Characterization and Bioavailability Study of Ropinirole Hydrochloride Intranasal Mucoadhesive Thermoreversible In-Situ Gel

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Abstract: Ropinirole Hydrochloride (RHCl) is one of the most important highly selective Dopamine agonist drugs for the treatment of Idiopathic Parkinson's disease (PD). It is a potent drug (0.25-5mg) that is rapidly absorbed in humans after peroral administration. However, it undergoes extensive first-pass metabolism which is the cause for its low bioavailability (50-55%). Therefore, the intranasal delivery of Ropinirole is one of the effective strategies to improve bioavailability. Thermoreversible polymer pluronic F127 (PF127) (18% w/v) in addition to Mucoadhesive polymers Hydroxypropyl methylcellulose (HPMC E5), Hydroxypropyl cellulose (HPC), Sodium carboxymethylcellulose (NaCMC), Sodium alginate (NaALG) at different concentrations (0.3, 0.5, 0.7% w/v) were used to develop mucoadhesive PF127 in-situ gels for intranasal delivery system of Ropinirole. Full factorial design was constructed using Design-Expert® 7 software. In-vitro characterization, permeation study and histological evaluation of the prepared formulations as well as the pharmacokinetics study of the best formula were determined. F3 (18% w/v PF127, 0.7% w/v HPMC E5) was the optimized formula having the highest rheological properties, gel strength, mucoadhesiveness and safety for intranasal administration. The percentage of cumulative amount permeated through sheep nasal mucosal membrane was 95.4 % after 45 mins. Short term stability studies for 6 months indicated that 4 °C is the appropriate storage condition for the formulations. The pharmacokinetic study in rabbits revealed that the relative bioavailability of RHCl intranasal optimized formula F3 compared to the peroral reference tablets was 168.89%, with significant increase in C_{max}, $AUC_{0-\infty}$ and significant decrease in T_{max}.
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1. Introduction

Idiopathic Parkinson's disease (PD) is a neurodegenerative progressive disorder that affects dopaminergic neural systems. "Parkinsonism" is the used term for a motor syndrome whose main symptoms are tremor at rest, slowing of movement, stiffness and postural instability **(Savitt *et al.*, 2006)**.

Ropinirole Hydrochloride is non-ergoline D2/D3 Dopamine agonist having a direct action on the dopamine receptors. It is indicated for the treatment of symptoms and signs of PD, either alone or as an adjunct to levodopa. RHCl is a highly selective agonist for the dopamine D2-like receptor subtype, with very low affinity for the D1-like receptor subtype. On comparing with L-dopa, RHCl has more neuroprotective effect and slower progression of PD **(Whone *et al.*, 2003)** where it could modify oxidative cell damage and preserve dopamine neurons **(Iida *et al.*, 1999)**. Also, Ropinirole was the first medication approved by the US Food and Drug Administration (FDA) for the treatment of moderate-to-severe primary restless legs syndrome (RLS) **(Kushida, 2006).**

RHCl is a potent drug (low dose 0.25-5mg) rapidly absorbed after oral administration, reaching peak plasma concentration within 1-2 hours. However, its bioavailability is (50-55%) indicating a first-pass effect (Anon, 2014). It is extensively metabolized in the liver via CYP1A2 cytochrome P450 isoenzyme and none of its major circulating metabolites have pharmacological activity where its' mean elimination half-life is about 6 hours ($t_{1/2}$ = 6 hrs) (Kaye and Nicholls, 2000). Patients with Parkinson's disease have low compliance with swallowing oral dosage forms due to loss of muscle tone.

In order to overcome hepatic metabolism, poor bioavailability of RHCl and increase patient compliance, intranasal route is an attractive alternative route of administration than the peroral route. Intranasal route has large absorptive surface with rich vasculature and highly permeable mucosal structure with avoidance of hepatic first-pass elimination **(Merkus, 1994)**, gut wall metabolism in the gastrointestinal tract **(Arun Kumar Singh, 2012).** Also, the simplicity of this route and the ease of drug

application improve patient compliance when compared to parenteral route **(Merkus, 1994).** Moreover, intranasal route offers a non-invasive promising route of drug delivery with high permeability to both hydro- and lipophilic drugs as well as direct drug targeting to the brain **(Arun Kumar Singh, 2012)**. The nose to brain delivery is beneficial in conditions such as Parkinson's disease where a rapid and/or specific targeting of drugs to the brain is required **(Kisan *et al.*, 2007)**. So recently, different attempts were done to formulate intranasal Ropinirole Hydrochloride nanoemulsion **(Gulam *et al.*, 2012)**, nanoparticles **(Jafarieh *et al.*, 2014)**, microemulsion **(S. Mantry & Balaji, 2015**), microspheres **(S. Mantry & Balaji, 2017).**

Intranasal gel formulation is more attractive and advantageous than other intranasal formulations as powder, inserts and solution. It can prolong the residence time at the nasal absorption site and decrease the rapid mucocillary clearance and so increase drug absorption and enhance bioavailability compared with peroral delivery **(D'Souza *et al.*, 2005).**

Nowadays, intranasal in-situ gel of ropinirole becomes more convenient and attractive than ordinary gels since it is fluid-like prior to nasal administration allowing accurate drug dosing, reduced frequency of administration and so improved patient compliance. In-situ gelation can be achieved by using thermosensitive smart polymers.

Pluronic F127, a tri-block copolymer that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units (Giulia Bonacucina, 2011), is one of the excellent thermosensitive polymers known for its excellent gelling property with suitable gel strength within the nasal physiological temperature range**.** It forms negative temperature-sensitive hydrogels **(Swamya and Abbas, 2012)**. It has high solubilizing capacity, high water solubility, low irritation and toxicity with good drug release characteristics. Also, it does not require organic solvents or copolymerization agents because of which it has gained increasing attention **(Ruel-Gariepy and Leroux, 2004)**. Meanwhile, its' comparatively low molecular weight and nonionic nature makes it a weak mucoadhesive agent **(Ankita et al, 2015).**

So, combination of thermo-sensitive in-situ forming polymers as pluronic F127 with other mucoadhesive polymers promotes their properties, optimize sol-gel transition temperature, increase bioadhesive properties **(Dumortier *et al.*, 2006)** and provide firmer platform of drug delivery to the nasal cavity. Also, addition of mucoadhesive polymers can increase the viscosity and so the residence time at the site of absorption by interacting with the mucus layer covering the mucosal epithelial surface and so enhance bioavailability compared with peroral delivery **(Majithiya *et al.*, 2006).** These mucoadhesive systems - using different polymers other than the forgoing polymers used in this study - were very effective, with higher bioavailability for intranasal drug delivery of Ropinirole **(Khan *et al.*, 2010).**

The purpose of the present study is to formulate RHCl intranasal mucoadhesive in-situ gel delivery system with optimized properties using thermoreversible polymer PF127 and different mucoadhesive polymers HPMC E5, HPC, NaCMC, NaALG at different concentrations to enhance its bioavailability. To understand the variables and their interactions, statistical experimental factorial design has been constructed. In-vitro characterization is done for selection of the best formula for bioavailability study with respect to physical compatibility, gelation temperature, rheological properties, gel strength and mucoadhesiveness. Permeation study using synthetic and natural sheep nasal mucosal membrane is done. Short term stability studies are also conducted. Possible histological effects of different prepared intranasal formulations on sheep nasal mucosa are investigated. Furthermore, RHCL pharmacokinetic parameters have been studied after intranasal administration in rabbits compared to the commercially available Requip® tablets in the international market.

