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Development and Correlation between *in vitro* Drug Release and *in vitro* Permeation of Thermally Triggered Mucoadhesive *in situ* Nasal Gel of Repaglinide PVP K30 Complex

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ABSTRACT

The aim of the investigation was to develop and evaluate thermoreversible *in situ* nasal gel formulations of repaglinide (REP) and to establish correlation between its *in vitro* release and *ex vivo* permeation profiles. The solubility of REP was enhanced by preparing solid dispersions (SDs) with hydrophilic carriers (PVP K30/ PEG 6000/ poloxamer 188) in different weight ratios. REP: PVP K30 (1:5) was selected as the optimized SD as it showed highest enhancement in solubility (405%). The optimized SD was characterized by SEM and DSC and incorporated into a blend of thermoreversible and mucoadhesive polymers (poloxamer 407 and carbopol 934 P) by cold technique to form *in situ* gels (F1-F6). The prepared *in-situ* gels were evaluated for various pharmacotechnical features and the formulation F3 exhibited least gelling time of 6.1± 0.20, good mucoadhesive property to ensure sufficient residence time at the site of application and a %CDR of 82.25%. The *ex vivo* permeation characteristics across goat mucosa can be summarized as CDP of 78.7%, flux = 6.80 mg/cm^2/h; permeability coefficient of 2.02 mg/h and zero order kinetics. On correlating the CDR profile of F3 with that of its CDP profile, a R^2 value of 0.991 (slope= 0.921) was observed. The value of slope approximating one, suggested that almost entire amount of drug released from F3 was capable of permeating across the nasal mucosa, *ex-vivo* indicating that *in-situ* nasal gels of REP for systemic action can be successfully developed for the management non-insulin dependent type-II diabetes mellitus.

Keywords: Solid dispersion, nasal *in-situ* gel, in vitro release, permeation characteristics, correlation.

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INTRODUCTION

The nasal mucosa is considered as an administration route that favours faster and higher level of drug absorption due to its porous endothelial membrane, large surface area, and high total blood flow. [1-2] The nasal cavity offers a number of advantages such as easy accessibility, optimal permeability for lipophilic and low molecular weight drugs, and avoidance of both harsh gastrointestinal milieu and hepatic first pass metabolism. [3-4]

Repaglinide (REP) is a meglitinide phenylalanine analogue (2-ethoxy-4-({[(1S)-3-methyl-1-[2-(piperidin-1-yl) phenyl] butyl] carbonyl} methyl) benzoic acid) used an oral antidiabetic for the treatment of type II (non-insulin dependent) diabetes mellitus. It acts primarily by decreasing insulin resistance. A BCS class II, is a highly lipophilic molecule with partition coefficient (log P) of 5.9, poor dissolution properties, presents poor oral bioavailability of 50%. [5-6] Although REP is rapidly absorbed after oral administration, it is critical to improve the dissolution rate of REP in order to enhance bioavailability due to its low solubility. [7] REP induces rapid onset of short-lasting insulin release. It is administered before each meal to control postprandial hyperglycemia. [8] Poor solubility in gastrointestinal fluids causes variations in its dissolution rate which leads to incomplete bioavailability. [9] Hence in the current project an attempt was made to increase the solubility of the drug by solid dispersion technique. The effects of hydrophilic carriers on enhancement in solubility of the drug were investigated and the optimized SD was selected. The optimized SD was then incorporated into the mucoadhesive *in-situ* gel for nasal delivery with aim to overcome the limitations associated with oral administration.

REP is commercially available as tablets and capsules, alone and in combination with metformin. [8] A plethora of reports on developing effective delivery systems can be found in the scientific literature. Polymeric nanoparticles of REP were developed using ethyl cellulose by solvent evaporation method in order to obtain a novel delivery system adequate for the treatment of diabetes. [10] Floating multiparticulate system of beta cyclodextrin complexed REP was prepared by melt granulation method using gelucire 43/01 as a binder. [11] In another research report solid matrix tablet of REP was formulated using sodium alginate, sodium CMC and PVP and evaluated for controlled drug release of the drug. [12] Though these reports claim superiority over the conventional formulations, the drug related gastrointestinal adverse effects and extensive hepatic metabolism on oral administration cannot be ignored.

Nasal route can serve as an alternate delivery route to obviate the gastrointestinal side effects. *In-situ* nasal gel formulations offer an alternative for achieving systemic drug effects of parenteral route. It also promotes the easy and convenient deliverance of accurate dose as well as prolongs residence time of drug in contact with mucosa. The favorable features of the drug and not a single report on its intranasal delivery guided us for developing an *in-situ* nasal gel of REP that would avoid hepatic metabolism as well the adverse effects associated with drug on oral administration.

MATERIALS AND METHODS

REP was a kind gift from Sun Pharmaceuticals, Andaman and Nicobar, India. Poloxamer 407 and Carbopol 934 were procured from BASF Corporation, USA and CDH Pvt. Ltd., New Delhi, India. The chemicals supplied by SD Fine Chem Ltd., Mumbai, India are PVP K 30, poloxamer 188, polyethylene glycol (PEG) 6000, methanol and potassium dihydrogen orthophosphate.

