

PREPARATION AND CHARACTERIZATION OF THERMOSENSITIVE MUCOADHESIVE IN-SITU GELS FOR NASAL DELIVERY OF ONDANSETRON HYDROCHLORIDE

BY

Ghada A. Abdel Bary, Amal Y. Abdel Reheem*, Amira A. Boseila*

FROM

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

*Department of Pharmaceutics, National Organization for Drug Control and Research (NODCAR), Giza, Egypt

ABSTRACT

A nasal mucoadhesive thermo reversible in-situ gel appears very attractive since it is fluid-like prior to nasal administration and can thus easily be installed as a drop allowing accurate drug dosing. The feasibility of developing an efficacious intranasal formulation of the potent antiemetic drug Ondansetron HCL has been undertaken in this work. The ultimate goal is to circumvent the first-pass elimination of the drug when taken orally. Poloxamers P407 and P188 (20/5% w/v) were used using cold method to prepare thermo reversible gels as they have excellent thermo-sensitive gelling properties, water solubility, good drug release, low toxicity and irritation. Mucoadhesive polymers like chitosan high molecular weight (HMW), sodium carboxymethyl cellulose low molecular weight (LMW) and polyvinylpyrrolidone K30 (PVP) were used at concentration of 0.5 % (w/v) to form thermo reversible gels. Three nasal in-situ gels with desirable $T_{sol-gel}$ in the range of 30-35°C were developed. pH, mucoadhesion, rheological measurements, in vitro release and ex-vivo permeation studies were performed to evaluate the prepared gels. The incorporation of chitosan to poloxamer polymers showed significant increase in the mucoadhesion ability. The prepared gels exhibited non-Newtonian shear thinning behavior at 35°C. Drug contents were in the range of 97.8-100.1%. The release pattern was enhanced by the PVP polymer, in opposition; chitosan and NaCMC retarded it. Concerning permeation through sheep nasal mucosa, the steady state flux (J_{ss}) of the three formulae was found to be 3.57, 5.64 and 3.81 $\mu\text{g}/\text{cm}^2\cdot\text{min.}$, respectively. No marked alteration in the histological structure of the nose epithelial cell membrane of male Wister rats after application of the formed gels was observed to confirm their safety. The bioavailability for the optimized formulation was 86.98% providing that intranasal route could be promising for Ondansetron HCL delivery.

INTRODUCTION

Nasal delivery is increasingly considered to be an alternative route for drugs that currently require parenteral administration. As a site for systemic absorption the nasal route provide means of avoiding first pass metabolism (Ullarwar et al., 2012). The development of in situ gel systems has received considerable attention over the past few years. These systems possess potential advantages like simple manufacturing process, reduced frequency, ease of administration, improved patient compliance and comfort (Miyazaki et al 2003). In-situ gel forming drug delivery is a type of mucoadhesive drug delivery system. In contrast to very strong gels, they can be easily applied in liquid form to the site of drug absorption, swell to form a strong gel capable of prolonging the residence time of the active substance. Both natural and

synthetic polymers can be used for the production of in-situ gels. In-situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and ionic cross-linking (**Kant et al., 2011**). Poloxamers or pluronics are the series of commercially available copolymers of non-ionic nature. They were used as an in situ gel forming polymer together with mucoadhesive polymers like NaCMC, chitosan and PVP to ensure long residence time at the application site (**Alexandridis and Hatton, 1995**).

Ondansetron hydrochloride (ON) has been used to prevent and control both nausea and vomiting after cancer chemotherapy, radiotherapy and surgery.

Unlike metoclopramide, (ON) is known not to induce the undesirable side effect such as extrapyramidal reactions. It should be administered 30 min before chemotherapy, and it tends to be discharged by vomiting (**Rolia and Del Favero, 1995**). It has been used by oral and injectable administration. It is rapidly absorbed orally, but extensively metabolized by the liver (**Figg et al., 1996**). Based on this, the feasibility of developing an effective intranasal formulation of the potent antiemetic drug (ON) has been undertaken in this study.

The aim of this study was to formulate (ON) in a mucoadhesive in-situ gelling system to increase the residence time of the drug in the nasal cavity. The system would allow accurate drug dosing. The poloxamer 407/188 gel was used as the base whereby its gelation temperature was modulated so as to be liquid at 25°C and gels at 32°C. Additionally, different mucoadhesive polymers were used together with poloxamer to fortify the adhesion of the in-situ gel to the nasal mucosal surface.

MATERIALS AND METHODS

Materials

Ondansetron hydrochloride dihydrate (ON) was kindly supplied from (Ameriah pharmaceutical company, Cairo, Egypt). Risperidone (pharo, Egypt), Zofran 8mg tablets, Danset ampoules 4mgm/2ml (glakso, Egypt), Chitosan (Cs) high Mol.Wt, Mucin from porcine stomach, Poloxamer 407 and 188 (Sigma–Aldrich Company, St. Louis USA). Haematoxylin–eosin (Bark Scientific Limited, UK), thiopental (Epico, Egypt), 0.9% saline solution (Haydelina, Egypt), sodium carboxy methyl cellulose (NaCMC), poly vinyl pyrrolidone K30 (PVP), sodium hydroxide, sodium chloride, potassium chloride, calcium chloride dihydrate, propylene glycol (Fluka Chemika-BioChemika, Switzerland), benzalkonium chloride, iso-propyl alcohol, formalin, acetonitrile, zinc sulphat, ammonium acetate, glacial acetic acid, acetonitrile were purchased from (Sigma-Aldrich Chemicals St. Louis, MO, USA). Freshly prepared phosphate buffer saline solution pH 6.4. All other chemicals were of reagent grade and were purchased from (EL-Nasr Company Cairo, Egypt).