2. Materials and Methods

1. Materials

RHCl was kindly supplied from Eva Pharma Pharmaceuticals Co., Egypt. Requip® 1mg Tablets, Glaxo Smith Kline, Ireland. PF127, HPMC (Methocel E5), HPC, Na CMC & NaALG, Cilazapril, Methanol and Acetonitrile (HPLC grade) were purchased from Sigma-Aldrich Co., St. Louis, USA. Methyl-t-butyl-ether (MTBE) was purchased from Merck, Germany. Spectra/Pore© dialysis membrane 12,000-14,000 molecular weight cut off was purchased from Spectrum Laboratories Inc., USA.

2. Methods

2.1. Preliminary study for Ropinirole Hydrochloride Mucoadhesive Thermoreversible Nasal In-Situ Gels

Primary formulation trials were carried out to select the concentration of PF127 and the types and concentrations of the mucoadhesive polymers that gave sol to gel transition temperature within the acceptable range for intranasal administration (25-37°C). PF127 polymer in concentrations from 16% to 20% w/v was screened preliminarily to decide lowest possible concentration for thermoreversible gelling within the stated range either alone or after addition of RHCl **(Majithiya *et al.*, 2006)**. Then, different

concentrations (0.3-1 % w/v) of mucoadhesive polymers HPMC E5, HPC, NaCMC, NaALG, Carbopol 934, carbopol 940 and chitosan were then added for screening to select the types and concentrations of the mucoadhesive polymers.

2.2. Optimization of the in situ gels using factorial design

Based on primary formulation trials and physicochemical compatibility studies results (not mentioned) using Differential scanning calorimetry (DSC) and Fourier Transform-Infrared (FT-IR) Spectroscopy, it was desirable to develop an acceptable pharmaceutical formulation in shortest possible time. This was achieved using Design-Expert® 7 Software (Stat-Ease Inc., Minneapolis, MN, USA) where a $4^1 \times 3^1$ two factors full factorial experimental design was constructed. Two independent variables were evaluated, which were namely; type of mucoadhesive polymer (X_1) at four levels and concentration of mucoadhesive polymer (X_2) at three levels. The design layouts, coded value of independent factor as well as the selected Responses (dependent variables) were shown in Table (1A). The composition of the prepared formulae is shown in Table (1B). Desirability was calculated for the selection of the optimized formulae. The application of the desirability function combines all the responses into one variable and leaves the possibility to predict the optimum levels for the independent variables (**Holm *et al.*, 2006).**

Table (1A): Full factorial design used for optimization of RHCl mucoadhesive thermoreversible in-situ gel formulae

Factors (Independent variables)	*Levels*			
X_1: Type of mucoadhesive polymer	HPMC	HPC	NaCMC	NaALG
X_2: Concentration of mucoadhesive polymer	0.3	0.5	0.7	
Responses (Dependent variables)	***Desirability Constraints***			
Y_1: $T_{sol-gel}$	In range (25-37 °C)			
Y_2: Consistency index (m)	Maximize			
Y_3: Flow index (n)	Minimize			
Y_4: Gel strength	Maximize			
Y_5: Mucoadhesive strength	Maximize			
Y_6: Permeation Coefficient (Kp)	Maximize			
Y_7: Q120 permeated (µg/cm²)	Maximize			

2.3. Preparation of Ropinirole Hydrochloride Mucoadhesive Thermoreversible Intranasal In-Situ Gels from the chosen polymers

In- situ gels were prepared according to cold method described by Schmolka **(Schmolka, 1972)** after minor modification, as PF127 was added slowly with continuous stirring on a magnetic stirrer (stuart UC152, Stuart Scientific, USA). The prepared solution was then stored at 4 °C until a clear solution was obtained. RHCl solution of concentration of 5.7mg/ml (equivalent to 5mg free base) and the calculated amount of mucoadhesive polymers (0.3, 0.5, 0.7% w/v) were slowly added to the PF127 polymer with continuous agitation till complete dissolution. Prepared samples were stored at 4 °C till further analysis.

2.4. In-Vitro Characterization Of The Prepared Ropinirole Hydrochloride Mucoadhesive Thermoreversible Nasal In-Situ Gels:

2.4.1. Drug Content, Clarity and pH measurement

Samples of 0.25 ml from each gel formula were added to 100 ml volumetric flask containing 75ml phosphate buffer Saline (PBS) pH 6.4 and stirred overnight over magnetic stirrer to ensure complete dissolution of the gel. Final volume was adjusted to 100ml with buffer then filtered. Samples were then analyzed for drug content determination by HPLC (Agilent 1260 infinity series, USA).

The clarity of all formulae after gelation was determined by visual inspection against white and black background. It was grade coded as follows: turbid +, clear ++ and very clear (glassy) +++. PH of each formulation was determined using digital pH-meter (Jenway 3510, USA) after calibration **(A. Geethalakshmia, 2013)**.

2.4.2. Measurement of the Sol-Gel Transition Temperature ($T_{sol-gel}$)

The temperature at which the liquid phase undergoes transition to gel is measured. Samples of 2 ml of the prepared formulae in clear glass vials were transferred to a digital water bath (Julabo, USA) at 15°C. The temperature of the bath was increased in increments of 2 °C and then 0.1 °C in region of sol-gel transition temperature (25- 37 °C) and left to equilibrate at each new setting. Then, samples were examined for gelation to occur when the meniscus would no longer move upon tilting through 90 °C. The actual temperature was recorded by immersing inside the sample solution a probe of digital thermometer (Alpha Technics, USA) with accuracy of 0.1°C. Measurements were done in triplicates **(Madhu Vadnere, 1984, Gilbert JC, 1987).**

2.4.3. Rheological properties Characterization

All formulae were left unagitated overnight in refrigerator and then equilibrated to room temperature before measurement (to prevent entrapment of air bubbles on gelation). The rheological properties of the formulations were measured as described by Zaki **(Zaki *et al.*, 2007)** with simple modification using cone and plate viscometer (Brookfield Programmable Ultra Rheometer, Model DVIII, Engineering Laboratories, Inc., USA) using spindle 52, at 35 °C ± 1°C. Samples of 0.5 ml were applied in the centre of the lower plate of the viscometer. Viscosity measurement protocol was started after 2 minutes of solution application at a shear rate (γ) gradually increased in the range of 1- 250 rpm. Power law constitutive equation (Tung, 1994) was used to determine the two dimensionless quantities; the

consistency index (**m**) and the flow index (**n**) characteristic for each formulation where:

$\eta = m \gamma^{n-1}$

η is the viscosity in centipoises (cps), **γ** is the shear rate (s^{-1}). If **n** = 1, this indicates Newtonian behavior of the formula. The lower the value of (**n**), the more shear thinning of the formulation. Measurements were done in triplicates.

2.4.4. Gel strength Evaluation

The gel strength is an indication for the viscosity at physiological temperature of the nasal in-situ gel. The TA.XT plus Texture Analyser (Stable Micro Systems LTd., UK) was used for determination of gel strength by penetration technique with its attached 10 mm gel penetration cylindrical probe. Samples of each formula were put in 25 ml beakers and left in an ultrasonic water bath at 37 °C to remove air bubbles for 20 min before testing. Samples were then positioned centrally under the probe at 35 ± 1°C. Probe was then preceded with a trigger force of 5g to penetrate into the gel sample at a region of flat surface to a depth of 5mm at a defined rate of 0.1mm/s. From the resultant force-time plot performed by the Texture Exponent Software (version 6.1.4.0), the maximum peak force reading (i.e. the resistance to penetration) during the first compression cycle required to attain a given deformation was obtained and translated as 'Hardness' or the 'Gel Strength' of the sample **(David S Jones, 1997)**. Measurements were done in triplicates after creating a fresh smooth flat surface.