Table 1: Composition and evaluation parameters of solid dispersions of REP

Code	Carrier	Drug: carrier (by weight)	Solubility (mg/ml)	Enhancement in solubility (%)	Drug content (%)
REP	--	--	0.037	-	-
SD1	Poloxamer 188	1:1	0.087	136	90.1 ± 1.50
SD2	Poloxamer 188	1:3	0.125	237	86.0 ± 1.32
SD3	Poloxamer 188	1:5	0.162	337	82.3± 1.04
SD4	PEG 6000	1:1	0.050	35	82.0 ± 1.32
SD5	PEG 6000	1:3	0.112	202	78.8 ± 1.25
SD6	PEG 6000	1:5	0.125	237	80.8 ± 0.76
SD7	PVP K 30	1:1	0.100	170	94.5 ± 1.80
SD8	PVP K 30	1:3	0.150	305	96.5 ± 0.45
SD9	PVP K 30	1:5	0.187	405	98.4 ± 0.51

Preparation of solid dispersions

The solid dispersions of REP with PVP K30 were prepared by solvent evaporation method while the solid dispersions with PEG6000 and poloxamer 188 were prepared by melting method. For the former, REP and PVP K30 were taken in different weight ratios (1:1, 1:3, and 1:5). The mixtures were triturated and dissolved in methanol. The solution was evaporated at 40°C and the residual mass was scraped and sifted through sieve # 120 and stored in a dessicator for drying. The composition is given in the Table 1. In melting method, PEG 6000 was melted in a water bath at 70°C. REP was added in the solid state and the mixture was stirred well until homogeneity was attained. The mixture was allowed to cool slowly at room temperature 25°C, and then pulverized in a glass mortar. The pulverized mass was sifted through a sieve No. 120 and kept in a dessicator for drying. The same procedure was repeated for Poloxamer 188, where the polymer was melted at temperature of 55°C. The solid dispersions were stored in a dessicator until use for drug content analysis and equilibrium solubility.

Drug content

Solid dispersion theoretically equivalent to 2 mg of REP was weighed accurately and dissolved in 2 ml of methanol. The stock solution was diluted with distilled water to make up the final volume up to 10 ml and analyzed spectrophotometerically (UV spectrophotometer, Shimadzu Pharmaspec 1700, Kyoto, Japan) at 281 nm.

Equilibrium solubility

To assess the solubility of drug, an excess amount of REP and its solid dispersion(s) with PVP K 30/ PEG 6000/ poloxamer 188 were added separately to 10 ml of distilled water in a conical flask and agitated on a water bath shaker (Hicon Enterprises, New Delhi, India) maintained at 37± 0.5°C. The flasks were shaken for 72 h and thereafter, the samples were withdrawn, filtered through membrane filter (0.45µ) and analyzed spectrophotometrically. The optimized solid dispersion

was selected on the basis of maximum enhancement in the solubility of REP and characterized by scanning electron microscopy and differential scanning calorimetry.

Scanning Electron Microscopy

The surface morphology was studied by scanning electron microscope (JEOL 5400 Tokyo, Japan). The samples were adhered on a double-sided tape stuck to aluminum stub. The stubs were then coated with gold ion by sputter coater unit under an argon atmosphere in order to make them conductive. The micrographs were visualized under 2.96 KX magnification.

Differential Scanning Calorimetry

Differential scanning thermograms of REP, PVP K 30, and optimized solid dispersion were obtained using a DSC Q-200 V 24.4, USA. Thermal behaviours were studied with samples sealed (5 to 10 mg sample) in aluminium pans and under a nitrogen gas flow of 20 ml/min. The heating rate of samples was 10°C/min over a temperature range of 60-200°C.

In situ nasal gel

The optimized solid dispersion was developed as *in situ* nasal gel. A formulation design comprising of six formulations was used to optimize thermosensitive mucoadhesive *in-situ* nasal gel formulation. The amount of poloxamer 407 (thermosensitive), and carbopol 934 P (mucoadhesive polymer) was varied at three levels (low, medium and high) to analyze the gelling and mucoadhesive characteristics of the formulated *in situ* gels. As the *in situ* gel was aimed at sustaining the drug release for 12 h, accordingly the REP dose to achieve the same was calculated.

Dose calculation for sustained release

The dose for sustained release of the drug from *in situ* nasal gel was calculated using the following equation:

$$D_T = Do\ (1 + 0.693\ t/t_{1/2}) \quad \ldots\ldots Eq.1$$

Do = conventional dose of REP (2- 4 mg 4 times a day); t = Time duration for sustained release (12 h) and $t_{1/2}$ = half-life of REP (1 h). D_T was calculated as 18.6 mg that was rounded off to 19 mg for handling purpose.

Preparation of thermally triggered mucoadhesive *in-situ* nasal gel

In-situ nasal gel gels were prepared by the cold method described by Schmolka. [13] An amount of SD9 equivalent to 19 mg of REP was mixed with carbopol 934 P and stirred with 50 ml of distilled water at room temperature. The dispersions were then cooled to 4°C by storing in a refrigerator for 3 h. Poloxamer 407 was added slowly with continuous stirring on a thermostatically controlled magnetic stirrer maintained at 4°C. The dispersions were then stored in a refrigerator overnight to get clear sols.The formulations were stored in a refrigerator so that they remain in sol form and evaluated for the parameters detailed below.

Evaluation

Clarity

The clarity of all formulations (F1-F6) was determined by visual inspection under black and white background.

pH and viscosity of sol

One ml of each formulation was transferred to the 10 ml volumetric flask and diluted with distilled water to make volume up to 10 ml. The pH of the resulting solution was determined by calibrated pH meter. The viscosity of each sol was measured using Brookfield viscometer DVII+ Pro coupled with S-94 spindle at 100 rpm at 4 ± 1°C.

