery system in which the preparation is in a solution form before administration in body, but it converts into a gel upon responds to external stimuli at the site of administration due to its polymeric components (6, 7), such as intranasal mucoadhesive thermosensitive in situ gel of loratidine containing poloxamer 407, carbopol 934 p (8).

The mucociliary clearance is one of the major barrier of the nasal drug route. To overcome this problem there is so many adverts, one of this is utilize of the polymer adhesive to the nasal mucosa to raise the nasal permanence time as chitosan and HPMC (9), and kolliphor 407 as thermoreversible polymer. The change of solution to gel in respond to temperature is dependent on the concentration of the kolliphore (10).

The aim of this study was to develop intranasal mucoadhesive in situ gel (IG) formulation containing OND-HCl for immediate and sustain prevention of nausea and vomiting through nasal route. This may lead to improve drug absorption and its plasma concentration through avoiding hepatic metabolism leading to enhancement in drug bioavailability, reducing its dosing repetition and prolong its action.

2. Materials and Methods:

2.1 Materials:

OND-HCl powder, HPMC E15 polymer, kolliphore 407 polymer and chitosan was purchased from Hangzhou Hyper chemicals, China, Methanol, ethanol and polysorbate 20 were purchased from J.T Baker, China.

2.2 FT-IR study:

To study any sign of complexation and interactions, if any, between OND-HCl and excipients, FT-IR was used. The FTIR spectra of samples of the in situ gel formulation in comparison with pure drug were recorded using an FT-IR spectrophotometer (FTIR -8300 Shimadzu, Japan).

2.3 Construction of Calibration Curve:

Standard stock solution of OND-HCl (1mg/mL) was prepared by dissolving (100 mg) of the drug in 100 mL methanol. The ultraviolet absorption spectrum (λ_{max}) of standard stock solution of OND-HCl was obtained using a Perkin Elmer Lambda 2 UV visible spectrophotometer and 5 cm quartz cells, over a wavelength range of 200 to 400 nm. The working standard solution was scanned against methanol as a blank (3, 5).

Calibration curve of OND-HCl in methanol was obtained by preparing serial dilutions of the drug from the standard stock solution (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 mL) up to 10mL with methanol in 10 ml volumetric flasks. The absorbance of these diluted solutions were determined spectrophotometrically at the previously estimated λ_{max} and plotted against concentration to get a calibration curve. The R^2 value and calibration curve equation were obtained.

2.4 Preparation of Nasal Mucoadhesive In situ Gel for OND-HCl (IG):

A weighed amount of kolliphor 407 was lingeringly added to a beaker contain 15 mL of the cool water (at $4 \pm 2^\circ\text{C}$) with constant stirrer speed (500 rpm) for 2 hr. The temperature of water was remained cool at $4 \pm 2^\circ\text{C}$ throughout the preparation. The prepared solution was preserved cool overnight in refrigerator. Later, chitosan and HPMC E15 was/were added to kolliphor dispersion with permanent stirring. The weighed amount of drug (0.5% w/v; 500 mg) was dissolved in the mixture of polysorbate 20 and ethanol (1: 2). The drug

solution was added to kolliphor dispersion. The final volume was made up with water

(30 mL) (11). Six formulas have been prepared for IG as shown in table-1.

Table-1: Composition of the drug loaded IG formulations

composition	F1	F2	F3	F4	F5	F6
kolliphore 407%w/v	15	20	15	20	15	20
Chitosan %w/v	0.5	0.5			0.3	0.3
HPMC E15%w/v			1	1	0.2	0.2
OND-HCl %w/v	0.5	0.5	0.5	0.5	0.5	0.5
Polysorbate 20-ethanol (1:2) (mL)	6	6	6	6	6	6
DW (mL)	30	30	30	30	30	30

2.5 Determination of Sol-Gel Temperature (Tsol-gel):

The sol-to-gel transition temperature was determined by placing a test tube containing 2 mL of the refrigerated IG formulas (F1-F6) to a test tube (10 mL), with a diameter of 1.0 cm and sealed with a parafilm, the tube was kept in a water bath at low temperature about 15°C. The temperature of water bath was increased slowly in increment of 1°C (equilibration was allowed for 10 min after each temperature increment) until gelation occurred and the preparation would no longer move upon slanting the test tube at 90° (12).