2.4.5. Mucoadhesive Strength Evaluation

The Mucoadhesive Strength of each formula was determined by measuring the force required to detach each formula from the nasal mucosal tissues **(Jones *et al.*, 2000).** The TA.XT plus Texture Analyser (Stable Micro Systems LTd., UK) was used with 5 Kg load cell equipped with the gel mucoadhesion probe. Briefly, Sheep nasal mucosa was utilized as the model membrane for mucoadhesion determination. Tissues (about 20 x 25 mm) were used after separation and were stretched on the mucoadhesion rig and fixed in place with aperture support ring. The gel formulae were applied on the mucoadhesion probe at 35 ± 1°C. The probe was lowered to get contact with the membrane.

Before testing, effect of varying contact time (1, 2, 3, 4 and 5 minutes) was investigated for some of the gel formulae with different mucoadhesive polymers to determine the optimized initial contact time needed **(Majithiya *et al.*, 2006).**

After contact time, the probe was subsequently withdrawn upwards at a constant speed of 0.1 mm/s to a distance of 10 mm. The maximum detachment force or peak force for adhesiveness (F_{max}) in newton required to separate the probe from the model membrane was obtained directly from the Exponent Software (version 6.1.4.0) to determine mucoadhesive strength according to the following equation:

Mucadhesive Strength (dyne / cm^2) = F_{max} * **g** / **A** (1)

Where F_{max} is measured in grams; **g** is the acceleration due to gravity (980 cm s^{-1}) and **A** is the area of the exposed mucosal tissue (Bhandwalkar and Avachat, 2013).

Each experiment was carried out in triplicates using fresh sheep nasal tissue and new gel sample.

2.5. In-vitro & Ex-vivo permeation Study

This study was conducted using Franz Diffusion Cell Hanson 57-6M (Hanson Research Corporation, USA). The study was first conducted using Synthetic membrane (molecular weight cut off 12,000 - 14,000 Da) stored in PBS pH 6.4 for 24 hrs before use **(Himawan, 2016)**. Then, the ex-vivo permeation study was conducted on the promising formulae from the preceded in-vitro permeation step using fresh sheep nasal mucosa obtained from the local slaughter house. The separated mucosa was preserved in normal saline solution during transportation and freezed at -20 °C until utilized. The nasal mucosa was separated from septum, connective tissue and most of the adhering cartilaginous tissue with forceps and scissors without scratching the mucosa. The mucosal membrane was soaked for one hour in PBS pH 6.4 before stretching it between donor and receptor chamber of the diffusion assembly with diffusion area of 1.77 cm^2. The donor and receptor chambers were clamped together carefully to ensure absence of air bubbles that might interfere with the permeation process. Receptor chamber filled with 7ml de-aerated PBS pH 6.4 at 34 °C was kept constantly stirred throughout the experiment using a magnetic bar.

Samples of 0.25 ml of gel containing 5.7 mg of RHCl equivalent to 5 mg of Ropinirole free base was placed in the donor chamber. Samples of 0.5 ml was withdrawn from the cells' sampling outlets at 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 minutes and replaced with equal volumes of fresh de-aerated PBS pH 6.4 maintained at 35 °C. Blank samples of the corresponding plain gel formulae were run throughout the experiment to check for any interference **(Bhandwalkar and Avachat, 2013**). The withdrawn samples were diluted and then filtered. The cumulative amount of RHCl permeated was quantified by HPLC (Agilent 1290 Infinity series, Agilent Tech., USA) at 249 nm, calculated by calibration curve method and expressed as cumulative amount permeated (μg/cm^2) versus time. Permeation test was run in triplicates.

2.5.1. Data Analysis Of Permeation Study

The cumulative amounts of RHCl penetrating per unit area (Q) were plotted against time. From the slope of the linear portion of the plot a steady state

flux (SSF) was determined. The permeability coefficients (Kp) (cm s^{-1}) under steady-state conditions across synthetic membrane & excised mucosa can be calculated according to equation:

Peff = (dC /dt)ss V/ACD (3)

where **(dC /dt)ss** (µg mL^{-1} s^{-1}) is the time-dependent change of concentration in the steady-state; **A** (cm^2) is the permeation area; **V** (mL) the volume of the receiver compartment; and **CD** (µg mL^{-1}) is the initial donor concentration **(Lang *et al.*, 1996, Mital S. Panchal, 2012).**

2.6. Histological Evaluation of Nasal Mucosal Integrity

Histological effects in sheep nasal mucosa were investigated after exposure to intranasal formulations as described by Karasulu **(Karasulu *et al.*, 2008)**. Fresh sheep nasal mucosal tissues that were carefully removed from the nasal cavity of sheep (of approximately 20 ± 5 kg, aged 4 to 8 months) were used to conduct this study. Formulae F3, F6, F9 and F12 with higher concentration of mucoadhesive polymers were chosen. Samples of 2 ml of each formula were applied on the separated right nostril of the mucosal tissues and incubated at 35 °C for 8 hrs. Control tissues were taken from the separated left nostril of the same animal for each formula to reduce biological variability between tissues and incubated at 35 °C in PBS pH 6.4 for 8 hrs. The experiment was conducted using at least three animals for each formula. Tissues treated with the prepared formulae as well as control tissues were fixed in a 10 % buffered formalin solution (pH 6.4), then washed, dehydrated, immersed in xylene and finally, embedded in paraffin wax. Paraffin sections (7µm) were cut on glass slides and stained with hematoxylin and eosin (H & E). Sections of treated and control nasal mucosal tissues were examined under a light microscope (Olympus CX31RTSF, Philippines) by a histologist blinded to the study.

2.7. Short term stability studies

Stability testing of prepared pharmaceutical products is done to ensure the quality, effectiveness as well as safety of active drug ingredients in products during storage. RHCl Optimized gel formula (F3) was subjected to stability studies according to ICH guidelines for 6 months (Food and Drug Administration, 2001). F3 was kept at refrigeration temperature (4°C), room temperature (25°C) and at elevated temperature (40°C ± 2°C / 75% RH ± 5% RH) (AS Chinchole *et al.*, 2014). Samples were withdrawn after 30, 90,150 & 180 days and evaluated for parameters such as pH, drug content, gelation temperature and viscosity. The experiment was performed in triplicates at each sampling time.

2.8. Pharmacokinetic Study And Comparative Bioavailability Of Ropinirole Hydrochloride Intranasal In-situ Gel & Peroral Commercial Tablets:

– Calibration Curve Of Ropinirole Hydrochloride In Rabbit Plasma

Stock solution of 50 µg /ml of Ropinirole Hydrochloride was prepared using deionized water. Plasma standards for calibration curves were prepared by spiking 1.0 mL aliquots of pooled drug free plasma with 100 µL of the above mentioned working solutions, to make Ropinirole hydrochloride concentrations in the range from 0.25 to 50ng/ml. Then, the peak area ratio (PAR) of rabbit plasma containing different concentrations of RHCL and fixed concentration of internal standard (IS) Cilazapril (50 ng/ml) after analysis was plotted versus the concentration of RHCL standard solutions.