Equipment

Electric balance (Sartorius GMBH, Germany), Brookfield DV-III ultra programmable cone and plate rheometer fitted with a spindle number 52 and controlled with rheometer operating software (Brookfield, USA), dissolution tester apparatus II (Hanson research test, USA), UV 240 double beam spectrophotometer (Schimadzu Corporation, Kyoto, Japan), pH meter (Genway ltd, UK), Ultra Centrifuge (Jouan, France), magnetic stirrer (Jenway, UK), Fridge (Toshiba, Egypt), light microscope (Euromex, The Netherlands), HPLC equipped with G1311A quaternary pump and UV detector (VWD-G1314A, agilent, Germany). Thermostatic water bath (Poly Science, USA), diffusion cell (designed as per the dimensions given by (Pisal et

al), ultrasonic sonicator (Crest Trenton, U.S.A), vortex (snijders, Holland), 0.45 μ m membrane filter (nupore, India).

Preparation of (ON) thermo- reversible gels

The formulations were prepared on the weight ratio according to cold method (**Pisal et al., 2004**). Medicated in-situ gelling formulations composed of 20/5% w/v P407/P188 were prepared with the addition of mucoadhesive polymers namely: NaCMC (LMW), Chitosan (HMW) and PVP K30. The drug, benzalkonium chloride and the polymers were stirred in the calculated amount of distilled water with proper amount of propylene glycol (10% v/v) at room temperature. The dispersions were cooled; the poloxamers were added slowly and then left to hydrate at 4°C. (chitosan was dissolved in 0.1N Acetic Acid). Table (1) illustrates composition ratio of in-situ gel components.

Visual appearance, clarity and pH of in-situ gel

The clarity and color of the formulated solutions determined by visual inspection under black and white background (**Mahadlek 2008**). The pH of the medicated formulations was determined by bringing the electrode of the pH meter in contact with the surface of the formulation and allowing it to equilibrate for 1 min. The experiments were run in triplicates.

Gelation temperature determination

The gelation temperatures of formulations were determined using a modified “Visual Tube Inversion Method” (**Ur-Rehman et al., 2011**). Approximately 4 g of thermo-sensitive gel was transferred to vials and incubated in a thermostatic water bath with an increasing rate of 1°C/min; an equilibration period of 5 min was applied after each temperature raise. Observation of the gel surfaces was taken at every temperature point by tiling the vials to the horizontal position, the temperatures at which the surfaces remained immobile within 30 sec were measured by an inserted thermometer and were recognized as the gelation temperatures. The measurements were performed in triplicate.

Measurement of steady shear viscosity

The rheological properties of the in-situ gelling formulations were studied using (cone and plate Brookfield viscometer) (**Zaki et al., 2007**). The measurements were made at 35 \pm 0.1°C using spindle 52 at a shear rate ranging from 0.5 to 100 rpm. The shear rate ($\dot{\gamma}$) in S⁻¹ and the viscosity (η) in cps were determined and fitted to the power law constitutive equation: $\eta = m \dot{\gamma}^{n-1}$ (**Tung and Fang, 1994**) where m is the consistency index and n is the flow index. If n=1, this indicates Newtonian behavior while n<1 indicates shear thinning flow and the lower the value of n the more the thinning the formulation (**Asasutjarit et al., 2011**).

Mucoadhesion measurement

The mucoadhesive behavior was evaluated according to the method described by (**Hassan and Gallo, 1990**) based on the idea that the chemical interaction and entanglements between the polymer and glycoproteins in mucus causes a rheological synergism. Dried mucin was hydrated with simulated nasal electrolyte fluid (SNEF) by stirring for 3 hrs at room temperature. Six grams of mucin dispersion were mixed for 15 min with 2 gm of each polymer solutions before measurement. The viscosity of mucin (15% w/w) was measured in absence and presence of polymer solution to evaluate the mucoadhesion properties of the tested polymer solution. The

measurement was done at 35 ± 1 °C and shear rates (D) of 10, 20, 50 and 100 s^{-1} . All measurements were performed in triplicate. The viscosity of mucoadhesion component (η_b) was calculated from the following equation: $\eta_b = \eta_t - \eta_m - \eta_p$. Where η_t is viscosity of mucin with polymer, η_m is viscosity of mucin without polymer and η_p is viscosity of corresponding in-situ gelling prepared solution. The mucoadhesion index M [cp] was calculated using the shear rate D [s^{-1}] and the viscosity of mucoadhesion component (η_b) [cp] according to the equation: $M = \eta_b * D$ where (η_b) was calculated from previous eq. and D is the shear rate per second. Since (η_b) may decrease with the increase in the applied shear rate D, it was decided to use a high value of D to eliminate weakly mucoadhesive materials (**Hassan and Gallo, 1990**). The SNEF was composed of 7.45 mg/ml NaCl, 1.29 mg/ml KCl and 0.32 mg/ml $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and pH were adjusted to 5.5 (**Pund and Borade, 2013**).

Drug content

Accurately measured 1ml of each formula was shaken with 100 ml SNEF until drug completely dissolved. The solutions were filtered through whatmann filter paper. Drug content was estimated spectrophotometrically at 310 nm using plain SNEF as a blank and was calculated using standard calibration curve. The mean percent of drug content was calculated as an average of 3 readings.

In-vitro drug release study

Drug release was monitored by the USP dissolution test apparatus type II (**Zaki et al., 2007**). A dialysis tube containing 1 ml gel formulation was immersed in 500 ml of SNEF as a dissolution medium at $35^\circ\text{C} \pm 0.5^\circ\text{C}$ and rotation at 50 rpm. Aliquots of 1 ml were withdrawn at time intervals at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480 min and each aliquot was replaced by 1 ml of fresh SNEF. The samples were measured spectrophotometrically as mentioned earlier. The experiments were run in triplicates.

Ex-vivo permeation studies using diffusion cell

The freshly excised sheep nasal mucosa, except septum part was collected from a local slaughter house. The superior nasal membrane was identified and separated from the nasal cavity and made free from adhered tissues. Maintaining the viability of the excised nasal tissues during the experimental period is crucial. Within 10 min of killing the animal, the mucosa was carefully removed, then immediately immersed in phosphate buffer saline solution pH 6.4 for 15 min and was aerated (**Pund and Borade, 2013**). The membrane was mounted in between the donor and the receptor compartment of the diffusion cell. The nasal diffusion cell was designed as per the dimensions given by (**Pisal et al., 2004**) as seen in fig(1).

