NANOPARTICLE LOADED THERMOSENSITIVE NASAL IN-SITU GELS FOR DELIVERY OF LORATADINE: IN- VITRO & IN-VIVO EVALUATION STUDIES

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ABSTRACT

To investigate nanoparticle loaded thermosensitive *in situ* gel (NPLTIG) for effective treatment of allergic rhinitis. Intranasal thermosensitive *in situ* were prepared by combination of poloxamer 188 and poloxamer 407 (55:45%) with mucoadhesive polymers carbopol 940 (upto 0.1%). Nasal drug delivery systems are better suitable for loratidine particularly for allergic rhinitis. The viscosity of NPLTIG system was found to be in the range (239 to 3659 cps) for the sol, whereas for the gels it was up to (49898 cps). The maximum gel strength and mucoadhesion was found to be (131 seconds) and (6562 dynes/cm^2) respectively. The spreadabilty of NPLTIG formulations was found to be (27.41 gm.cm/sec). Different techniques, FTIR, DSC, SEM and HPLC were used to estimate the incompatibility. Pharmacokinetic study in rabbits showed significant ($p<0.05$) improvement in bioavailability (six-folds) of the drug from NPLTIG than oral solution.

Keywords: Bioavailability, Nanoparticle loaded *in situ* gel, HPLC

INTRODUCTION

Loratadine (LOR) is a second-generation antihistaminic drug used in treatment of allergies such as hay fever (allergic rhinitis), urticaria (hives), upper respiratory tract infections, and other skin allergies [1]. Loratadine, once given orally, is well absorbed from the gastrointestinal tract and reaches peak plasma levels within 1–1.5 h [2]. It experiences rapid first-pass hepatic metabolism which tips to poor oral bioavailability of 40% [3]. Consequently to bypass the liver, an alternative route of administration would be preferred.

Nasal route of drug delivery offer the advantages of potential bypass of first-pass effect and avoidance of presystemic elimination of gastrointestinal tract. Therapeutic effect may be achieved in smaller dose of a particular drug [4].

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Intranasal drug delivery is a favorable nasal route for administration of drugs as it possesses all the above-listed advantages, has large absorptive surface area with high vascularity [5], and is considered equivalent to i.v. route. Nevertheless, the disadvantage connected with the nasal route is rapid elimination of the instilled drug from the nasal cavity by mucociliary beating (clearance half-life of 15 min). This parameters the time available for drug absorption from the applied dosage form and thus consequences in poor nasal bioavailability. In order to prevent rapid mucociliary clearance and improve the bioavailability, a mucoadhesive system may be utilized. These systems adhere onto the mucus and increase the residence time within the nasal cavity. This intensifies contact between nasal mucosa and the drug and facilitates the drug absorption which results in increased bioavailability [6].

The major focus on novel drug delivery systems during the past two decades is to improve the therapeutic efficacy and safety profile of the drug substances. Colloidal drug delivery systems are considered to be more popular than the matrix or reservoir drug delivery systems. Among all the

colloidal systems, Nanoparticles hold promise as drug delivery through various routes due to their greater stability and easier manufacturing ability. These systems are used for specific drug delivery, controlled drug delivery and also for the improvement of bioavailability of the hydrophobic drugs [7].

Recently, in situ-gelling liquids have been investigated as a more convenient dosage form of topical applications. The liquids applied to the topical areas such as eyes can make transition to gels as a result of a chemical/physical change induced by the physiological environments. The transition could be induced by temperature as for poloxamers [8,9]. Poloxamer, a block copolymer made of polyoxyethylene and polyoxypropylene, is known for its excellent compatibility with other chemicals, high solubility capacity for different drugs, and good drug release characteristics [10].

Mucoadhesive nanoparticle loaded *in situ* gel of loratidine for nasal delivery have been reported where the researchers ensured longer retention time of ethylcellulose nanoparticle at the site of deposition and avoidance of hepatic metabolism resulting in improved bioavailability. The nanoparticle loaded mucoadhesive *in situ* system has potential mucoadhesive properties and retention nasal airway pathway. Thus, the present project was aimed at avoidance of multiparticulate system for nasal delivery to avoid the listed complications and consequently to develop a thermally triggered mucoadhesive *in situ* nasal gel in which the drug (Nano form) is directly dispersed in the polymeric dispersion of termosensitive gel. This delivery system is expected to overcome the limitation of earlier report and couple the advantages of mucoadhesion along with the nasal delivery. Poloxamer 188 and 407 was selected as a thermosensitive gelling polymer because it has low toxicity and irritation, excellent water solubility, and compatibility with most of the proposed formulative excipients [11]. For the mucoadhesive property, carbopol 940 was used which increases viscosity of the formulation and forms hydrogen bond with the mucosa that increases the nasal residence time of the formulation [12].