- Development Of Sensitive LC-MS/MS Assay Method Of Ropinirole Hydrochloride In Rabbit Plasma

A sensitive, selective and accurate LC-MS/MS method was developed and validated before the study for determination of Ropinirole Hydrochloride concentrations in vivo. Cilazapril internal standard (IS) stock solution was prepared by dissolving 10 mg in methanol and serially diluted with mobile phase to give a final working concentration of 50 ng/ml. The method was validated following the international guidelines (Shah *et al.*, 1991). A shimadzu Prominence (Shimadzu, Japan) series LC system equipped with degasser (DGU-20A3), solvent delivery unit (LC-20AB) along with auto-sampler (SIL-20 AC) was used to inject 30 µl aliquots of the processed samples at room temperature on a Luna C18 (phenomenex, USA) (50x4.6) mm, 5 µm particle size. The Guard column was phenomenex C18 (5x4.0) mm, 5 µm particle size. The isocratic mobile phase (pH 4.5) consisted of acetonitrile and (0.02 M) ammonium acetate buffer (70%: 30% v/v) and 0.1% formic acid which was delivered at a flow rate of 1ml/min into the mass spectrometer's electrospray ionization chamber. Quantitation was achieved by MS/MS detection in positive ion mode for both Ropinirole Hydrochloride and IS, using a MDS Sciex (Foster City, CA, USA) API-3200 mass spectrometer, equipped with a Turbo ionspray TM interface at 300°C. The ion spray voltage was set at 5500 V. The common parameters, viz., nebulizer gas, curtain gas, auxillary gas and collision gas were set at 14 psi, 25 psi, 30 psi and 11 psi, respectively. The compound parameters, viz., declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CEP) were 46 V, 31 V, 6 V, 22 V for Ropinirole Hydrochloride and 26 V, 25 V, 7.5 V, 30 V for Citazapril (IS), respectively. Detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of

the m/z 261.277 precursor ion to the m/z 114.200 for Ropinirole and m/z 418.342 precursor ion to the m/z 211.106 for IS. Quadrupoles Q1 and Q3 were set on unit resolution. The analytical data were processed by Analyst software (Version 1.6) (Bhatt *et al.*, 2006).

Study Design

The study was a single dose, two-treatment, One -period, parallel design comparing equal doses (1.14 mg of RHCL equivalent to 1mg Ropinirole base) of the optimized prepared gel formula (formula F3), and the reference commercial product Requip® 1mg Tablets.

Twelve healthy new zealand albino male rabbits were obtained from animal house of the National Organization Of Drug Control And Research (NODCAR), Cairo, Egypt. The selected weight range was (2 - 2.5 Kg). The study protocol was approved by the institutional review board; Research Ethics Committee-Faculty of Pharmacy, Cairo University (REC-FOPCU), Egypt. The treatment groups' weights were balanced, and rabbits were randomly divided into two groups. Rabbits were kept under appropriate housing conditions. The animals were maintained at controlled temperature (25 ± 2 °C), and humidity (60 ± 5 %).

Following an overnight fast of 18 hours, six rabbits was randomly assigned to each one of the two treatment groups (F3) and Reference product (Sanford Bolton, 1997). The animals were conscious throughout the duration of the experiments. The reference product (commercial tablet) was administered by the aid of an oral feeding 3ml syringe. About 100 µl of the Gel formula F3 (containing 1.14 mg of RHCL equivalent to 1mg Ropinirole base) were administered intranasal with the help of special intranasal applicator fitted to 1ml syringe at the right nostril of rabbits' nose ((CDER), 2005).

Approximately 1ml blood samples for RHCl analysis were drawn at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes after dosing from the marginal ear veins of the rabbits into heparinised glass tubes. Blood samples were centrifuged at 3500 rpm for 10 min at 4 °C. Then, plasma was frozen at -20°C till drug analysis.

All frozen plasma samples were thawed at ambient temperature. Plasma samples (0.5 ml) were placed in 7 ml glass tubes, and 100 µl of IS solution (50ng/ml cilazapril) was added to each and vortexed for 1 min. The extraction solvent (4 ml Methyl-t-butyl-ether (MTBE)) was then added, and samples were then vortexed for 2 minutes. The tubes were then centrifuged at 4000 rpm for 10 min. Then, upper organic phases were transferred to glass tubes for evaporation to dryness using centrifugal vacuum concentrator Vacufuge® 5301 (Eppendorf, Germany) at 40°C. Dry residues were dissolved in 200 µl of mobile phase and vortexed for 1 min for reconstitution of residues, and 30 µl was injected using the autosampler.

Pharmacokinetic and Statistical Analysis

Plasma concentration-time data of Ropinirole was analyzed for each subject by non-compartmental pharmacokinetic models using kinetica® software (version 4.4.1). The peak plasma concentrations ($\mathbf{C_{max}}$) and the time of their occurrence ($\mathbf{T_{max}}$) were obtained from the concentration-time data. The area under the plasma concentration-time curve (**AUC**) from time zero to last measured concentration after 6 hrs ($\mathbf{AUC_{0-6}}$) was calculated according to the linear trapezoidal rule while the $\mathbf{AUC_{0-\infty}}$ was calculated by extrapolation to infinity. The terminal elimination rate constant (K) was estimated by linear regression of the terminal portion of the ln (concentration)-time curve, and the elimination half life was calculated. Results were Expressed as mean values ± S.D (Gibaldi, 1984).

One-way analysis of variance (ANOVA) was performed using SPSS ® software (Version, 19.0) for a parallel design to assess the effect of formulation, and subjects on $\mathbf{C_{max}}$, $\mathbf{T_{max}}$, $\mathbf{AUC_{0-6}}$ and $\mathbf{AUC_{0-\infty}}$. Differences between two related parameters were considered statistically significant for p-value equal to or less than 0.05.

3. Results and Discussion

3.1. Formulation of Ropinirole Hydrochloride Mucoadhesive Thermoreversible Intranasal In-Situ Gels

From primary formulation trials, it was observed that 18% w/v of PF127 solution after addition of RHCl was the lowest possible concentration for thermoreversible gelling within the acceptable range for intranasal administration (25-37°C). Thermoreversible polymer PF127 in addition to mucoadhesive polymers HPMC E5, HPC, Na CMC, Na ALG at different concentrations (0.3, 0.5, 0.7% w/v) were used to develop mucoadhesive in-situ gels Formulae as shown in table (1B).

3.2. In-Vitro Characterization Of The Prepared Ropinirole Hydrochloride Mucoadhesive Thermoreversible Intranasal In-Situ Gels:

3.2.1. Drug Content, Clarity and PH measurement

Drug content was in the range of 95.02 to 98.45% as shown in table (2A) indicating uniform distribution of RHCl. Pluronics are widely used in gel formulations due to their ability to form clear water-based gels. All the prepared formulae were free flowing liquid at room temperature (25 °C), clear (glassy), and free of any particulate matter except sodium alginate formulae where all are pale yellow in color as shown in table (2A).

The normal physiological pH of nasal mucosa ranges from 4.5 to 6.5. However, the nasal mucosa can tolerate solutions within pH range of 3-10. The pH of all formulation was in the range of 6.01-6.55 as shown in table (2A) which was within the physiological range of the nose.

Table (1B): Experimental runs, independent variables and measured responses of the full factorial experimental design of RHCL mucoadhesive thermoreversible in-situ gel formulae

Formulae	X_1 Type of mucoadhesive polymer	X_2 Concentration of mucoadhesive polymer (% w/v)	Y_1 $T_{sol-gel}$ (°C)	Y_2 Consistency index (m)	Y_3 Flow Index (n)	Y_4 Gel strength (g)	Y_5 Mucoadhesive strength (dyne / cm^2)	Y_6 Permeation Cefficient Kp (10^{-3}) (cm/sec)	Y_7 Cumulative Q 120 permeated (µg /cm^2)	Desirability
P1	HPMC	0.3	31.05	132434	0.113	15.54	1497	1.463	438	0.149
P2	HPMC	0.5	30.54	152756	0.101	16.07	1497	2.091	604	0.334
P3	HPMC	0.7	30.49	166724	0.092	19.91	1788	5.032	805	0.726
P4	HPC	0.3	27.64	148936	0.146	19.55	1622	0.488	97	0.001
P5	HPC	0.5	27.34	157036	0.112	17.77	1996	1.013	189	0.048
P6	HPC	0.7	27.25	200000	0.083	16.41	1996	1.793	225	0.090
P7	Na CMC	0.3	31.20	127938	0.145	14.71	2704	0.982	121	0.004
P8	Na CMC	0.5	31.15	140281	0.144	14.64	1747	3.231	628	0.291
P9	Na CMC	0.7	30.91	174180	0.138	13.26	1664	2.097	346	0.108
P10	Na ALG	0.3	33.31	183231	0.123	15.48	1913	0.946	181	0.041
P11	Na ALG	0.5	33.14	185780	0.102	18.24	2013	1.416	467	0.278
P12	Na ALG	0.7	32.85	205116	0.099	18.67	2038	3.972	741	0.690