Drug content

One ml of each formulation was extracted with 1 ml of methanol by vortexing. Appropriate dilutions were made with phosphate buffer, pH 6.4 and assayed.

Gelling temperature and time

The gelling temperature of the formulation was determined by method described by Miller and Donovan. [14] Two ml of aliquot of each formulation was taken in a test tube, immersed in a water bath. The temperature of water bath was increased in increments of 1°C and left to equilibrate for 5 min at each new increment. The samples were observed for gelation which was said to have occurred when the meniscus would no longer flow upon tilting through 90°. Gelling time was recorded as the time for first detection of gelation.

Gel strength

The gel strength was measured by the method described in literature. [15] The property was quantitated determined by placing 50 g of formulation in a 100 ml graduated cylinder and gelled at 37°C using thermostat. A weight of 35 g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by the weight to penetrate 5 cm was measured.

Ex-vivo mucoadhesive strength

Ex-vivo mucoadhesive strength was determined by modified balance method using the freshly excised goat nasal mucosa. The two sides of the balance were balanced by placing a beaker on the right hand pan and 5 g weight on the left hand pan. A piece of (1×1 cm^2) of goat nasal mucosa was fixed with cyanoacrylate glue over inverted beaker which was covered with inert aluminium surface. The assembly was lowered into the glass beaker filled with phosphate buffer, pH 6.4 to keep the mucosa moist and kept underneath the left hand side of the balance. One gram of the gel was spread as a thin film (thickness 2 mm) on the lower surface of the left hand pan of the balance. Beaker was removed from the right hand pan which lowered the left hand pan along with the gel. The balance was kept in this position for 2 min contact time. The beaker was replaced on the right hand pan and water was gradually added from burette at the rate of 100 drops/min to the beaker over right hand pan until the mucosal membrane detached from the gel surface. [16] The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

Mucoadhesive strength (dynes/cm^2) = m g / A ….Eq. 2

Where, m = mass required for detachment in gram, A = Area of mucosa exposed, and g = Acceleration due to gravity (980 cm/s^2). The nasal mucosa was changed for each measurement

In vitro release

The *in vitro* release drug from sol was carried out using fabricated Franz diffusion cell consisting of donor and receptor compartment separated by dialysis membrane of pore size 0.22µm (Himedia Ltd., Mumbai,). Formulation equivalent to 2 mg/ml REP was placed in donor compartment and receptor compartment was filled with phosphate buffer, pH 6.4 and constantly stirred using magnetic stirrer. The donor and receptor chambers were separated by dialysis membrane soaked in receptor medium overnight prior to experiment. The temperature was maintained at 37 ± 1.0^{o}C in order to simulate body conditions. One ml sample from receptor chamber was withdrawn at predetermined time intervals replacing the withdrawn sample with fresh phosphate buffer, pH 6.4 after each sampling for 12 h. The samples were analyzed spectrophotometrically at 281 nm. Graph was plotted between the cumulative drug release versus time to analyze the release characteristics.

Ex vivo permeation

The *ex vivo* permeation study of all the formulations and pure drug suspension was performed using Franz diffusion cell using goat nasal mucosa. The nasal mucosa was obtained from local butcher shop immediately after the animal was slaughtered and transferred into phosphate buffer, pH 6.4 within one hour of slaughtering. The nasal conch was collected and washed with phosphate buffer, pH 6.4. The extraneous tissues were removed to get the mucosa. The donor compartment was filled with formulation equivalent to 2 mg of REP and the receptor compartment was filled with 10 ml of freshly prepared phosphate buffer, pH 6.4 and stirred continuously with magnetic bar. The donor and receptor compartment was separated by nasal mucosa (area 0.785 cm^2) in such a way that its epithelial surface faced the donor compartment. The whole assembly was maintained at 37 ± 0.5^{o}C. One ml sample was withdrawn from the receptor chamber and replaced with an equal volume of phosphate buffer, pH 6.4 at predetermined time points of 0, 1, 2, 4, 6, 8, 10, 12 h. The samples were analyzed for the amount of drug permeated at 281 nm. Graphs were plotted between percent cumulative drug permeated against time. Flux (Jss) was calculated as the slope of steady state portion of the plot (mg/h) between cumulative amount of drug permeated versus time.

Permeability coefficient (Kp) = Jss/ Co ……Eq. 3

Where; Jss = steady state flux (µg/cm^2/h), Co = total donor concentration of the formulation (mg)

Target flux (J_{Target}) = Css Cl BW/A ….Eq.4

BW represents the standard human body weight (70 kg), A represents the surface area of nasal mucosa used for the permeation study, Css is the therapeutic concentration of REP (2-4µg/L) and Cl the total clearance. For calculation the average values of both Css and Cl were taken. [17] The calculated J_{Target} for REP was calculated as 0.78 (µg/cm^2/h). Permeation kinetics of each formulation was determined by subjecting the data to various models (Zero, First, Higuchi model). Additionally, graphical correlation between *in-vitro* drug release and *ex-vivo* drug permeation was established by linear regression.

Selection of optimized gel

The optimized formulation was selected on the basis of least gelling temperature, highest mucoadhesive strength and highest percent cumulative drug permeated after 12 h.