2.6 Determination of pH:

The apparent pH of the all prepared IG formulas (F1-F6) was measured using digital calibrated pH meter at room temperature using 5 mL sample in a 10 mL beaker.

2.7 Viscosity of Formulation at Gel State:

The viscosities of the prepared IG formulas (F1-F6) were measured using viscometer (Brookfield-DVE-USA) and were read directly after 30 seconds. Measurements were performed in triplicate using suitable

spindle number 64 and sheared at a rate of 3, 4, 5, 6, 10, 12, 20, 30, 50, 60, 100 rpm, at gelation temperature of each IG formula (13).

2.8 Drug Content:

Accurately, one mL of liquid IG solution (equivalent to 5mg/ml OND-HCl) from each IG formulas (F1-F6) was transferred to 100 mL volumetric flask and 70 mL of methanol was added. The sample was sonicated for 30 minutes until clear solution obtained. The volume was diluted up to 100 mL with methanol and centrifuge at 3000 rpm for 15 minutes then filtered using millipore filter 0.22µm. One ml sample was withdrawn from prepared solution and diluted to 10 mL with methanol. Contents of OND-HCl was determined spectrophotometrically using double beam UV-Visible spectrophotometer (Shimadzu 1650 PC-Japan) at λ max= 246nm (14, 15).

2.9 Mucoadhesive Force Determination:

The mucoadhesive strength of each nasal IG formulas (F1-F6) was determined by measuring the detachment force of the in situ gel formula from fresh sheep nasal mucosal tissue (detachment stress). This test was done by using mucoadhesive

measuring device modified in our laboratory and the tissue specimen obtained from the mucosal side of sheep nasal cavity. The device consist of two-arm balance. One of the arms (right hand pan) is tied to glass plate using string and the nasal mucosa tissue is supported on other glass plate of same size, to allow the smooth surface of nasal mucosa face the upper side of the glass. One gram of the prepared IG formulas (F1-F6) was placed on the mucosal tissue. The right pan was lowered (by adding extra weight in the right pan) and attach the IG formula for 2 minutes where the IG will be spread over mucosal tissue, water was slowly added to the left pan using a burette until the nasal mucosa was detached from the IG film. The mucoadhesive force was calculated by determining the weight of water required to separate the mucosa using the following equation (16, 17):

$$DS = w \times gr / a$$

Where; DS = Detachment stress (dyne/cm²), w =weight required for detachment of two glass plates in grams, gr = Acceleration due to gravity (980cm/s), a = Area of tissue exposed in rectangle shape (width× length) which is 3 cm² in all preparations.

2.10 Dissolution Release:

OND-HCl release was measured using dialysis membrane (MWCO 12000 Da). Rotating paddle dissolution apparatus type II was used to measure the in vitro drug release from all prepared IG formulas (F1-F6). The sealed dialysis bag containing 6 gm of IG was immersed into a dissolution media containing 600 mL phosphate buffer pH 6.4 (equivalent to 1.5 gm gel for 150 ml dissolution media) with a speed of 50 rpm (18). The temperature of the medium was preconditioned and maintained at

34±0.5°C. Five mL aliquots withdrawn at time intervals (5, 15, 30, 45, 60, 90, 120, 150, 180, 240 and 360 min) and immediately replaced with fresh dissolution medium (19). The drug content in the withdrawn samples was determined spectrophotometrically at 246 nm using a UV/Vis spectrophotometer.

2.11 Release Mechanism Analysis:

Four different models including zero order, first order, Higuchi model, and Korsmeyer's peppas were considered for characterization of the kinetics of OND-HCl release from in situ gel formulation (F1-F6) (20).

3. Results and Discussions:

3.1 FT-IR study:

The FT-IR spectrum of pure drug showed the distinctive peaks values which are 756 (o-disubstituted benzene), 1279 (C-N), 1458 and 1479 (CH₃), 1531 (C=C aromatic), 1638 (C=N, C=O in six member ring), 3410 (H₂O). The FTIR spectrum of OND-HCl after preparation in IG formulas displayed same functional groups band with very slight shifting indicating the compatibility of excipients with the drug.

3.2 Construction of Calibration Curve:

Scanning of the stock standard solution of OND-HCl (1 mg/ml) by UV-spectrophotometer at 200 - 400 nm gave the spectrum that have wave length of maximum absorption (λ max) at 246 nm in methanol. The calibration curves of OND-HCl in methanol showed a straight line obtained by plotting the absorbance versus concentration with high regression coefficient (R² =0.9987) which indicates that the calibration curves follow Beer's law within the range of concentration used (Figure 1).