Table (2A): Appearance, Clarity, pH & Drug Content and Permeation parameters of RHCl mucoadhesive thermoreversible in-situ gel formulae

Formulae	***Appearance***	***Clarity***	***pH*** *(mean ± SD)*	***Drug Content %*** *(mean± SD)*	Jss (µg/cm2.sec)	Kp (10-3) (cm/sec)	Q120mins (µg/cm2)	Time of max. permeation (mins)
F1	Free flowing liquid	+ + +, colourless	6.12±0.19	95.55±0.24	2.085	1.4632	438	360
F2	Free flowing liquid	+ + +, colourless	6.01±0.22	96.29±0.19	2.980	2.0912	604	180
F3	Free flowing liquid	+ + +, colourless	6.25±0.20	98.45±0.34	**7.170**	**5.0316**	**805**	**75**
F4	Free flowing liquid	+ + +, colourless	6.48±0.25	96.21±0.39	0.696	0.4884	97	360
F5	Free flowing liquid	+ + +, colourless	6.55±0.08	98.11±0.42	1.444	1.0133	189	360
F6	Free flowing liquid	+ + +, colourless	6.41±0.10	96.92±0.19	2.555	1.7930	225	300
F7	Free flowing liquid	+ + +, colourless	6.45±0.05	95.02±0.13	1.035	0.7263	121	360
F8	Free flowing liquid	+ + +, colourless	6.48±0.05	96.42±0.68	**4.604**	**3.2309**	**628**	**150**
F9	Free flowing liquid	+ + +, colourless	6.39±0.05	97.32±0.12	2.569	1.8028	346	360
F10	Free flowing liquid	+ +, pale yellow	6.35±0.21	98.41± 0.68	1.348	0.9460	181	360
F11	Free flowing liquid	+ +, pale yellow	6.31±0.25	95.31±0.39	2.018	1.4161	467	300
F12	Free flowing liquid	+ +, pale yellow	6.41±0.05	96.36±0.52	**5.660**	**3.9719**	**741**	**120**

3.3. Measurement of the Sol-Gel Transition Temperature ($T_{sol\text{-}gel}$)

In general, the $T_{sol\text{-}gel}$ of all formulae have been considered to be suitable if they are within the acceptable range for intranasal administration (25-37°C) (Majithiya *et al.*, 2006), as the temperature of the nasal cavity is about 34 °C (Keck *et al.*, 2000). If gelation occurs at room temperature (25 °C), so it will be very hard to handle or administered. If the gelation occurs at higher temperature than 35 °C, formulation will be still in the liquid form at body temperature, resulting in rapid nasal clearance of formulation (Majithiya *et al.*, 2006).

During primary formulation stage, it was observed that increasing PF127 concentration of plain gels decreased the sol-gel transition temperature ($T_{sol\text{-}gel}$). The optimal concentration of PF127 was 18 % w/v to be used in RHCl gels preparation. As stated in literature, gelation of PF127 results from the change in the micellar number with temperature where the number of micelles formed increases as a consequence of the negative coefficient of solubility of block copolymer micelles upon increasing temperature. When micelles become so tightly packed, the solution becomes immobile and turns to gel (Kabanov *et al.*, 2002). On increasing concentration of PF127 solution, larger number of micelles was formed with small water content to hydrate PF127 chains. As a result, cores dehydration of PF127 micelles occurs on rising temperature and $T_{sol\text{-}gel}$ occurred at lower temperature (Majithiya *et al.*, 2006). Another theory for gelation proposed that the mechanism of gelation is probably due to repulsive interactions among close-packed spherical micelles, rather than aggregation or transitions change in micelle morphology to rods or lamellae (Robert K.

Prud'homme 1996).

It was also observed that the addition of RHCl and other mucoadhesive polymers changed the $T_{sol-gel}$ of PF127. RHCl addition caused rise in the $T_{sol-gel}$ of the 18 % w/v PF127 gel. Tsol -gel of 18% w/v PF127 gels increased on addition of RHCl could be due to the water soluble nature of RHCl which may modify micelles association of PF127 gels and increase its sol-gel transition temperature ($T_{sol-gel}$) (Zaki *et al.*, 2007, Bhandwalkar and Avachat, 2013).

$T_{sol-gel}$ of all formulae was in the range of 30.49 ^{o}C to 33.31 ^{o}C which was a proper gelling range at the nasal cavity. The polymers showed varied gelation temperature which decreased on increasing polymers concentration as follows: NaALG > Na CMC > HPMC > HPC as shown in table (1B). The lowering effect of increasing mucoadhesive polymer concentration could be due to the ability of the polymers to bind to the polyoxyethylene chains present in PF127 molecules. This would promote its dehydration and increase in entanglement of adjacent molecules and so increase hydrogen bonding formation between molecules which result in gelation at low temperature with increase of mucoadhesive polymer concentration (Wei *et al.*, 2002, Zaki *et al.*, 2007).

3.4. Rheological properties Characterization

The rheological behavior of the thermoreversable systems was evaluated by constructing rheograms by plotting shear stress (dyne/cm^2) *versus* shear rate (sec^{-1}). Intranasal formulation must have an optimum viscosity that will allow easy instillation into the nose as liquid drops facilitate its instillation as liquid drops and which would undergo rapid sol-gel transition upon intranasal administration. Consistency index and Flow index data were shown in table (1B). All the formulation exhibited pseudoplastic behavior as shown in figure (1) where upon increasing shear rate viscosity is decreased. This may be attributed to the miceller formation of PEO/PPO ratio of PF127 at higher temperature as stated in literature. Another theory stated that Gels with an ordered structure (cubic packing of spherical micelles) were observed over a well-defined temperature window when the PF127 concentrations were greater than 17 %w/v (Robert K. Prud'homme 1996). This may be attributed to the miceller formation of PEO/PPO ratio of PF127 at higher temperature. This shear thinning behavior is responsible for uniform distribution of RHCl on the mucosal surface of nose. The high viscosity at lower shear rate helps to increase the contact time of in-situ gels formulae on mucosal surface.

The prepared gel formulae had consistency index (**m**) values ranging from 132434 to 205116, while flow index (**n**) values ranging from 0.083 to 0.146. The decrease in flow index (n) indicated the more shear thinning behavior of the formulation. The increase in the conc. of mucoadhesive polymer led to increase in consistency index and decrease in flow index.

3.5. Gel strength Evaluation

For characterization of mucoadhesive formulation, several criteria should be taken into consideration such as the ease of removal from the container, the ease of administration to the desired region and the retention at the administration site (Schwartz, 1975)**.** Gel strength can predict all these criteria.

Gel strength was determined at 35°C ± 1°C and data was shown in Table (1B). Generally, there was gradual increase in the gel strength of all formulae upon increasing concentration of the mucodhesive polymers.

For NaALG formulae, the increase in gel strength with increasing NaALG concentration may be due to the interaction of sodium alginate with PF127 leading to increase in the hydrophobic portion.

Figure (1) showed the Gel Strength performance graph of the optimized formula F3.

3.6. Mucoadhesive Strength Evaluation

Important factors such as the type, architecture and molecular weight of the mucoadhesive polymer and its' concentration in formulae can influence the mucoadhesive strength of the prepared formulae to the nasal mucosal tissue (Park *et al.*, 2003). High mucoadhesive strength of the formulation could increase its' retention time and its' absorption across mucosal tissues (Kunisawa *et al.*, 2000).