MATERIALS AND METHODS

Loratidine is a gift sample obtained from Kotra Pharma (M) Sdn Bhd, Malaysia. Ethylcellulose and poloxamer 407 procured from Sigma Aldrich USA. Poloxamer 188 was purchased from Merck (Germany). Carbopol 940, polyvinyl alcohol, span 20 and span 40 procured from SD Essex (UK). The experimental work was performed by using HPLC water. All other chemicals were of analytical grade.

Preparation of loraditine nanoparticles

Preparation of loraditine nanoparticles was prepared by emulsion solvent diffusion technique [13]. Drug and polymer (1:1, 1:2, and 1:3) were dissolved in ethyl alcohol by using sonicator (Table 1). This organic phase added (by using aerosol sprayer pressurized up to 200 PSI) to external aqueous phase containing various surfactant at a fixed concentration (1% w/v). The mixture was homogenized in the high pressure homogenizer at 10,000 rpm for 2 hours. Subsequently organic solvent from external aqueous phase was removed under reduced pressure. The formed loratidine nanoparticles were recovered by centrifugation at 6,000 rpm for 10 min followed by washing twice with distilled water and washed nanoparticles were subjected to freeze drying (Table 1).

Evaluation of loratidine nanoparticle: The prepared loratidine nanoparticle formulations were evaluated by particle size, polydispersity index, zeta potential and entrapment efficacy. All the parameters were evaluated by tripilicate [14].

Table-1: Composition of drug loaded nanoparticle

Ingredients	NP1	NP2	NP3	NP4	NP5
Drug and polymer ratio	1:3	1:2	1:1	1:1	1:1
Ethyl alcohol	10	10	10	10	10
Polyvinyl alcohol	1	1	1	-	-
Span 20	-	-	-	1	-
Span 40	-	-	-	-	1

Preparation of nanoparticle loaded in-situ gel: Preparations of temperature response mucoadhesive *in situ* gel were prepared by the

cold method [15]. Specified amount of poloxamer 188 (P188), poloxamer 407 (P407) and carbopol 940 (C940) were stirred in the calculated amount of cold distilled water. The dispersions was cooled to 4°C by keeping it in a refrigerator for overnight. Equivalent to 10 mg of nanoparticle (optimized formulation NP1) was added slowly in polymeric solution with continuous stirring (thermostatically controlled magnetic stirrer). Dispersions was stored in a refrigerator for overnight to get clear sol and eventually stored in a refrigerator so that it remains in sol form. Finally the soln pH was adjusted with triethanolamine.

Gelation temperature: The different formulations of in situ system combinations were evaluated for gelation temperature. The gelation temperature was determined by heating the solution (1-2 ºC) min in a test tube with gentle stirring until gel was formed. The gel was formed when there was no flow after container has overturned [16].

Determination of Spreadability: For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability [17].

Determination of mucoadhesive force: The experimental technique used for determining the bioadhesive force has been derived from a previously published method [18,19]. The experimental setup is presented in Figure. 4. The mucoadhesive force of the formulations was determined as follows; a section of sheep nasal mucosa was cut from the sheep local slaughter house and instantly fixed with mucosal side out onto each glass vial (E) using rubber band. The vial with sheep nasal mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan (A). NPLTIG was added onto the mucosa of first vial. Before applying the gel, 150µL of phosphate buffer pH 7.4 was evenly spread on the surface of the test membrane. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given to the vials. Then, the switch (C) of the infusion apparatus was opened to make the water drop into the glass vial (B) with a constant flow rate of 5 mL/min. The weight of the water in the glass vial (B) kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dyne/cm2), was determined from the minimal weights that detached the gel. The chicken mucosa pieces were changed for each measurement.

Determination of gel strength: Gel strength was measured by placing 50 g of formulation in a 100 cm^3 graduated cylinder and gelled at 37°C using thermostat. A piston of weight 35 g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by weight to sink 5 cm was measured [20].