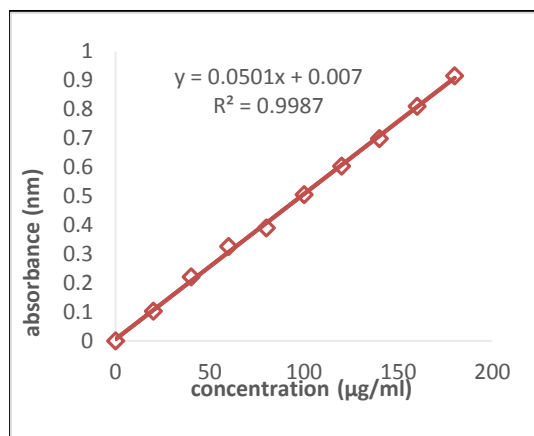


Figure-1: Calibration curve of OND-HCl in methanol

3.3 Determination of Gelation

Temperature:

In situ gel preparation was designed to be a liquid solution at room temperature and changed to gel near nasal temperature. Table 2, Figure 2 showed gelation temperature of the prepared IG formulas (F1-F6). Effects of kolliphore 407 concentration was studied on the gelation temperature and it was found that the IG formulas (F2, F4 and F6 containing 20% w/v of kolliphore 407) have gelation temperature range 30 – 34°C, while the IG formulas (F1, F3 and F5 containing 15% w/v of kolliphore 407) have gelation temperature range 35-43°C due to the consolidation of the network structure of the kolliphore 407 at higher concentration in the solution and turn to neatly overcrowded as a result higher volume and number conqueror by micelles to form the gel at low temperature (21).

The IG formulations F1 and F2 (containing chitosan as mucoadhesive polymer) showed lower gelation temperatures than IG formulas F3 and F4 (containing HPMC E15). This is because chitosan has greater capacity to propagate viscosity and to make more comprehensive intermolecular hydrogen bonding to yield a close constancy in the gel structure (21).

3.4 Determination of pH:

The pH of the nasal preparation is very important mainly to prevent the growth of

pathogenic bacteria, to avoid irritation of the nasal mucosa microorganism and to sustain normal physiological ciliary movement (22). The pH of all IG formulas (F1-F6) was measured using pH meter. The pH values were ranged (5.0-5.9) as shown in Table (2). All the formulas having pH within the range specified for nasal formulation (4.5-6.5) which is acidic pH. This indicating that the excipients used in the formulation does not affect pH. This pH range is useful in keeping the drug in its soluble form since this pH is below drug pKa.

3.5 Viscosity of Formulation at Gel

State:

Figure (3) shows the viscosity values for IG formulas (F1-F6) at gelation temperature. All the preparations show quite low viscosity at low temperature while there was a considerable increase in viscosity at the point of gelation temperature. The results also showed that as the concentration of kolliphore 407 increased from 15% (F1, F3 and F5) to 20% (F2, F4 and F6) the viscosity of formulations increased.

3.6 Drug Content:

The drug content of all IG formulas (F1-F6) results agreed with the requirements of USP, indicating high content uniformity of the prepared formulas. The results presented in table-2.

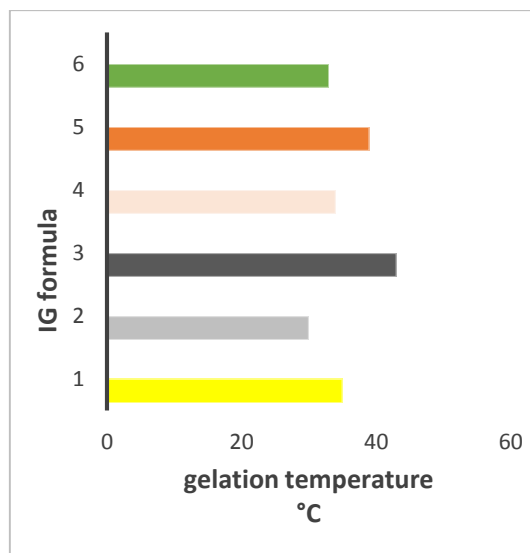


Figure -2: Sol-gel transition temperature of IG formulas.