Our study indicated that the contact time of 3 mins resulted in maximum bioadhesive strength was selected as optimum required contact time.

Mucoadhesive strength data showed that the PF127 preparations possessed good adhesive properties. The variation in concentration of PF127 and mucoadhesive polymer changes mucoadhesive strength that increased with the increase in mucoadhesive polymer concentration.

Figure (1) showing gel mucoadhesive strength performance graph of the optimized formula F3 (containing HPMC). This was due to wetting and swelling of HPMC, which permits close contact with nasal mucosa. The mucoadhesive force increased with the increase in concentration of HPMC as more hydroxyl groups were available for hydrogen bond formation with mucin molecules (Khan *et al.*, 2010).

4. In-vitro & Ex-vivo permeation Study

Skin permeation rate of drug depends on both the solubility and the diffusion of the drug. The permeation profiles of cumulative amounts of RHCL permeated through synthetic and nasal sheep mucosal membrane were shown in Figure (1 (d to h)). The best

permeation through synthetic membrane was 99.2% after 120 mins for F3 & was 98% after 150 mins for F12.

Table (2A) showed that the cumulative amount of drug permeated through synthetic membrane after 120 mins (**Q120**) as follows: F3 (805 µg/cm^2) > F12 (741µg/cm^2). Relevant to the steady state flux (***Jss***) of RHCl, highest ***Jss*** values were for F3 (7.17 µg/cm^2.sec) > F12 (5.66 µg/cm^2.sec).

Table (2B): Comparative pharmacokinetic parameters of RHCl following administration of intranasal mucoadhesive thermoreversible in-situ optimized gel formula (F3) and peroral Tablets (Requip®) in rabbits

Pharmacokinetic parameters	***Intranasal In-situ gel (F3)***	***Peroral tablets (Requip®)***
C_{max} (ng/ml) ± SD	*496.33 ± 364.93*	*110.16 ± 74.33*
T_{max} (mins) ± SD	*5.00 ± 0*	*90.00 ± 0*
AUC_{0-6} (min * ng/ ml) ± SD	*14184.17 ± 6302.12*	*7987.74 ± 5109.14*
$AUC_{6-\infty}$ (min * ng/ ml) ± SD	*217.35*	*539.22*
$AUC_{0-\infty}$ (min * ng/ ml) ± SD	*14401.52 ±4202.09*	*8526.96 ± 5148.55*
$t_{1/2}$ (min) ± SD	*110.38 ± 25.11*	*175.74 ± 66.76*
K (min^{-1}) ± SD	*0.0065 ± 0.0011*	*0.0058 ± 0.0057*
MRT (min)	*54.70 ± 15.66*	*171.56 ± 35.88*
Relative bioavailability (%)	*168.8939*	-

The formulae F3 & F12 that showed best permeation through synthetic membrane than other formulae were further subjected to permeation study across nasal sheep mucosal membrane. Figure (1h) showed that F3 was also superior than F12 in ex- vivo permeation where its permeation was 95.4 % after 45 mins for F3 & was 93.2% after 75 mins for F12.

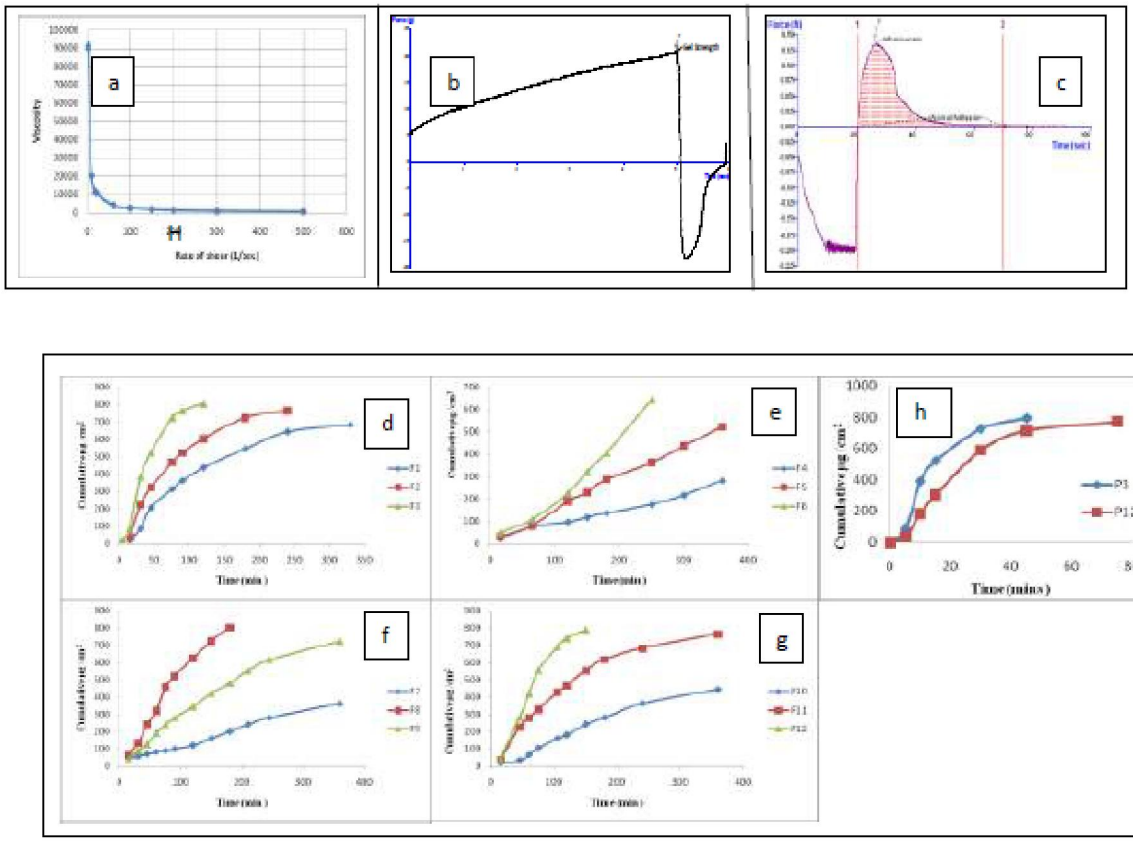

Figure 1: a-Rheological behavior of RHCL mucoadhesive thermoreversible in-situ gel Optimized formula (F3), b- Gel Strength performance graph of (F3), c- Gel Mucoadhesive strength performance graph of (F3).
In-Vitro Permeation profile of RHCL mucoadhesive thermoreversible in-situ gel through synthetic membrane; d-Formulae F1, F2, F3, e- Formulae F4, F5, F6, f- Formulae F7, F8, F9, g- Formulae F10, F11, F12 and h- RHCL mucoadhesive thermoreversible in-situ gel through sheep nasal mucosal membrane; Formulae F3 & F12.

RHCl diffused through the natural mucosal membrane was more as compared to RHCl diffused through synthetic membrane. This could be due to the nature of mucosa which acts as lipophilic–hydrophilic barrier for mucosal penetration while synthetic membrane acts as a mechanical barrier. Also, it could be due to the nature of Pluronic, as a non ionic surfactant, where it might increase RHCl transcellular transport either by reducing mucus viscosity and elasticity and so reducing barrier function of the mucus layer (F.W.H.M.Merkus, 1993) or by causing perturbation of lipid membranes leading to leakage of lipids and proteins from the membranes (Zaki *et al.*, 2007).

Hitendra *et al* (Hitendra *et al*, 2009) stated that the permeation of drug molecules was a factor of its diffusivity through the nasal mucosa as well as the rigid structure of the gel and that would influence its bioavailability.