The position of the donor compartment was adjusted so that the mucosa just touches the permeation medium. Formulation equivalent to 0.5 ml of prepared in-situ gel was taken in the donor compartment which was in contact with the mucosal surface of the membrane, while the receptor compartment was filled with 67 ml of SNEF and its temperature was maintained at 37°C . The content of the receptor compartment was stirred using a magnetic stirrer. An aliquot of 1 ml was withdrawn at suitable time intervals and replaced with the same volume of fresh medium. These samples were analyzed spectrophotometrically at 310 nm (**Samson et al., 2012; Nisha et al., 2012**). The experiments were run in triplicates.

In-vivo nasal irritation test

Briefly, male Wister rats weighing 250–300 g were sedated with an intra-peritoneal injection of thiopental (~45 mg/kg) before each dosing to facilitate nasal administration. The rats were divided into 5 groups 3 rats in each group. Group I received 0.9% saline solution in the right nostril (-ve control), group II received iso-propyl alcohol in the right nostril (+ve control), group III, IV and V received in-situ gel formulae M1, M2 and M3 respectively once daily for 14 consecutive days, after which, the rats were sacrificed. The nasal septum with the epithelial cell membrane on each side was carefully separated from the bone. The septum was fixed with 10% formalin, sliced on a microtome and stained with haematoxylin–eosin. The left nostril (un-dosed) was used as a control. The slides of control and treated nasal mucosal tissues with the in-situ gels were examined using a light microscope (**Banchroft and Stevens, 1996**).

In-vivo pharmacokinetic study

Animal handling and drug administration

Nine male albino New Zealand rabbits weighing about 2.5 ± 0.1 kg were used. The rabbits were housed individually in stainless steel cages, fed a commercial laboratory rabbit diet. The rabbits were fasted for 18 hrs prior to and during the pharmacokinetic study. The animals were conscious during experiments and held in restrainers during withdrawing blood samples. The animals were randomly divided into three groups of three rabbits each. One group received 400 μ l (equivalent to 8mg ON) of the selected developed nasal in-situ gel formula deposited into both nostrils. The second group received the commercial oral product (Zofran 8mg tablets) administered at the back of the pharynx using gastric intubation silicone rubber with one tablet set on the tip of the tube and immediately 5ml water was administered through the tube to ease swallowing. Finally, the third group received the commercial I.V (2 ampoules of Danset 4mg each) injected into the animal marginal ear vein. (**Mahajan and Gattani, 2010**). The study was conducted according to a 2-period, 2-sequence crossover design with one week wash out period between the phases. All animal procedures were approved by the Ethics Committee of Faculty of Pharmacy, Cairo University.

Sample collection and analysis

After administration of the previously described three different dosage forms, blood samples were collected at time intervals of 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16 and 24 hrs. Blood samples were centrifuged at 4000 rpm for 15 min to separate plasma which were stored at -20°C pending HPLC analysis. Risperidone (internal standard) was added to 0.5 ml plasma and the sample was then deproteinized with mixture of 1ml acetonitrile and 50 μ l 10% w/v zinc sulphat solution. The treated samples were vortexes for 2 min, centrifuged at 10,000 rpm for 20 min. The supernatant then filtered through a nylon membrane filter (0.45 μ m) and was injected into the HPLC column, cyano (CN) (Phenomenex, 250x4.6mm ID, 5mm). The mobile phase consisted of a mixture of 50 mmole ammonium acetate adjusted to pH 3.5 with glacial acetic acid and acetonitrile (35:65 v/v), filtered through a 0.45 μ m membrane filter, and degassed by sonication prior to use. The flow rate was 1ml/min, and the detection wavelength was 310 nm. All measurements were performed at ambient temperature (**Shelsha et al., 2011**). The experiments were run in triplicates.

Data treatment and statistics

The maximum plasma drug concentration (C_{\max}) and the time to achieve this peak (t_{\max}) were determined directly from the data. The area under the concentration-time curves from 0 to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The ($AUC_{0-\infty}$) was summation of area under plasma concentration-time curve from 0 to time t (AUC_{0-t}) and area under plasma concentration-time curve from time t to infinity ($AUC_{t-\infty}$). ($AUC_{t-\infty}$) was calculated by dividing the last measurable plasma concentration with the terminal elimination rate constant (K_e). The value of (K_e) was calculated using the least-squares regression analysis of the terminal portion of the log plasma concentration vs time curve. The elimination half-life ($t_{1/2}$) was calculated by dividing 0.693 with (K_e). The pharmacokinetic parameters, ($AUC_{0-\infty}$), (C_{\max}), ($t_{1/2}$), (K_e) were analyzed statistically using one way analysis of variance (ANOVA) (**Wagner, 1975**). The values of $AUC_{0-\infty}$ and C_{\max} were logarithmically transformed before analysis. The (t_{\max}) values were analyzed using Wilcoxon Signed Rank test for paired samples. A statistically significant difference was considered when $p < 0.05$. All plasma concentrations data were dose and weight normalized and analyzed using Wagner-Nelson Method for determination of (ON) pharmacokinetics.

The absolute bioavailability F (%) of intra nasal administration (IN) was calculated using the following equation: $F (\%) = (AUC_{0-t IN} \times Dose_{IV}) / (AUC_{0-t IV} \times Dose_{IN}) \times 100$. The relative bioavailability F (%) of nasal administration was calculated using the following equation: $F (\%) = (AUC_{0-t IN} \times Dose_{Oral}) / (AUC_{0-t Oral} \times Dose_{IN}) \times 100$. (**Charlton et al. 2007**).

RESULTS AND DISCUSSION

Visual appearance, Clarity and pH of in-situ gel

Table (2) illustrates the appearance, clarity and pH. All prepared in-situ gels were in acceptable range of pH for nasal administration. Greater drug permeation is usually achieved at a pH lower than the drug pKa because under such conditions the penetrating molecules exist as unionized species. Because the pH of the nasal cavity can alter that of the formulation and vice versa, the ideal pH of a formulation should be within 4.5-6.5 (**Nishan et al., 2012**).