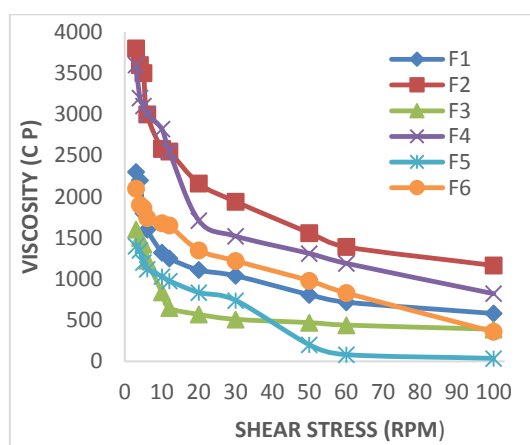


Figure-3: Viscosity of IG formulas at gelating temperature of each IG formula for OND-HCl.

3.7 Mucoadhesive Force Determination:

The IGs mucoadhesive force is the result of the hydrogen bond formation between the polymer in IG and the oligosaccharide chain of mucin glycoprotein in mucus membrane. Figure 4 showed the mucoadhesive force of the all prepared IG formulas (F1-F6) and it showed that as the amount of kolliphore 407 increased (F2, F4 and F6) there was increase in the mucoadhesive force of the IG due to formation of more compact lattice structure as well as increase of density. The mucoadhesive of IG formulas (F 1 and F2) containing chitosan are higher than those containing HPMC E15 (F3 and F4)

due to the ability of chitosan to comprise trimmer hydrogen bonding:

3.8 Dissolution Release:

The ondansetron HC release from different IG formulas (F1-F6) was shown in figure (5). The IG formulas (F1, F3 and F5) containing 15 % poloxamer-407 showed higher % release after 4 hr than the IG formulas (F2, F4 and F6) containing 20% kolliphore 407, this is because the kolliphore 407 delays the drug release due to the lessening in proportion of water channels resulting for reinforcement micellar structure As the kolliphore 407 consists of very large amount of micelles in aqueous phase, the incorporated drug may be released by diffusion through gel

matrix [23]. Drug release can also be affected by the gel viscosity, aqueous channel size, and drug distribution between the micelles and the aqueous phase. The increase in kollophore concentration causes slight increase in viscosity and thus

slightly decreases the release of the drug from the gel [22]. Therefore, F6 showed 100% release after 6 hrs, so it is recommended as best optimized mucoadhesive IG for OND-HCl

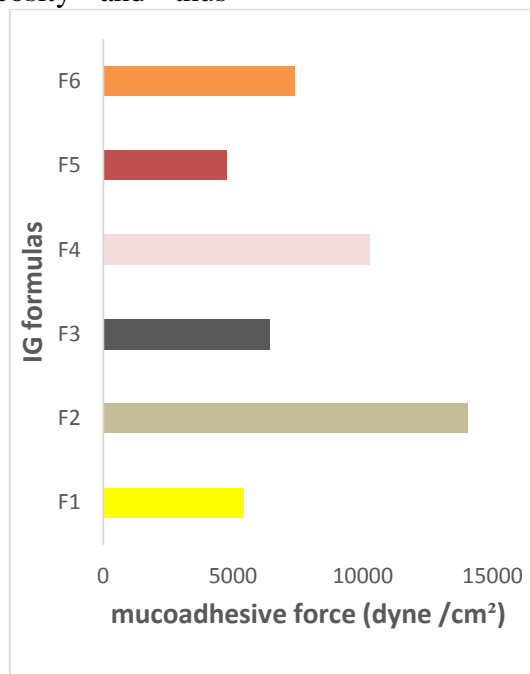


Figure-4: Mucoadhesive force of IG formulas.

3.9 Release Mechanism Analysis:

In-vitro release data were fitted to various mathematical models such as Zero order, First order, Higuchi and Korsmeyer-Peppas model in order to understand the mechanism of drug release and the release rate from prepared formulations.

Table 3 illustrated the correlation of dissolution data to different models of release kinetic. The IG formulations

follows Higuchi kinetics and zero order indicated by highest regression value (R²). For Korsmeyer-Pappas model, the value of release exponent (n) defines the release mechanism; the n value of IG formulas are between 0.45 to 0.89 indicating anomalous (non-Fickian) transport, which refers to combination of drug diffusion, and matrix erosion mechanism drug release [25].