5. Factorial Design Analysis

The $4^1 \times 3^1$ full factorial design with statistical analysis was used for planning and analysis of experimental trials. For selection of optimized formulae, it was almost impossible to achieve all the desired responses simultaneously due to the possibility of interference occurrence. The optimum condition reached in one response may possess an opposite impact on another response. Fortunately, the desirability function combines all the responses into one variable to predict the optimum levels for the studied factors (Arun Kumar Singh, 2012)**.** So, desirability was calculated to select the optimized formulae with the highest permeation coefficient, Q120 min permeated, mucoadhesive strength, gel strength, consistency index, and the least flow index. The highest desirability values were 0.708 and 0.656 for formulae F3 (containing 0.7%w/v HPMC) and F12 (containing 0.7%w/v Na Alginate) respectively (figure 3 and table 1B).

6. Histological Evaluation of Nasal Mucosal Integrity

Successful mucoadhesive formulation doesn't depend only on bioadhesive efficacy of mucoadhesive polymers but also on their biosafety. Figure 2 (a and b) showing some of the histological examination of sheep nasal mucosa of the self control groups treated with PBS pH 6.4 and after application of F3 (HPMC) (figure 2 c), F6 (HPC) (figure 2 d), F9 (NaCMC) (figure 2 e) and F12 (Na ALG) (figure 2 f) respectively. For the examined control group; olfactory epitheliums, mucosal structures, olfactory glands, inter cellular spaces were completely normal. No signs of inflammation or erosion were noticed.

Histological examination of olfactory organs of F3 treated mucosal tissues proved that mild histological changes after application could be seen as mild inflammation. Olfactory glands displayed high activity intact nuclei. On comparing F3 treated mucosa with PBS at pH 6.4 with treated mucosa (control), the epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosal tissues.

Histological examination of olfactory organs of F6 & F9 treated mucosal tissues revealed sever erosion of olfactory epithelium, Lamina propria displayed mild thickness, with inflammatory cells infiltration in interstitial spaces. Vasculature showed thickening in their walls. Serous glands with signs of degenerative changes, however some glands displayed desquamated cells in their lumen. Meanwhile, mild degenerative changes in nerve fibers could be seen with F6.

Severe histological changes could be seen after application of F12 in form of erosion of olfactory epithelium, widening of lamina propria with signs of inflammation, hemorrhagic areas in intercellular spaces. Olfactory glands display moderate degenerative changes; some of them display desquamated cells in their lumen.

Thus, the results of histological examination indicated that formula F3 had no serious effects on the microscopic structure of mucosa and was biocompatible and so could be considered the most safe prepared formula for the intranasal administration.

7. Pharmacokinetic Study and Comparative Bioavailability of Ropinirole Hydrochloride intranasal in-situ gel & peroral commercial tablets:

The calibration curve of ropinirole hydrochloride prepared in rabbit plasma was found to be linear over the concentration range of 0.25- 50 ng/ml ($r^2 = 0.993$).

The LC-MS/MS assay has been validated with high selectivity and sensitivity and acceptable within and between day accuracy and precision. The lower limit of RHCl quantification in plasma was 0.25 ng/ml.

RHCl mean plasma concentration-time profiles following single dose administration of intranasal gel formula F3 and peroral Requip® 1mg tablets (Glaxo Smith Kline) to six male rabbits is shown in Figure 4. Corresponding pharmacokinetic parameters were summarized in Table 2B.

After intranasal administration, RHCl was absorbed and reached C_{max} 496.33 ± 364.93 ng/ml at T_{max} 5.00±0 min. The mean AUC_{0-6}, $AUC_{6-\infty}$, $AUC_{0-\infty}$ values after intranasal treatment were 14184.17 ± 6349.42, 217.348 ± 82.62 and 14404.52 ± 6302.12 min * ng /mL respectively.

While after peroral administration, RHCl was absorbed and reached C_{max} 110.16 ± 74.33 ng/ml at T_{max} of 90.00±0 min. Mean AUC_{0-6}, $AUC_{6-\infty}$, AUC_{0-}

$_{\infty}$values after peroral treatment were 7987.74 ± 5109.14, 536.22 ± 276.9 and 8526.69 ± 5148.55 min * ng /mL respectively.

The time required to reach the maximum plasma concentration (T_{max}) values revealed that the reference product (commercial tablet) spent a longer T_{max} (90 mins) to reach the maximum drug concentration in the systemic circulation. While; shorter T_{max} (5 mins), higher C_{max} (496.33 ± 364.93 ng/ml), AUC_{0-6} (14184.17 ± 6349.42 min * ng /ml) and $AUC_{0-\infty}$ (14404.52 ± 6302.12 min * ng /ml) values were obtained in case of intranasal formula F3. The rapid absorption of ropinirole from intranasal gel may be attributed to the permeation enhancement to some extent and increased retention at the mucosa by HPMC.

Statistical analysis showed that there was a significant difference in the rate of drug absorption from the intranasal dosage form comparative to the peroral one. The calculated relative bioavailability of RHCL after the intranasal administration compared to the peroral administration of tablets was approximately 168.89 %. Obviously, the improvement of bioavailability of the intranasal route was also due to the elimination of the first path effect of this route.

These results were in accordance with Khan *et al (*Khan *et al, 2010*). The in- situ gels formulation using chitosan and hydroxyl propyl methyl cellulose enhanced intranasal delivery of ropinirole to the blood and brain where the AUC 0–480 min in brain after intranasal administration of ropinirole in situ gel was 8.5 times higher than that achieved with I.V. administration and also considerably higher than intranasal ropinirole solution. The maximum blood concentration was achieved at 30 min for nasal ropinirole in situ gelling formulation whereas it took 60 min to reach the peak concentration for control ropinirole HCl solution. The enhanced bioavailability of in-situ gels was due to accumulation of the drug in the brain. Ropinirole can cross the blood brain barrier via the direct nose-brain pathway after nasal delivery was also confirmed.

As well, Monica et al (Monica Rao *et al.*, 2017) observed that there was a five-fold increase in bioavailability of intranasal administration of in-situ ropinirole Hydrochloride gel as compared to IV route.

From the *In-vivo* study, we can conclude that bioavailability of RHCl from intranasal in-situ gels prepared from PF127 and HPMC was much higher than the peroral route.