In vitro permeation studies: The in vitro permeation study was performed using nasal mucosa collected from slaughter house. The nasal conch was collected in phosphate buffer pH6.4 and washed three times with phosphate buffer pH6.4 and extraneous tissues were removed [21]. The prepared nasal mucosa was mounted on Franz diffusion cell to get a permeation area of 3.14 cm^2. Sixteen milliliters of phosphate buffer pH6.4 was added to the acceptor chamber maintained at 34°C. Formulation equivalent to 10 mg of loratidine was placed in the donor chamber. At predetermined time points (0, 0.15,0.30, 1, 2, 3, 4, 5, 6, 8, 16 and 24 hours), 2-ml sample was withdrawn from the acceptor compartment and replaced with an equal volume of the phosphate buffer pH6.4. The sample was then filtered and drug release was determined by RP HPLC method. RP HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, water (pH 4.5) (75:25, v/v), and detection was made at 254 nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation. The drug content was calculated from the calibration curve and expressed as loading efficiency.

Animal handling and administration of nasal gel: For this study, prior clearance from institutional animal ethics committee (Approval number: AUHAEC-22/FOP/2014) was obtained. Briefly, female New Zealand white rabbits (n=3), weighing 2.4±0.2kg were housed individually in stainless steel cages, fed with commercial rabbit diet and provided drinking water ad libitum. The rabbits were fasted for 18 h before and also during the pharmacokinetic study. All the animals were conscious throughout the duration of experiment. A cross-over study protocol (with 1 week washout period) was followed. A micropipette (10–100 mL capacity), with its tip placed 1 cm deep inside the right nostril of rabbits, was used to administer 200 mL of NPLTIG (equivalent to dose of 4 mg/kg of loratidine gel). For oral pharmacokinetic study, 5mL oral solution of loratidine in water (equivalent to dose of 10 mg/kg of loratidine) was administered to rabbits by oral gavage [21].

Collection of blood samples and analysis: Post dosing, blood samples (1.5 mL) were collected by marginal ear vein puncture at following time intervals: 1, 2, 3, 6, 12 and 24 hours. Blood samples were collected into 2mL sample collection tubes containing 4% w/v sodium citrate solution as an anti-coagulant. Plasma samples were obtained by centrifuging blood samples in a cooling centrifuge at 905 × *g* for 10 min at 4ºC. Supernatant clear plasma was carefully collected and frozen at -20ºC till further analysis. For analysis of loratidine plasma samples, a validated HPLC method developed in our lab was used. Briefly, frozen plasma samples were thawed to room temperature; to 100 mL of each sample, 150 mL of ACN was added as a protein precipitating agent. Further, vortex-mixing (5 min) followed by centrifugation at 8500 × *g* for 20 min at 4ºC gave clear supernatant; 100 mL of clear supernatant was collected and 20 mL of this was injected into HPLC.

RESULT AND DISCUSSION

In this paper, different loratidine loaded matrix-type ethyl cellulose nanoparticle formulations were prepared by solvent diffusion technique. This technique works best for water-insoluble drugs like loratidine to provide high drug loading and suitable particle size. Nanoparticle was prepared fixed ratio of different surfactant to investigate the particle size on the physicochemical characteristics of the prepared nanoparticles.

The particle size of prepared nanoparticles were analysed by scanning electron microscopy analysis. The particle sizes of loratidine nanoparticles were found to be 128.8 to 256.2 nm (Table 2). Particle size of nanoparticle play a key role in the transport of drugs to the receptor level and the size of less than 200nm is best suited for increasing the nasal uptake of nanoparticle. All the prepared nanoparticles were appeared spherical in shape (Figure 1 and 2). Zeta potential of loratidine nanoparticles were -15.49 mV to -31.8mV (Table 2). The zeta potential of nanoparticle was reduced due to conjugation of positive charge of loratidine. Entrapment efficiency is the important parameter to optimize the nanoparticle formulations. In the loratidine nanoparticle preparation, ethyl cellulose nanoparticle showed 51.0% and 91.6% (Table 2). The prepared nanoparticle NP1 was higher than other NP formulation code, due to over coating of (increased polymer concentration).

Gelation temperature of nanoparticle loaded temperature response *in situ* gel decreased with increase in concentration of poloxamer 188 from 50.1^{o}C to 35.5^{o}C for a concentration of 43% to 55% (F1 to F4). Gelation temperature of the F5 formula which contains 55% of P188 and 43% of P407 was found to be 33.4^{o}C and to have the required viscosity P407 added to the formulation, it was taken for further studies.