Gelation temperature determination

It could be concluded from table (2) that, the addition of mucoadhesive polymers generally reduced the gelation temperatures. This was in good agreement with that reported by (**Choi et al. 1998**). The gelation temperature-lowering effect of the mucoadhesive polymers used could be explained by their ability to bind to the polyoxyethylene chains of the poloxamer molecules, which promotes dehydration and causes an increase in the entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding, thus producing gelation at lower temperature (**Ryu et al., 1999**).

Measurement of steady shear viscosity

As seen in fig.2-5, all gels exhibited non-Newtonian flow and exhibited shear thinning behavior as n value < 1 . The lower the value of (n), the more shear thinning of the formulation (**Owen et al., 2000**). Mucoadhesive polymers had a viscosity-enhancing effect as revealed by the values of the consistency index (m) as shown in table (3).

Mucoadhesion measurement

Use of polymers with strong mucoadhesive capacities can significantly limit the total clearance of the formulation from the nasal cavity (**Dodou et al., 2005**). Findings show that the polymer/mucous mixtures exhibit synergistic rheological profiles, as the viscosity value of the mixture is higher than the sum of the corresponding values of separate components at all the shear rates investigated (**Alsarra et al; 2009**). Table (3), represents the extent to which the viscosity of the polymer mucin mixture differs from the expected value, based on the addition of polymer and mucin contributions which is the viscosity component (η_b). As seen in table 3 the mucoadhesive force (F) calculated at $D = 40 \text{ S}^{-1}$ were 6520, 11000 and 3920 (dyne/cm²) for M1, M2 and M3 respectively and their viscosities and mucoadhesion forces are listed in Table (3).

The values and forces of mucoadhesion for formula M2 are significantly higher than for M1 ($P < 0.01$) and M3 ($P < 0.001$). This indicated that formula M2 is able to interact more strongly with mucin due to hydrogen bonding between mucin and chitosan and the electrostatic interaction between the amine function of chitosan and sialic acid and sulfonated residues of mucin may be possible (**Suknuntha et al., 2011**).

Drug content

As seen in Table (2), the drug content was found to be in acceptable range for all three formulated in situ gels. It was in the range 97.8-100.1% that indicates uniform distribution of (ON) in the gels.

In-vitro drug release study

The release profiles of (ON) mucoadhesive nasal in situ gels (formulae M1, M2, and M3) in Fig. 6 Show that in-situ gel with PVP K30 enhanced drug release when compared to in-situ gel without mucoadhesive polymers (formula M). This enhancement was attributed to the water soluble nature of PVP which allowed more rapid penetration of dissolution medium into semisolid matrix and initiated surface dissolution/erosion (**Jones et al., 1999**). The retardation of drug release with NaCMC and Chitosan could be due to the possible squeezing effect on the aqueous channels of poloxamer micelles through which drug diffuses as well as to an increase in overall product viscosity (**Desai and Blinhard, 1998**). The correlation coefficient value R^2 was found to be >0.95 indicating goodness of fit of the data in the Korsmeyer–Peppas equation. When n is equal to 0.5, the fraction of drug released is proportional to the square root of time (Higuchi kinetics) and the drug release is solely diffusion controlled (Fickian diffusion kinetics). If $n = 1$, indicates drug release is swelling controlled (zero-order kinetics), while if $0.5 < n < 1$ indicates anomalous transport and superposition of both phenomenon (non-Fickian kinetic) (**Zaki et al., 2007**). The results of the in vitro dissolution study revealed the non-Fickian ($n = 0.5977$) or anomalous behavior of release of (ON) from the in situ gel (M2) as shown in table (4). This indicates that the dissolution of the gel controlled the (ON) release. The decrease in the diffusion rate of drug with time due to decrease in the concentration gradient can be due to gel dissolution.

Ex vivo permeation studies

To be successfully delivered through the nasal route, drug candidates should have adequate permeability. Fig. (7) and table (5) illustrate profile of (ON) permeation through sheep nasal mucosal membrane. Linear regression analysis of pseudo-steady state diffusion data allowed calculation of the steady state flux (J_{ss}) and were found to be 3.57, 5.64 and 3.81

$\mu\text{g}/\text{cm}^2\cdot\text{min}.$, respectively. The apparent permeability coefficients (P_{app}) were 0.06, 0.0945 and 0.0638 $\text{cm}\cdot\text{min}^{-1}$. Diffusion coefficient (D) were 0.55, 1.566 and 0.7162 $\text{cm}^2\cdot\text{min}^{-1}$. Ranking the three formulae in descending order according to percent amount of drug permeated/ cm^2 after 300 min, we find $M2 \geq M1 \approx M3$. Formula M 2 containing chitosan as a mucoadhesive polymer showed higher percent of drug permeated and this is related to higher mucoadhesion ability of Chitosan. The pKa of (ON) is 7.4 (Rolia and Del Favero, 1995), so it will be ionized at physiological pH (5-6.5) and hence will polar. As polar drugs with molecular weight less than 1000D_a they generally pass the membrane by paracellular route (Pires et al., 2009).

In-vivo nasal irritation test

The successful use of mucoadhesive nasal delivery systems is not only limited to their mucoadhesion efficacy, but of equal importance is their safety. After treatment the epithelial cell membrane of male Wister rats with the three prepared in-situ gels (M1, M2 & M3), no signs of irritation such as vascular congestion or sub-epithelial edema were observed and no marked alteration as compared to negative control from the histological structure as seen in Fig.8 (A-E).

In-vivo pharmacokinetic study

The concentration of ON in rabbits' plasma was determined by a validated HPLC assay. Fig. 9 shows a representative chromatogram for rabbit plasma containing (ON) and risperidone (IS) that were well separated at retention times 4.94 and 5.7 min. respectively. The mean percentage recovery of (ON) from spiked plasma samples was 97.85% and the mean correlation coefficient of the standard curve was 0.9973. The bioavailability of in situ (ON) gel was determined for the optimized formulation M2 (composed of 2% (ON), 20% poloxamer 407, 5% poloxamer 188, 10% PG and 0.5% chitosan) due to its high drug contents, slow release rate and highest permeation. In situ gel formula M2 was compared to commercial oral tablets and intravenous solution having the same (ON) dose. The mean plasma drug concentration-time profiles after administration of the IV, oral as well as the in-situ gel of (ON) are illustrated in fig 10. From the profile Plasma data of mucoadhesive nasal in-situ gel two peaks at 0.5 and 2 hr are seen. The first one corresponded to direct absorption from nasal cavity and the second to oral drug absorption that might have occurred due to portion of drug solution swallowing before conversion into gel following nasal instillation. Table 5 shows Plasma pharmacokinetic parameters for different routes formulations.