Histological evaluation

The tissue used for *ex vivo* permeation study was compared with freshly collected nasal mucosa incubated with phosphate buffer, pH 6.4 to assess histological change if any. After permeation study, the nasal mucosa was cleared off the gel, fixed in 10% buffered formalin, pH 7.2, routinely processed and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin. The samples were observed through photomicroscope (Hicon enterprises, New Delhi, India) at 1000X magnification.

Stability

For the assessment of stability of the optimized *in-situ* gel of REP (F3), was stored in a sealed glass vial at 40°C/75% relative humidity in the stability chamber for 3 months. The samples were withdrawn at predetermined time intervals of 0, 1, 2 and 3 months and observed for physical stability, drug content and *ex-vivo* drug permeation characteristics. The results were supported by statistical analysis using ANOVA (significance level $p<0.05$).

RESULT AND DISCUSSION

Solid dispersion

REP, a BCS class II drug is a poorly soluble drug with good permeability characteristics. Poor solubility causes the drug to dissolve very slowly in the gastrointestinal tract, thereby leading to poor dissolution and hence poor bioavailability. Solid dispersion is a firmly established as a platform technology for improving solubility of poorly soluble drugs. The solid dispersions of REP were prepared using inexpensive and easily available hydrophilic carriers PEG 6000, Poloxamer 188 and PVP K 30 to enhance its solubility. The solid dispersions were free flowing, white powders with drug content in the range of 78.8 ± 1.25 to 98.4 ± 0.51 (Table 1). Highest drug content was recorded for solid dispersions prepared with PVPK30 (94.5 ± 1.80 - 98.4 ± 0.51%) followed by those prepared with poloxamer188 and least for PEG6000 solid dispersions. Clearly the solid dispersions prepared by solvent evaporation method showed higher drug content than fusion method.

Low values of standard deviation indicated that the drug was uniformly dispersed in the formulation.

Equilibrium solubility

The solubility of REP in double distilled water was found to be 0.037 mg/ml whereas the solubility of its solid dispersions ranged from 0.100 to 0.187 mg/ml. The enhancement in solubility widely ranged from 35 to 405% as given in Table 1. The reasons attributable are the inherent differences between the carriers in terms of hydration, dissolution and complexation mechanisms of drug with different carriers. [18] The rank order in descending manner was PVPK30> poloxamer 188> PEG6000. Furthermore, the enhancement in solubility of REP was corelatable to the amount of carrier used. Thus it increased with increasing amount of carrier by a factor of ≥ 1.86) in the levels used in the study. As explained in the literature the solubility enhancement in solid dispersion is based on the phenomenon of drug entrapment within the dispersion matrix at molecular level. Since a solid dispersion is a drug–polymer two component system, the drug–polymer interaction is the determining factor in its performance. [19] Based on the maximum enhancement in the solubility of REP with PVP K30 in the ratio of 1:5, SD9 was selected as the optimized formulation.

Scanning Electron Microscopy

In order to deduce the physical state of the optimized solid dispersion scanning electron microscopy was carried out against reference sample. REP appeared as regular three dimensional crystals (Fig 1a) while PVP K 30 appeared as three dimensional globular particles (Fig 1b). In the micrograph of SD9 (Fig. 1c) the original morphology of both the individual components disappeared and an amorphous product was generated which proved the formation of solid dispersion.

Differential Scanning Calorimetry

The DSC thermogram of REP displayed a sharp endothermic peak at 127°C (ΔH of 118.57 J/g) due to melting of REP (Fig. 2a) which is consistent with literature report. [17] For PVP K 30 one broad endothermic peak at 118°C (ΔH of 258. 26 J/g) was observed due to dehydration (Fig. 2b). In the thermogram of SD9 (Fig. 2c), the peak of REP did not disappear rather a slight shift towards the lower temperature at 123°C (ΔH of 106.03 J/g) was seen with reduced intensity of the peak along with the peak of PVP K 30 at 78°C (ΔH of 101.65 J/g). The shift toward the lower temperature was the result of mixing of two components implying that REP was included into the PVP K 30 and partial amorphization. This confirmed the formation of solid dispersion of REP, a product of reduced crystallanity.

Thermally triggered mucoadhesive *in situ* nasal gel

Six formulations (F1-F6) of thermally triggered mucoadhesive *in situ* were successfully prepared by cold method using poloxamer as gelling agent and carbopol as mucoadhesive agent. The formulations appeared as translucent sols with faint odour of raw materials. The formulations were evaluated for various parameters and the results are discussed below.

Clarity and Drug content

All the formulations were found to be free from solid residue matter that could potentially harm the nasal mucosa and affect the syringability of the formulations. The drug content of all the formulations ranged between 83.24 ± 0.43 - 94.38 ± 0.25 % (Table 2).

pH and viscosity of sol

Nasal mucosa being a delicate organ, pH is a very important consideration for formulations intended for nasal application. The normal physiological pH of the nasal mucosa ranges from 4.5-6.5. But the nasal mucosa has the capability to tolerate extreme pH range from 3 to 10. [20] The pH of all the formulations (F1-F6) narrowly varied from 5.42-5.79 (Table 2) which is well within the desirable range and thus the formulations can be presumed to be free from nasal irritation. While an alkaline pH inactivates lysozyme secreted by the nasal cells making the nasal tissue susceptible to microbial infection; lower pH acts as hypertonic solution that causes shrinkage of epithelial cells and also inhibits ciliary activity in the nasal cavity. [21] The viscosity of the formulations ranged from 27.44 ± 0.34 to 42.17 ± 0.11 cp. Increase in the carbopol 934P content increased the viscosity of sols. The sol formulations should have an optimum viscosity, which would facilitate easy instillation into the nasal cavity wherein it will then undergo rapid sol to gel conversion. Viscosity plays an important role in a nasal formulation as it helps in increasing the residence time of the formulation by decreasing the mucociliary clearance. [22]