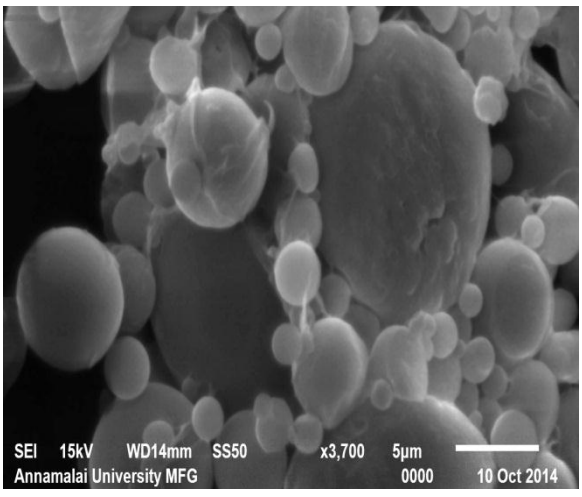

Figure-1: SEM image of loratidine nanoparticle using span 20

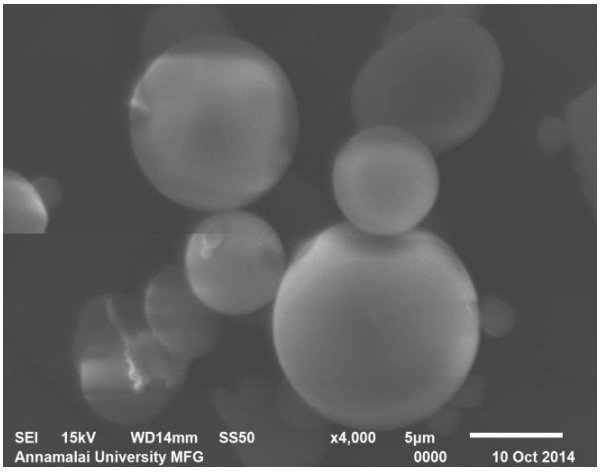

Figure-2: SEM image of loratidine nanoparticle using polyvinyl alcohol

However the optimized concentration of P188 (55%) and P407 (45%) showed a gelation temperature of 35.1ºC which is near to the body temperature and it's suitable for nasal drug delivery. The gelation temperature was decreased in the F7 formulation (33.0ºC) due to 0.1% of mucoadhesive polymer concentration. The optimum concentration of carbopol 940 was found to 0.1% and further addition led to an increase in gelation temperature more than that of body temperature. So, the formula F5, F6, F7 was considered to be optimized formulae, which are gelled at body temperature with considerable viscosity (Table 3).

Gelation time was defined as the time when the elasticity modulus became higher than the viscosity modulus. The gelation time of nanoparticle loaded temperature response *in situ* gel P188/ P407/C940 (55/45/0.1) was observed at 25s, but it took longer time for P188 (43%) which began to show the viscoelastic property of a gel at 62s. At 50.1 °C, the formulation with 47% of P188 gelled within 27s. The higher gelation rate of the F5 formulation with P188/ P407 (55/45) might have resulted from the stronger association of P188 with other components via hydrogen-bonding and ionic interaction. After intranasal application, the shorter gelation time observed in the formulations F5, F6 and F7 would be advantageous in that the rapidly gelled formulation might face the less change of drainage from the site of application, leading to a prolonged retention of nanoparticle loaded gel in the nasal cavity (Table 3).

The rheological properties of formulations F5, F6 and F7 exhibited an increase in viscosities (45979, 47979, 49898 cps) at body temperature. But the other formulations (F1, F2, F3 and F4) have not exhibited considerable increase in viscosity at body temperature which may be due to the increasing concentration of P407 (Figure 3).

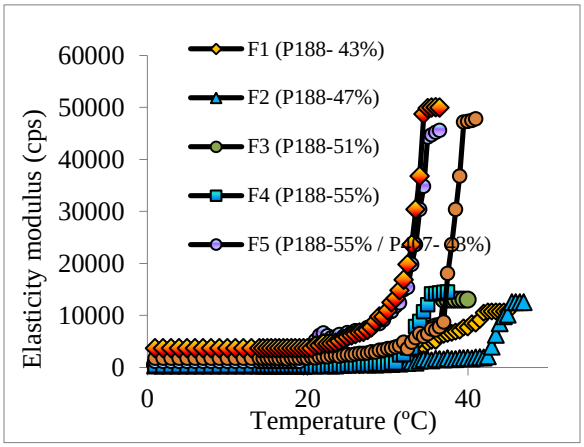

Figure-3: Temperature-dependent changes of the elasticity modulus.