The C_{max} values were 165.4 ± 15.15 ng/ml, and 324.1 ± 20.18 ng/ml for oral tablets and nasal in-situ gel respectively. Statistical analysis revealed that the C_{max} was significantly higher in case of the nasal in situ gel ($P < 0.001$). Concerning the rate of absorption, the results show that t_{max} values were 2 hr and 0.5 hr for oral tablets and nasal in-situ gels respectively. Statistical analysis revealed that the t_{max} was significantly higher in case of nasal in situ gel when compared to oral tablets ($P < 0.001$). The high values of plasma mean residence time (MRT) of (ON) obtained from nasal in-situ gels (6.38 hr) than in case of IV solution (2.46 hr.) indicates a sustained drug release. Statistical analysis revealed that the (MRT) was significantly higher in case of nasal in situ gel when compared to oral tablets ($P < 0.001$). The calculated rate of elimination (K_{el}) values of nasal in-situ gel (0.119 hr^{-1}) was significantly lower than that of IV (0.375 hr^{-1}) ($P < 0.01$). Significant difference was found between plasma ($\text{AUC}_{0-\infty}$) of nasal in-situ gel (1026.815 ng.hr./ml) and oral tablets (695.76 ng.hr./ml) ($p < 0.05$) indicating the nasal route achieves excellent absolute and relative bioavailability of (86.98%) and (147.5%) respectively for nasal in-situ gel. Improved nasal over oral bioavailability has been previously reported for verapamil

chitosan microspheres (Abdel Mouez et al., 2014). Chitosan has been reported to improve bioavailability by achieving dual effect: its ability to increase the epithelial permeability and its mucoadhesive nature (Hinchcliffe et al., 2005).

CONCLUSION

Taken together, the mucoadhesive nasal in situ gel was developed in the present study, so as to have favorable gelation, rheological and release properties in vitro. It has demonstrated an adequate safety to the nasal mucosa of rats. Nevertheless, the most prominent advantage of the in situ gel over the silent gel is that it is fluid-like prior to contact with the nasal mucosa: a feature that is warranted for convenience of administration for patients, accuracy of drug dosing and avoidance of the bitter taste of the antiemetic drug. Intranasal (ON) in situ gel could be considered as an alternative route for both oral and intravenous administration.

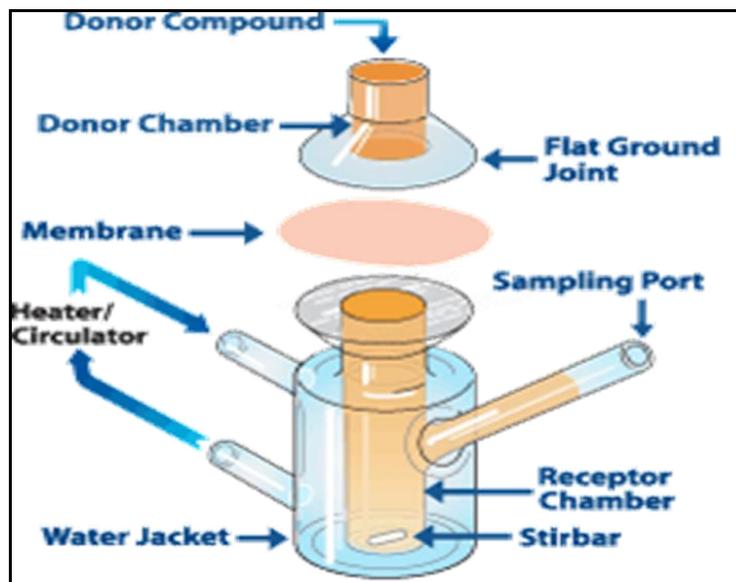


Fig. (1): Designed nasal diffusion cell

Table (1): Composition of prepared (ON) in-situ gels

Form	Drug %w/v	P407/P188 %w/v	Muco-polymer %w/v	PG %v/v	Benzalc-Cl %w/v	solvent
M	2	20/5	---	10	0.002	Dist .H ₂ O
M1	2	20/5	0.5NaCMC	10	0.002	Dist .H ₂ O
M2	2	20/5	0.5 chitosan	10	0.002	0.1N acetic a.
M3	2	20/5	0.5 PVPK30	10	0.002	Dist .H ₂ O

Table (2): physical characters of prepared (ON) in-situ gels

Form	Appearance	pH	Mean T _{sol-gel} (°C)	Drug content (%)
M	Turbide-white	6.6± 0.1	34±1	98.6±1.0
M1	Turbide-white	6.2±0.1	32±1	100.1±1.2
M2	Clear-pale yellow	5.4±0.1	33±1	99.9±1.8
M3	Turbide-white	6.4±0.1	31±1	97.8±1.7

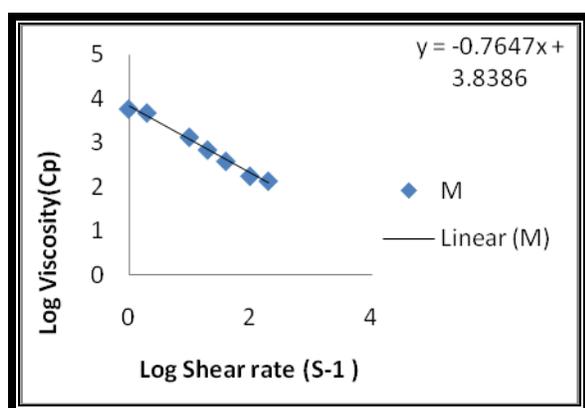
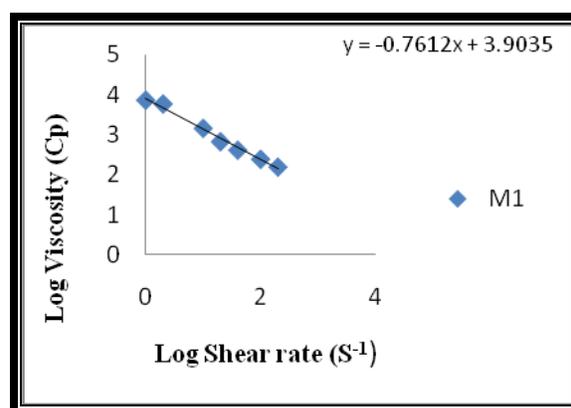
Mean ± SD, n = 3