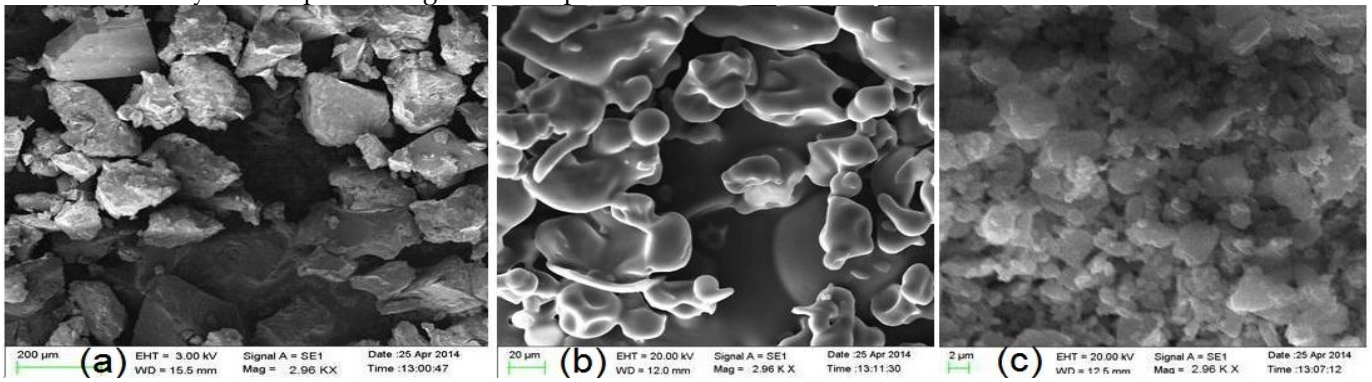

Fig. 1: Scanning electron micrographs of (a) REP (b) PVP K 30 and (c) SD9.

Table 2: Formulation design and evaluation parameters of thermoreversible mucoadhesive formulations of in-*situ* gel of REP solid dispersion containing 140 mg of SD9.

Code	Poloxamer 407 (%w/v)	Carbopo l 934P (%w/v)	pH ± S.D	Gelling temp. (°C) ± S.D	Gelling time (sec) ± S.D	Viscosity of sol (cps) ± S.D	Drug content (%) ± S.D	Gel strength (sec) ± S.D	Ex vivo mucoadhesive strength (dynes/cm²) ± S.D
F1	15	0.25	5.79 ± 0.01	35.24 ± 0.04	16.61 ± 0.25	29.63 ± 0.34	85.46 ± 0.25	29.54 ± 0.50	3295.13 ± 0.71
F2	25	0.50	5.61 ± 0.04	34.00 ± 0.01	13.73 ± 0.25	28.23 ± 0.11	89.16 ± 0.37	25.86 ± 0.76	3550.24 ± 0.34
F3	30	1.0	5.42 ± 0.02	29.42 ± 0.11	6.13 ± 0.20	42.17 ± 0.11	94.38 ± 0.25	47.02 ± 0.45	6432.44 ± 0.83
F4	15	1.0	5.69 ± 0.01	31.48 ± 0.02	11.86 ± 0.2	38.36 ± 0.17	90.56 ± 0.41	40.72 ± 0.11	6104.92 ± 0.56
F5	25	0.25	5.74 ± 0.01	30.60 ± 0.02	10.14 ± 0.26	27.44 ± 0.34	83.24 ± 0.43	32.44 ± 0.11	4386.78 ± 1.26
F6	30	0.50	5.67 ± 0.01	32.01 ± 0.01	12.47 ± 0.11	37.15 ± 0.15	88.73 ± 0.40	38.76 ± 0.15	4738.59 ± 0.76

Table 3: *Ex-vivo* permeability parameters of *in situ* nasal gel of REP across goat nasal mucosa

Code	% Cumulative drug permeated (12 h)	Flux (J_{ss}) (mg/cm²/h)	Enhancement ratio	Permeability coefficient (K_p) (cm/h)	Best fit model	R^2
REP	18.84 ± 0.34	3.36	-	1.68	Higuchi	0.9492
F1	58.94 ± 0.91	6.05	1.80	2.43	Zero order	0.9690
F2	63.42 ± 0.84	6.80	2.02	3.40	Higuchi	0.9766
F3	78.73 ± 0.37	9.90	2.94	5.01	Zero order	0.9913
F4	74.26 ± 0.48	4.05	1.20	2.02	Zero order	0.9902
F5	67.53 ± 0.71	5.85	1.74	2.92	Zero order	0.9931
F6	71.58 ± 0.53	5.37	1.59	2.68	Higuchi	0.9904