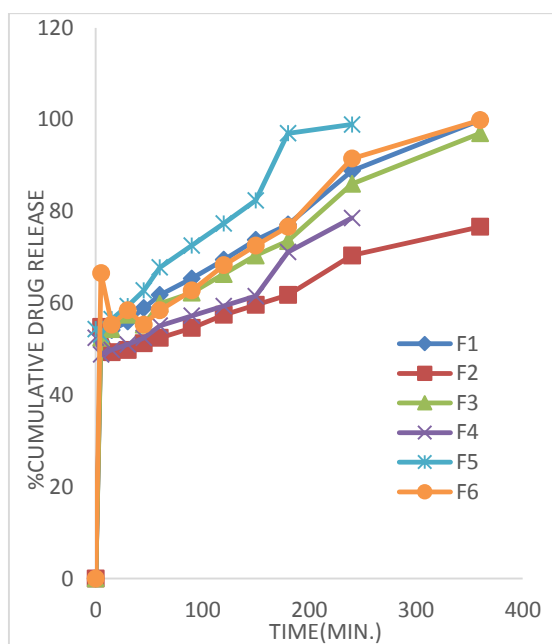


Figure-5: In-vitro drug release profile of OND-HCl from IG formulas in phosphate buffered solution (pH 6.4) at 34±1°C.

Table 2: Evaluation data of IG formulation

Evaluative data	F1	F2	F3	F4	F5	F6
Gelation temperature °c	34	30	43	36	35	32
pH	5.7	5.3	5.2	5.0	5.9	5.8
Content uniformity %	99.3	99.2	99.7	99.8	99.6	99.2

Table-3: Release mechanism analysis of drug release data of IG formulas.

IG	Zero order		First order		Higuchi model		Korsmeyer peppas model		Best fit model
	K ₀	R ²	K ¹	R ²	K _H	R ²	n	R ²	
F1	0.16	0.561	0.005	0.21	3.904	0.805	0.53	0.652	higuchi
F2	0.11	0.917	0.004	0.17	2.64	0.630	0.48	0.590	zero order
F3	0.18	0.97	0.005	0.20	3.698	0.782	0.515	0.625	zero order
F4	0.12	0.51	0.005	0.18	2.757	0.668	0.485	0.603	higuchi
F5	0.19	0.648	0.005	0.21	4.147	0.822	0.54	0.663	higuchi
F6	0.18	0.881	0.005	0.2	3.671	0.709	0.512	0.608	zero order

Conclusion:

Thermoresponsive in situ gel of OND-HCl (0.5% w/v) was luckily prepared by cold fashion using kolliphore 407,

chitosan, HPMC E15, polysorbate 20 ethanol (1: 2 ratio) as a drug dissolving solvent. The gelation temperature of all the formulations were within the range of 30–

43°C. It was observed that the lower the concentration of kolliphore 407 in the IG formulas increases its conversion temperature. By addition or increase in the concentration of hydrophilic polymer, viscosity, and mucoadhesion were increased. All the formulations exhibited uniform drug content. Drug release study of all the formulations showed burst release in first minutes. The release of drug through F6 followed and zero order mechanisms indicating sustained release profile. The nasal residence time has improved due to mucoadhesive polymers combination, and this can be viewed as acceptable alternative to conventional nasal drops. The ease of administration as drops coupled with its ability to provide sustained release thermosensitive gel could probably result in less frequent administration and better patient compliance.

References:

- 1- Chein YW, Chang SF. Intranasal drug delivery for systemic medications. *Critical Reviews in Therapeutic Drug Carrier Systems*. 1987; 4 (2): 67-194.
- 2- Chein YW, Su KSE, Chang S. *Anatomy and physiology of the nose*. New York, Marcel Dekker Inc: Chen YW. , Su KSE. , Chang SF., eds.; 1989; vol 39, pp 1-19.
- 3- USP DI. The United States Pharmacopoeial convention Inc. 15 edn. Rockville, MD; 1995; pp 2062-2063.
- 4- Britain HG. *Analytical profiles of drugs substances and excipients*. University of Granada, Spain: Salem II, Lopez JMR, Galan AC, Edi; 2001; vol 27: pp 301-338.
- 5- GlaxoSmithKline. Zofran (ondansetron hydrochloride) tablets, zofran ODT (ondansetron) orally disintegrating tablets and zofran Oral Solution: GlaxoSmithKline research Triangle Park. NC; 2011.
- 6- Shah H, Patel M. In situ gelling system: An insight, *inventi Impact*. 2012 ;(3):143-170.
- 7- Kute JU, Darekar AB, Saudagar RB. In situ gel-novel approach for nasal delivery. *World journal of pharmacy and pharmaceutical sciences*. 2013; 3(1):187-203.
- 8- Singh MP, Kumar A, Pathak K. Thermally triggered mucoadhesive in situ gel of loratadine: β -cyclodextrin complex for nasal delivery. *AAPS Pharm SciTech*. 2013; 14, (1).
- 9- Bhardwaj TR, Kanwar M, Lal R, Gupta A. Natural gums and modified natural gums as sustained- release carriers. *Drug Deliv Ind Pharm*.2000; 26: 1025-1038.
- 10- Ban E, Park M, Jeong S, Kwon T, Kim EH, Jung K, Kim A. Poloxamer-based thermoreversible gel for topical delivery of emodin: Influence of P407 and P188 on solubility of emodin and its application in cellular activity screening. *MDPI*. 2017; 22(246).
- 11- Chaudhary B, Verma S. Preparation and evaluation of novel in situ gels containing acyclovir for the treatment of oral herpes simplex virus infections. *Scientific World Journal Volume*. 2014; 2014:7.
- 12- Miller SC, Donovan MD. Effect of poloxamer 407 gels on the miotic activity of pilocarpine nitrate in rabbits. *Int J Pharm*. 1982; 12:142-152.
- 13- Xu H, Shi M, liu Y, Jiang J, Ma T. A novel in situ gel formulation of ranitidine for oral sustained delivery. *Biomolecules and therapeutics*. 2014; 22(2): 161-165.
- 14- Garg A, Aggarwal D, GARG S, Singla AK. *Pharmaceutical technology: spreading of semisolid formulations*. An update. 2002; vol 26: pp 84-105.
- 15- Sherafudeen SP, Vasantha PV. Development and evaluation of in situ nasal gel formulations of loratadine. *RPS*. 2015; 10(6): 466-476.

- 16- Ali FM., Rahi FA. And Yaseen A. Synthesis of Prodrug Ciprofloxacin Procain Male amide Polymer. Iraqi national journal of chemistry.2014; 56: 416-425.
- 17- Singh RM, Kumar A, Pathak K. Thermally triggered mucoadhesive in situ gel of loratadine: β -cyclodextrin complex for nasal delivery. AAPS.Pharm Sci Tech. 2013; 14:412-424.
- 18- Ravi PR, Aditya N, Patil S, Cherian L. Nasal in-situ gels for delivery of rasagiline mesylate: improvement in bioavailability and brain localization. informa healthcare,St John's University. 2014; 8:4.
- 19- Zaki NM, Awad GA, Mortada ND, Hady SSA. Enhanced bioavailability of metaclopramide HCl by intranasal administration of a mucoadhesive in situ gel with modulated rheological and mucociliary transport properties. Eur J Pharm Sci. 2007; 32:296-307.
- 20- Murthy PN, Dash S, Nath L,Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica—Drug Research.2010; 67(3): 217–223.
- 21- Anjan D, Subrata C, Arup M, Jayanta C, Souvik. Design and optimization of nasal in situ gel of ondansetron using factorial design. IJRPC. 2013; 3(3): 2231-2781.
- 22- Naisbett B, Woodley J. The potential use of tomato lectin for oral drug delivery: Lectin binding to rat intestine in vitro. Int J Pharm. 1994; 107: 223-230.
- 23- Patil SK, Dhage AN, Patil SV, Patil SS. Formulation and evaluation of nasal in situ gel for Alzheimer disease. IRJPBS. 2015; 2 (2):41- 58.
- 24- Riccia EJ, Lunardi LO, Nanclares DMA, Marchetti JM. Sustained release of lidocaine from Poloxamer 407 gels. Int J Pharm.2005; 288:235–44.
- 25- Nafi AT, Maraie NK. Development and in vitro/in vivo evaluation of floating in situ gelling oral liquid extended release formulation of furosemide. UK journal of pharmaceutical and bioscience. 2014; 2:1-11.