Table (3): Viscosity measurements of mucoadhesion and power law parameters of (ON) prepared thermo reversible in-situ gels

Form	m	n	Rheological behavior	Polymer Viscosity (η_p), (cps)	Mucoadhesion Viscosity Component (η_b) (cps)	Force of Mucoadhesion F (dyne/cm ²)
M		0.2353	Non-Newtonian	-	-	-
M1	8007.56	0.2388	Non-Newtonian	225±55	163±23	6520±920
M2	14057.24	0.1842	Non-Newtonian	450±68	275±36	11000±1440
M3	6586.67	0.2197	Non-Newtonian	96±13	98±18	3920±720

Consistency index (m), flow index (n), Polymer viscosity (η_p), the mucoadhesion Viscosity component (η_b), and force of mucoadhesion(F)

Mean ± SD, n = 3

**Fig. (2): The shear viscosity of M****Fig. (3): The shear viscosity of M1**

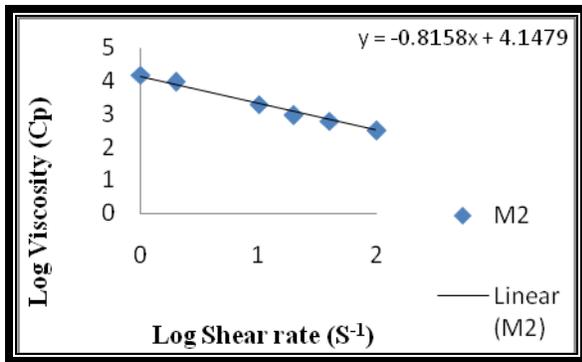


Fig. (4): The shear viscosity of M2

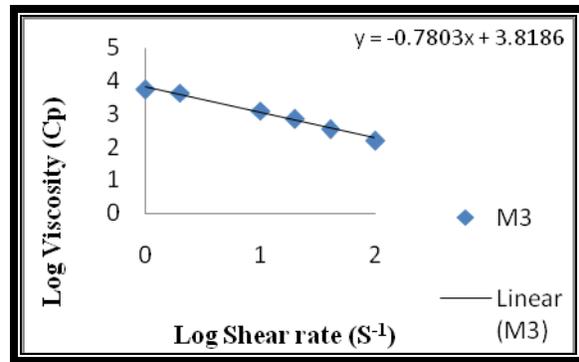


Fig. (5): The shear viscosity of M3

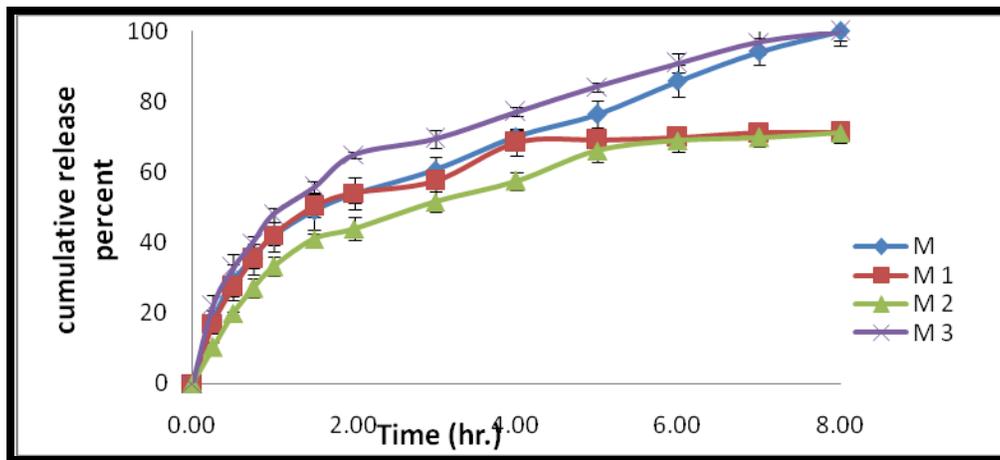


Fig. (6): Release profile of (ON) from M, M1, M2, M3 in SNES

Mean \pm SD, n = 3

Table (4): Data analysis of (ON) released from prepared nasal in-situ gels

Form	Zero order R^2	First order R^2	Higuchi R^2	Peppas R^2	Release exponent (n)	Release mech.
M	0.9583	0.8521	0.9924	0.9824	0.5225	Fickian
M1	0.7761	0.8615	0.9009	0.9542	0.501	Fickian
M2	0.8752	0.9484	0.9642	0.9512	0.5977	Non-Fickian
M3	0.9127	0.9237	0.9816	0.9952	0.5247	Fickian