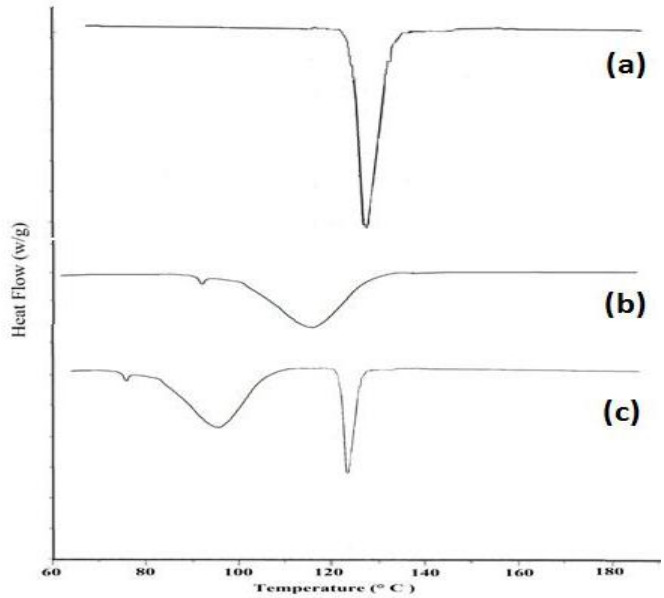

Fig. 2: DSC thermograms of (a) REP (b) PVP K 30 and (c) SD9.

Gelling temperature and time

The gelling temperature of the formulations ranged from 29.42 ± 0.11 - 35.24 ± 0.04°C (Table 2). A gelling temperature is considered to be suitable if it is in the range of 25°C to 37°C. At gelling temperature lower than 25°C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling and administering, and if it is higher than 37°C, it would not form gel at the temperature of nasal cavity so would result in rapid nasal clearance of administered formulation. [23] The formation of gel may be explained as formation of micelles. When the temperature is increased the micelles formation increases due to negative coefficient of solubility of block copolymer micelles. The micelles become so tightly packed that the solution becomes immobile and gel is formed. [24] Our results revealed that gelling temperature decreased with increase in the concentration of thermosensitive polymer poloxamer 407 due to formation of larger number of the micelles. [25] The mucoadhesive agent carbopol 934 P also causes lowering of gelling temperature because of its ability to bind to poly(ethylene) oxide chains present in poloxamer 407 molecule, thus promoting dehydration and causing an increase in entanglement of adjacent molecule with extensive intermolecular H-bonding thus enhancing micellar association. [23] Immediate gelling increases residence time and enhances bioavailability of drug. All the formulations gelled rapidly. The gelling time of the formulations ranged between 6.13 ± 0.20 to 16.61 ± 0.25. Lowest value was recorded for F3 that was constituted of maximum levels of poloxamer 407 and carbopol 934P.

Gel strength

The gel strength of the formulations was between 29.54 ± 0.50 and 47.02 ± 0.45 (Table 2) which is acceptable for nasal delivery as the gel strength values in between 25-50 are considered optimal. A gel strength of less than 25 would not be able to maintain gel integrity and may erode rapidly while gels with strength greater than 50 are too stiff and have potential to cause discomfort to the mucosal surfaces or may damage it. [26] Among the developed formulations, F3 showed highest gel strength of 47.02 while F1 showed least gel strength of 29.54. F3 composed of highest levels of both poloxamer 407 and carbopol 934P exhibited highest gel strength. On the other hand F1 was made of lower levels of both the polymers. Clearly the gel strength was directly dependent on the levels of constituting polymers.

Ex vivo mucoadhesive strength

The results in Table 2 revealed that amount of carbopol 934 P influenced mucoadhesive strength. On increasing the concentration of carbopol 934 P, mucoadhesive strength increased due to wetting and swelling of carbopol 934 P that permitted intimate contact with nasal tissue. As described in literature, interpenetration of bioadhesive carbopol 934 P chains with mucin molecules leads to entanglement and formation of weak chemical bonds between entangled chains. Furthermore, the carboxyl group of carbopol 934P undergoes hydrogen bonding with sugar residues in oligosaccharide chain of mucin glycoprotein in mucus membrane, resulting in strong network between polymer and mucus membrane. The strength of the

mucoadhesive force, is a prominent determinant of retentivity of the formulation in the nasal cavity and hence absorption across mucosal tissues. [27]

In vitro release

The *in vitro* release profiles of mucoadhesive *in-situ* nasal gels of REP (Fig. 3) were compared to the release profile of pure drug. REP depicted a cumulative drug release (CDR) of only 20.35% at the end of 12 h which may due to poor solubility of the drug in the release media that resulted in the poor dissolution of the drug. The CDR in 12 h from all the formulations designed using REP-PVP K30 dispersion was higher than pure drug indicating solubility limited dissolution of the drug. Consequently, the CDR ranged between 60.14 ± 0.65 and 82.25 ± 0.90%, almost 3-4 folds higher than pure drug formulation. Amongst these formulations, F3 revealed maximum CDR of 82.25% in 12 h followed by F4, F6, F5, F2 and F1.

Ex vivo permeation

Ex-vivo permeation profiles of the formulations in phosphate buffer, pH 6.4 (Fig. 4) were used to calculate *ex-vivo* permeability parameters (Table 3). The target flux was calculated as 8.02 mg/cm^2/h and pure drug was not able to reach the target flux. Though the formulations designed using REP solid dispersion, the flux was higher than pure drug formulation but except for formulation F3, none could achieve the target flux. Higher flux values of formulations F1- F6 than pure drug is attributable to the permeability affecting property of PVP K 30. Amongst all, formulation F3 composed of high levels of both poloxamer 407 and carbopol 934 P showed highest release and correspondingly highest permeation of drug (78.73 ± 0.37%) clearly demonstrating the role of levels of constituting polymers. F3 by virtue of its highest carbopol levels presented strongest mucoadhesion favouring permeation and consequently release.