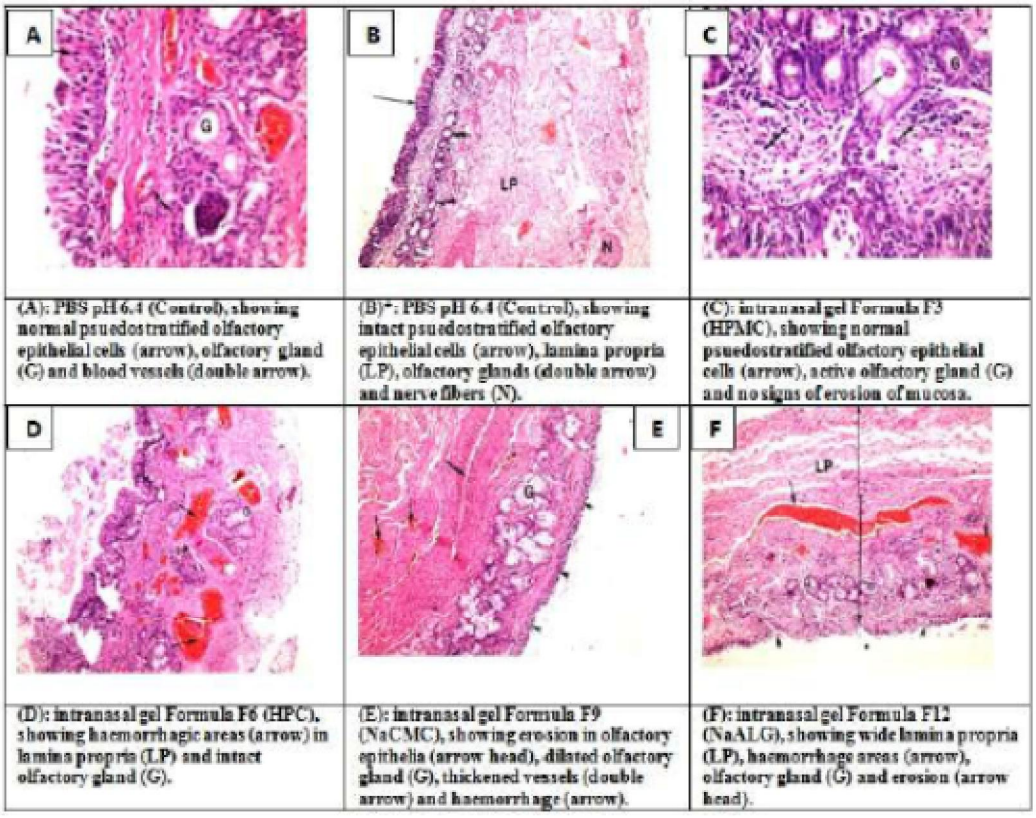

Figure 2: Histological examination of sections of sheep nasal olfactory mucosa ((H & E) (X=400)), after incubation at 35 °C for 6hrs

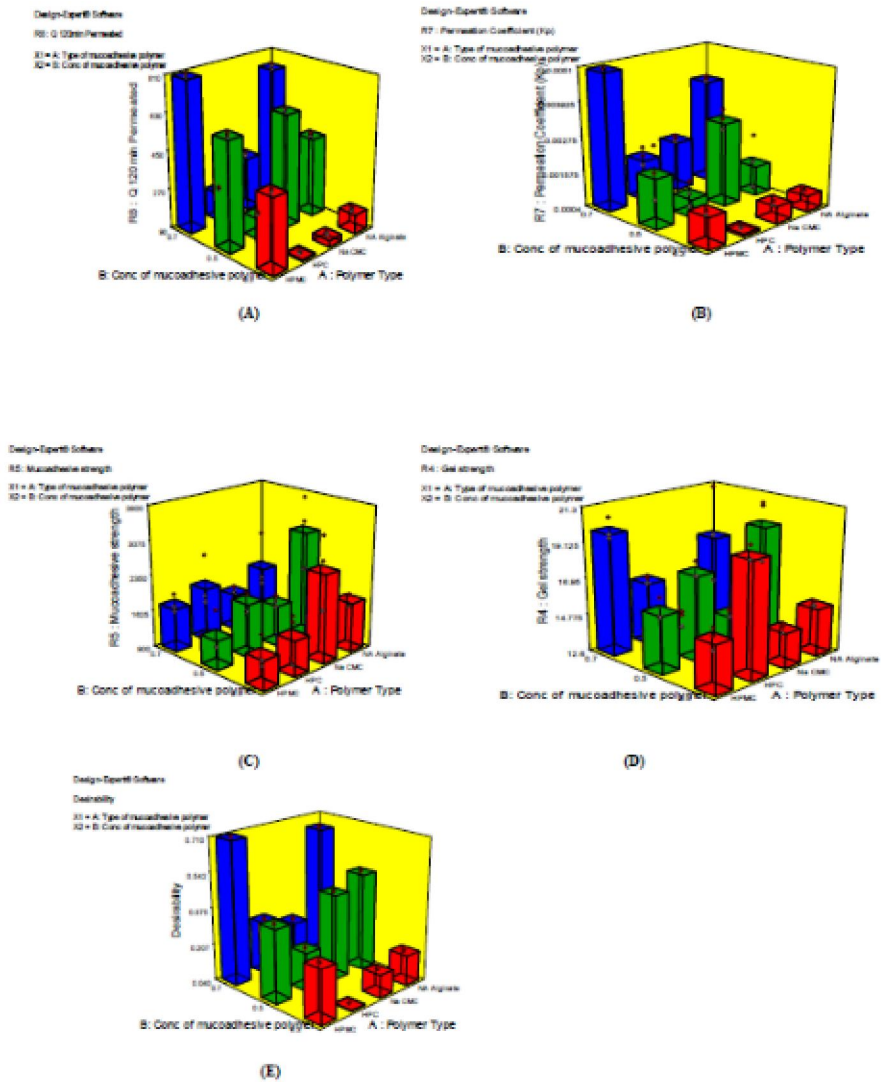

Fig. 3: Response 3D plots for the effect of type (X1) and concentration (X2) of mucoadhesive polymer on the Q120(A), KP (B), Mucoadhesive strength (C), and gel strength (D) and desirability of formulae (E)

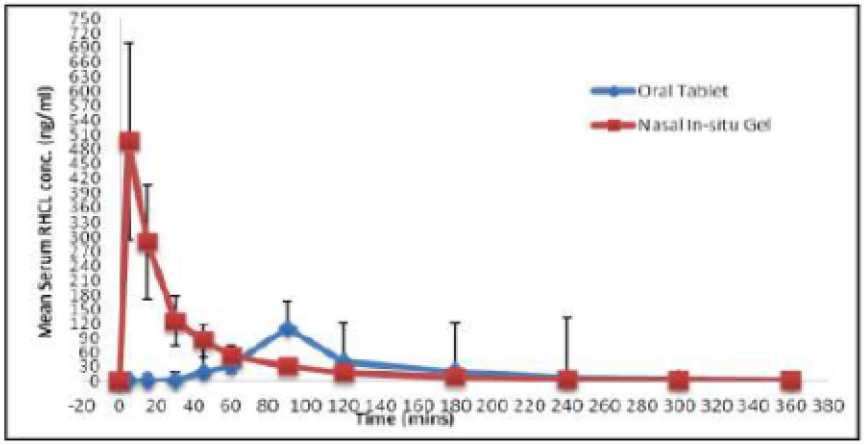

Figure 4: Mean serum concentration-time profiles in rabbit's plasma following administration of peroral Requip® Tablets and intranasal in situ gel of RHCl in rabbits. In-situ gel composed of 18% w/v PF127- 0.7% w/v HPMC.

Conclusion

The thermoreversible gel PF-127 can be considered as a suitable drug carrier system with unique characteristic as its micellar properties and gelation behavior to formulate systems with excellent solubility as well as suitable delivery rate. Combination of PF127 with mucoadhesive polymers increase the viscosity, bioadhesive properties and so the residence time of the dosage form at the site of absorption to provide firmer platform of drug delivery to the nasal cavity than other gel types.

RHCl intranasal mucoadhesive delivery system of thermoreversible PF127 in-situ gels proved to be successful promising biocompatible systems than the peroral route for enhancing the contact time and prolonging the residence time of RHCl at the application site to improve its bioavailability which in turn results in reducing its therapeutic dose with lesser side effects with high patient compliance. As this route is in direct contact with brain circulation, brain targeting and RHCL permeation through Blood Brain Barrier is expected to be increased.

Abbreviations

PD; Idiopathic Parkinson's disease, RLS; Restless Legs Syndrome, RHCL; Ropinirole Hydrochloride, PF127; Pluronic F127, HPMC E5; Hydroxypropyl methylcellulose Methocel E5, HPC; Hydroxypropyl cellulose, Na CMC; Sodium carboxymethylcellulose, NaALG; Sodium Alginate, PBS; Phosphated Buffer Saline, Na2HPO4; Disodium Hydrogen Phosphate, KH2PO4; Potassium Dihydrogen phosphate, NaCL; Sodium Chloride, KCL; Potassium Chloride, mins; minutes, IS; Internal standard, MTBE; Methyl-t-butyl-ether, fig; figure.

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