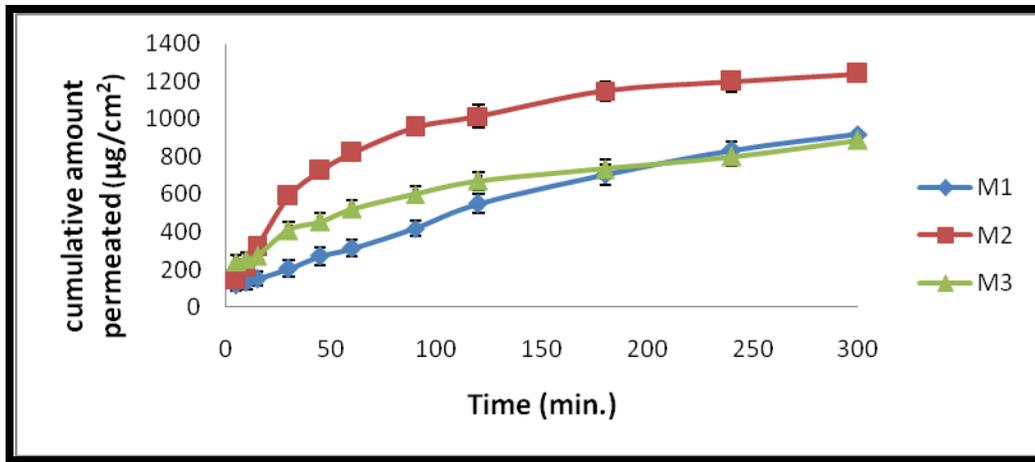
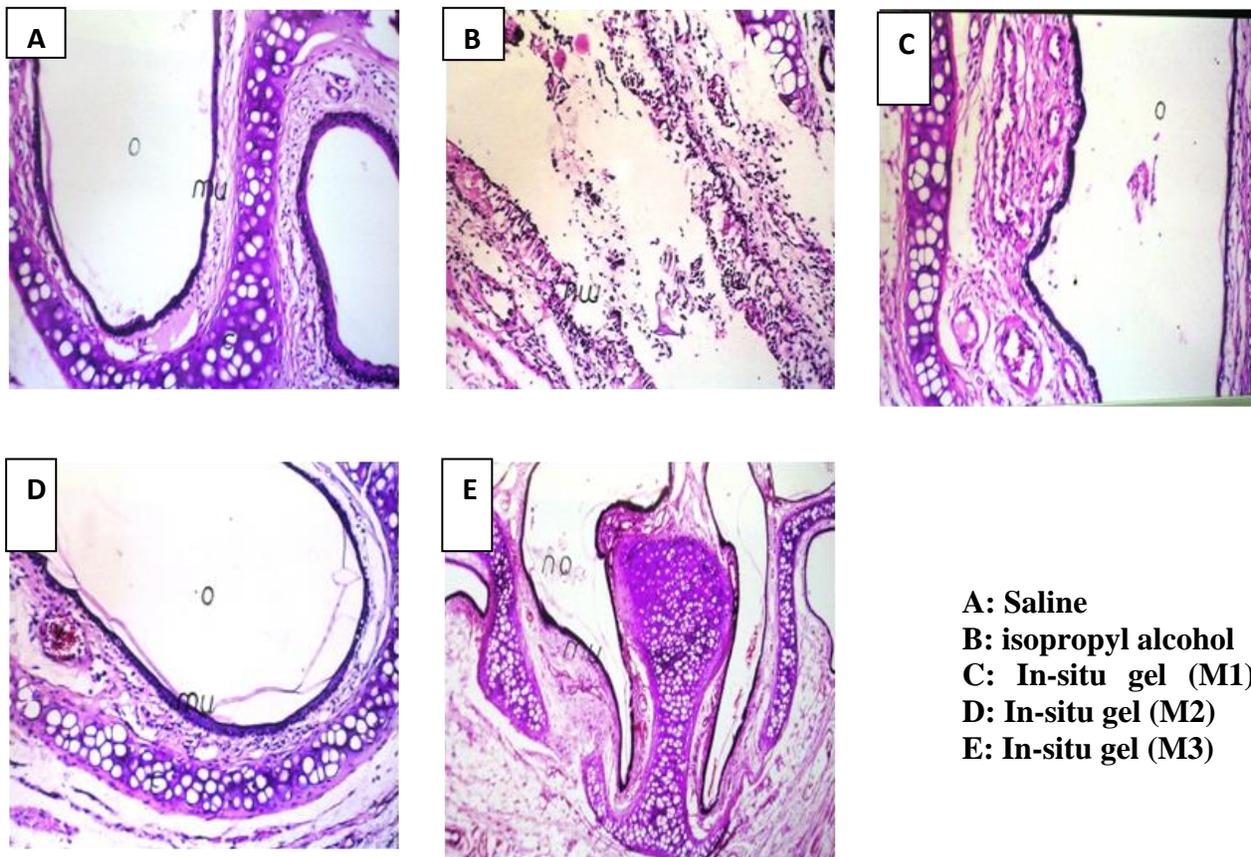


Fig. (7): Ex- vivo permeation of (ON) from in situ gels M1, M2, M3. Mean \pm SD, n = 3



A: Saline
 B: isopropyl alcohol
 C: In-situ gel (M1)
 D: In-situ gel (M2)
 E: In-situ gel (M3)

Fig. (8): Light photomicrograph of the anterior cross-section of the rat nasal cavity following administration of in situ gel formulations M1, M2 and M3

Table (5): permeability parameters of (ON) from prepared nasal in-situ gels (M1, M2, M3)

Formulae	FLUX (Jss) $\mu\text{g}/\text{cm}^2 \cdot \text{min}$	P_{app} $\text{cm} \cdot \text{min}^{-1}$	D $\text{Cm}^2 \cdot \text{min}^{-1}$	K	t_l Min.
M1	3.5668	0.06	0.553	0.011	0.00301
M2	5.6405	0.0945	1.566	0.006034	0.001064
M3	3.8069	0.0638	0.7162	0-008908	0.00233

Table (6): Plasma pharmacokinetic parameters

parameter	IV	Oral	Nasal In-situ gel (M2)
(ON)Dose	8mg	8mg	8mg
C_{max} (ng/ml)*	-----	165.4±15.15	324.1±20.19
t_{max} (hr.)	---	2	0.5
AUC_{0-24} (ng/ml.hr)*	1124.31±110.6	654.98±58.98	975.9±144.96
$AUC_{0-\infty}$ (ng/ml.hr)*	1180.58±158	695.76±55.21	1026.815±162
MRT (hr)*	2.46±0.36	4.25±0.4	6.38±0.23
K_{el} (hr ⁻¹)*	0.375±0.024	0.294±0.114	0.119±0.004