The study also depicts the importance of Ca^{++} in enhancing the permeation of REP across the mucosa. The anionic polymer carbopol is reported to demonstrate permeation enhancing properties by its ability to bind to the Ca^{2+} of the nasal mucosa. With the increase in carbopol concentration, the concentration of COO^- group is increased which leads to conformational changes in the polymeric chain. The polymer chain decoils due to electrostatic repulsion of the COO^- resulting in relaxation of polymeric network. At this stage, the drug is rapidly dissolved and diffused from the gels due to extensive swelling of the ionized carbopol. [23] Correspondingly, highest drug permeation was shown by F3 made of 1% w/v carbopol (highest levels) and the least permeation was shown by F1 (of 0.25 % w/v carbopol; lowest level). However, the presence of poloxamer in the gel tends to retard the release rate of drug due to reduction in dimensions of water channels. [28] The polymeric surfactant poloxamer 407, forms micelles in aqueous phase, and the incorporated drug may be released via diffusion through the gel matrix. Both the drug release and permeation can also be affected by the gel viscosity, aqueous channel's size and drug distribution between the micelles and the aqueous phase. [29] The increase in poloxamer causes slight increase in viscosity and hence slightly decreases the release REP from the gel and the permeation. [30] However, in our case the effect of poloxamer concentration was counteracted by the presence of carbopol 934P.

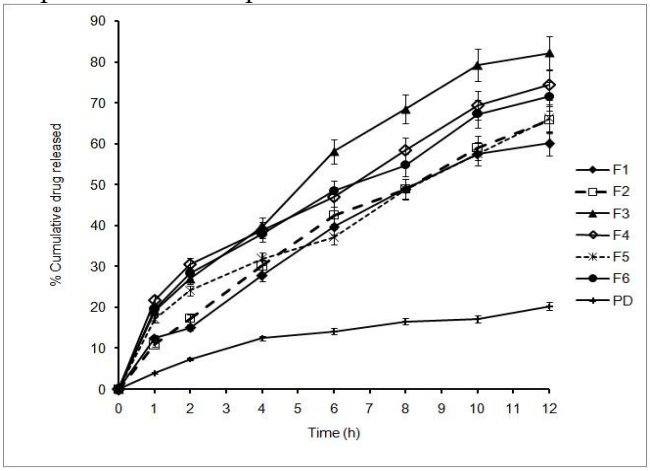

Fig. 3: *In vitro* release profiles of REP from mucoadhesive *in-situ* nasal gels compared with pure drug (PD)

The permeability coefficient for pure drug was 1.68 cm/h and for F1-F6 it ranged between 2.02-5.01 cm/h. The permeability coefficient was higher for the gels containing solid dispersion than the gel of pure drug REP and is co-relatable to the state of drug in the gel and the formulation variables. The drug in solubilized form exhibited higher permeability coefficient than pure drug. Furthermore, formulation F3 composed of highest levels of constituting polymers and highest flux value exhibited highest permeability. The *ex vivo* permeation data when modelled exhibited Higuchi kinetics for pure drug, F2 and F6.On the other hand F1, F3,F4 and F5 best fitted zero order model. The variation in kinetics is not explainable.

Data analysis

The *ex vivo* permeation plot of F3 was compared against the plots pure drug and fitted to various kinetic models to evaluate the role of solid dispersion in enhancing permeation across nasal mucosa. F3 was able to sustain the release and achieve 78.7 ± 0.37% in 12 h (Fig. 4) that was comparable to CDP of 18.84 ± 1.05% achieved by pure drug. This clearly indicates that the gel matrix did not impede the release of solid dispersion loaded *in situ* gel from F3 (Table 3). Compared to pure drug F3 exhibited 2.94 folds increase in *ex-vivo* permeation across goat nasal mucosa that was significantly higher than (P<0.05) pure drug suspension indicating the efficiency solid dispersion loaded *in situ* gel. On fitting the data to various kinetic models, F3 followed zero order kinetics (Table 3) which means that flux was independent of amount of drug permeated at various time points and showed maximum coefficient of permeability (2.02 cm/h) as compared to pure drug.