Mean ± SD, n = 3

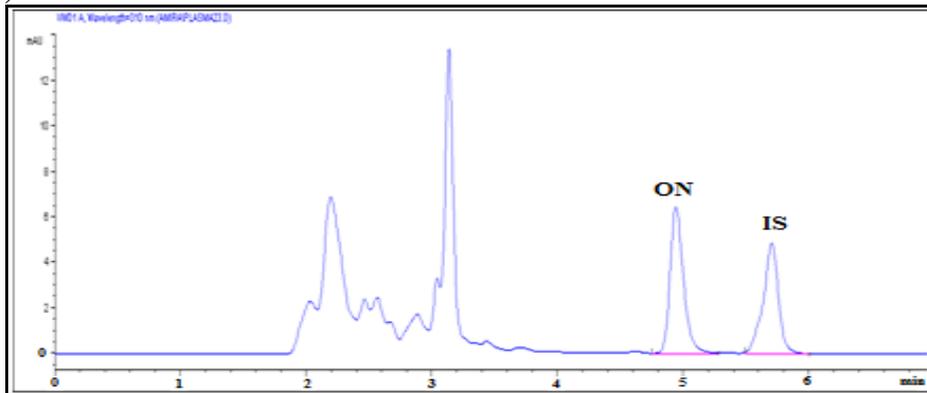


Fig. (9): Representative chromatogram of (ON) and Rispridone (IS)

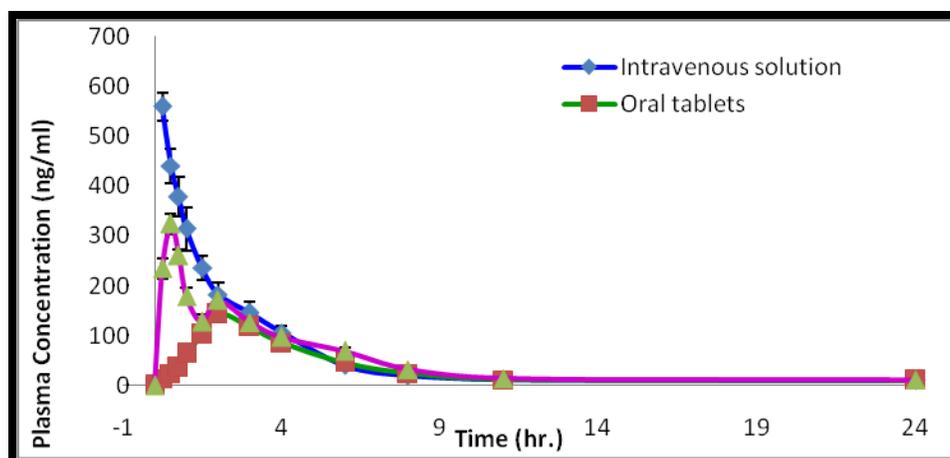


Fig. (10) Mean plasma conc.-time profile IV, Oral and

Nasal in-situ gel of (ON).

Mean \pm SD, n = 6

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الملخص العربي

صياغة و تقييم هلام موضعي معكوس الحرارة للأستخدام عن طريق الأنف يحتوى على أوندانسترون هيدروكلوريد ثنائى الهيدرات للسادة الدكتورة

غادة احمد عبد البارى - امال يوسف عبد الرحيم* - اميرة عبد الحليم بصيلة *

مــــن

قسم الصيدلانيات و الصيدلة الصناعية – كلية الصيدلة جامعة القاهرة.
* قسم الصيدلانيات - الهيئة القومية للرقابة والبحوث الدوائية

اثبتت دراسات سابقة قدرة الإيتاء الدوائى الأنفى أن يكون بديلا للإيتاء الدوائى عن طريق الحقن أو الفم وخاصة فى حاله الأدوية المضادة للقيء مثل الأوندانسترون هيدروكلوريد نظرا لسهولة تعاطى العقار عن طريق الأنف إضافة إلى ما للأنف من قدرة على التوصيل المباشر للعقار إلى المخ. وعلى ذلك فان الهدف من هذا العمل هو صياغة الأوندانسترون فى أنظمة ايتاء دوائية داخل الأنف على هيئة هلام يطيل من مدة بقائها فى التجويف الأنفى مما يؤدى الى زيادة اتاحتها الحيوية. تم استخدام هلام البولوكسامير 407 , 188 معكوس الحرارة و البوليمرات التى تلتصق بالمخاط مثل شيتوزان (وزن جزيئ مرتفع)، كاربوكسى ميثيل سليلولوز الصوديوم (وزن جزيئ منخفض) و بيروليدون (عديد الفينيل K30) بتركيز 0.5% و كان تركيز العقار فى الهلام الأنفى 10% .

تم تقييم هذه الأنظمة صيدليا و حيويأ بدراسة المعايير المختلفة مثل: درجة حرارة التحول الى هلام (درجة التهلم) من التركيزات المختلفة للبولوكسامير 407 و 188، وجد ان التركيز 5/20% له درجة تهلم فى نطاق 30-35 م و هو النطاق المنشود لاستخدام الهلام الأنفى. وكذلك تمت اختبارات اللزوجة و قوة الالتصاق بالمخاط و تعيين الانطلاق المعملى للعقار فى محلول ذائب كهربائى مماثل للأنف باستخدام غشاء السليلوز كنموذج معملى و كما أجريت دراسة لتقييم تخلل العقار من الهلام معكوس الحرارة خلال الأغشية المخاطية. اما بالنسبة لنفاذية الأوندانسترون خلال الغشاء المخاطى فقد كان مرتفع من الهلام الذى يحتوى على بوليمر الشيتوزان مقارنة بالهلام المحتوى على بوليمرات أخرى. كما ان هذه الانظمة الهلامية لم تُحدث اى تغيير مهبج ملحوظ فى التركيب النسيجى للأنف مقارنة بالمجموعة الضابطة السلبية و اخيرا أسفرت دراسة الإتاحة الحيوية للهلام الموضعى المختار باستخدام طريقة كروماتوجرافيا ضغط السائل العالى عن قيم 86,98% مقارنة بالايلاء عن طريق الحقن (المطلق) و 147.5% بالايلاء عن طريق الفم (النسبي) مثبتةً بذلك إمكانية إستخدام الطريق الأنفى كطريق فعال لتوصيل عقار الأوندانسترون هيدروكلوريد.