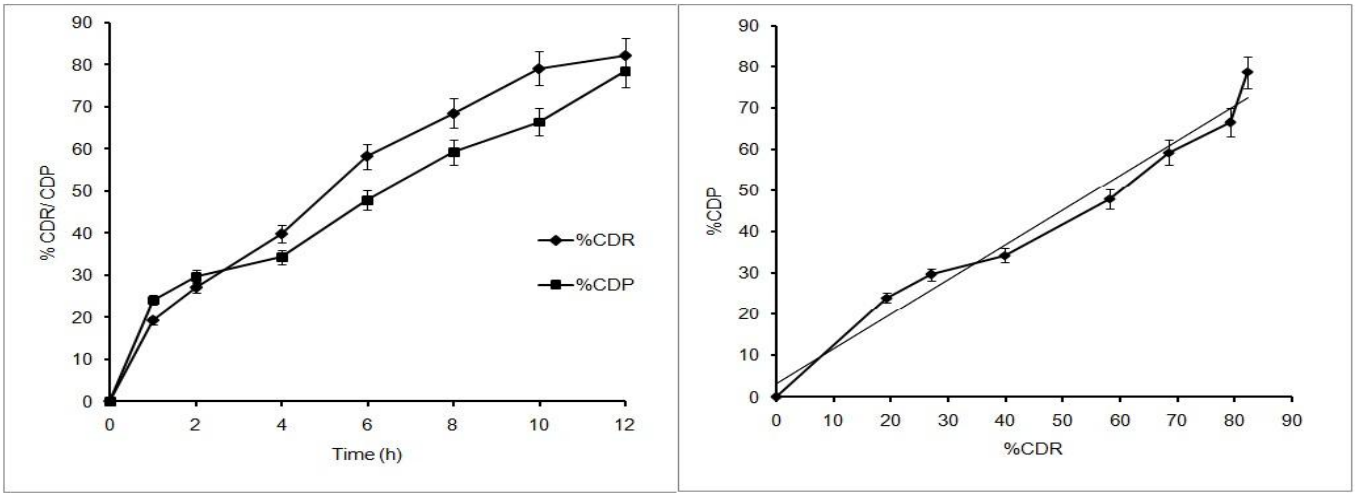

Fig. 4: Correlation plot of % CDR and % CDP for the optimized formulation

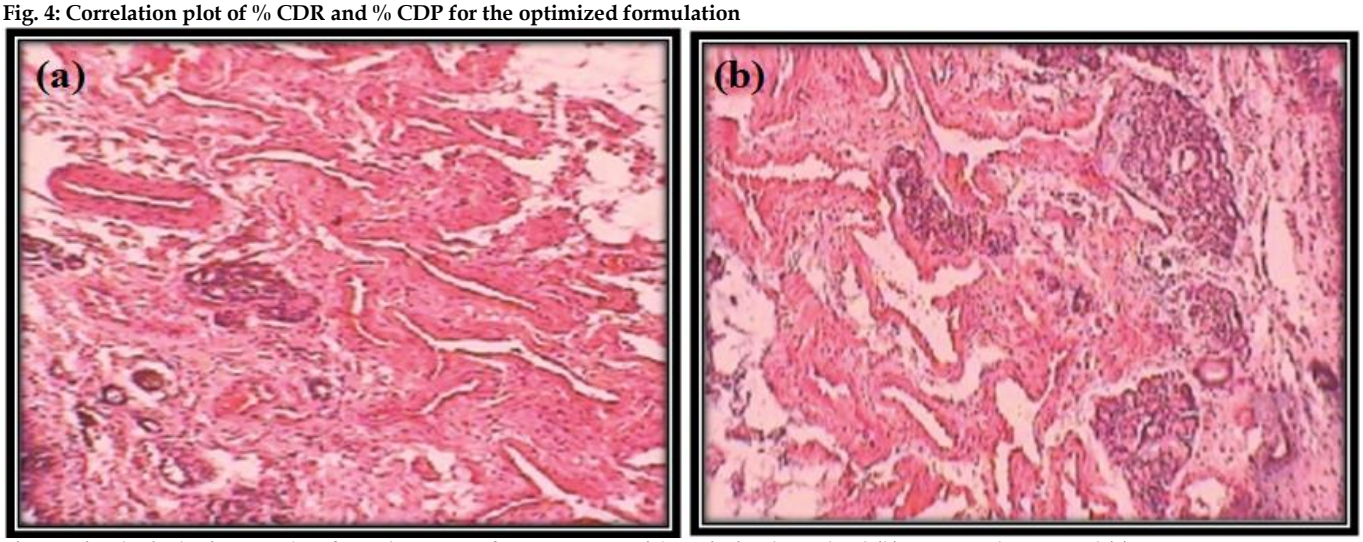

Fig. 5: Histological micrographs of nasal mucosa after treatment with optimized nasal gel (b) compared to control (a)

Selection of optimized gel

The selection of optimized formulation was done on the basis of evaluation parameters including gelling temperature, pH, gel strength, *ex-vivo* mucoadhesive strength and *ex-vivo* permeation study. Considering the mentioned parameters F3 was selected as the optimized formulation with least gelling temperature of 29.42°C. F3 was found to exhibit the maximum mucoadhesive strength of 6434.4 dynes/cm^2 along with maximum drug permeation of 78.7%. The optimized formulation F3 was further subjected to histological and stability study.

Histology

Photomicrograph of goat nasal mucosa was observed for histological changes after permeation studies and was compared with the control. As shown in Fig. 5 neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of the gel, consequently there were no alterations in epithelium layer, and basal membrane when compared with the control mucosa. Hence, it can be concluded that the gel formulation can be safely administered via nasal route.

Stability

The optimized formulation (F3) exhibited no significance difference in the clarity, pH, drug content and *ex-vivo* permeation characteristics at 0, 1, 2 and 3rd month**.** Hence the formulation can be concluded as a stable formulation.

Solubility of repaglinide was successfully increased by making its solid dispersion with PVP K 30. *In situ* nasal gels containing solid dispersion of drug reached the target flux easily. The optimized formulation F3 containing 30% Poloxamer 407, 1% Carbopol 934 P had sufficient mucoadhesive property to ensure longer residence time at the site of application and was able to permeate 78.7% drug through goat nasal mucosa within 12 hour. Controlled release of the incorporated drug was achieved, which ensured better patient compliance and higher therapeutic efficacy.

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