Table-2: Physiochemical parameters of loratidine loaded nanoparticle formulations.

Formulation code	Particle size* (nm)	PDI	Zeta potential (mV)	Entrapment efficiency* (%)
NP1	256.2±1.4	0.06	-15.49±1.33	91.6±1.7
NP2	224.0± 0.8	0.08	-17.01±1.01	83.4±6.1
NP3	128.8±2.1	0.69	-24.12±0.71	78.1±1.5
NP4	190.6±0.2	0.51	-18.04±4.10	52.2±3.3
NP5	170.0±1.1	0.09	-31.80±0.12	51.0±3.1

*Mean± SD, n=3.

Table-3: Characteristics of NPLTIG Formulations

Formulation Code	Clarity	pH*	Viscosity of sol (cps)	Gelation* Temp.(ºC)	Gelation Capacity	Gelation Time* (s)
F1	Clear	5.81±0.01	239.4	50.1±0.51	***	62.4±0.15
F2	Clear	5.61±0.01	284.9	47.4±0.20	***	27.2±0.35
F3	Clear	5.77±0.02	539.9	39.3±0.40	***	18.1±0.15
F4	Clear	5.86±0.01	644.9	35.5±0.32	***	20.4±0.20
F5	Clear	5.41±0.02	1710	33.4±0.48	***	19.4±0.18
F6	Clear	5.96±0.01	2984	35.1±0.50	***	22.2±0.15
F7	Clear	5.48±0.01	3659	33.0±0.50	***	25.1±0.11

* Mean± SD, n=3.

The gel strength of formulation F6 and F7 (130,131 sec) exhibited good gel strength among F4 and F5 code formulation which may due to increase in concentration of P188/P407 and its reversible gelation character at 37ºC.

The mucoadhesive force is an important physicochemical parameter of topical application in nasal cavity. The mucoadhesive force was significantly increased from 3536.76 dynes/cm^2 to 6562.30 dynes/cm^2 for the formula F7 which consists of 0.1% of Carbopol, 55% of P188 and 45% of P407. This also proved that carbopol 940 has better mucoadhesive property than poloxamer combination (Table 4).

The in vitro permeation studies conducted through the nasal mucosa from the formulae F5, F6 and F7 released 88.1%, 85.4% and 95.6% at the end of 24thhour (Figure 4b). Whereas the percentage of drug release (nanoparticle) through mucous membrane at the end of 8 h and 6 h for formulation NP1and NP4 was found to be 85.74% and 90.51% respectively (Figure 4a). The diffusion of drug from formulation F5 and F6 was less may be due to presence of P188/P407 in the gel which retards the drug release rate owing to reduction in dimension of water channel. While diffusion of drug through formulation F7 was found to be more which may be due to presence of carbopol 940, which undergoes rapid swelling and helps in faster diffusion**.**

FT-IR spectrum of loratidine and polymer combination formed the polymer active with no disturbance in the functional group; therefore a polymerized active constituent

Table-4: Characteristics of optimized NPLTIG Formulations
*Mean± SD, n=3.

Formulation Code	Mucoadhesive force (dynes/cm^2)	Spreadability (gm.cm/sec)	Gel strength (seconds)
F4	3536.76±0.41	20.56±1.10	80±0.55
F5	3621.10±0.12	27.41±0.98	120±0.31
F6	3740.79± 0.33	26.33±1.22	130±0.77
F7	6562.30±0.84	22.64±0.96	131±0.61

Table- 5: Comparative pharmacokinetic parameters of loratidine following the administration of oral solution and intranasal nanoparticle loaded in situ gel in rabbits.

S.no	Parameters	Oral Solution (10mg/kg)	Nanoparticle loaded nasal *in situ* gel (4mg/kg)
1	Cmax (µg/mL)	4.3	18.92
2	Tmax (h)	3	6
3	T1/2 (h)	10.81	14.79
4	AUC (µg/ml*h)	76.24	472.62
5	AUMC (µg/ml*h^2)	1220.22	11199.01
6	MRT (h)	16.00	23.69
7	Clearances ((µg/ml)/h)	10.82	1.74

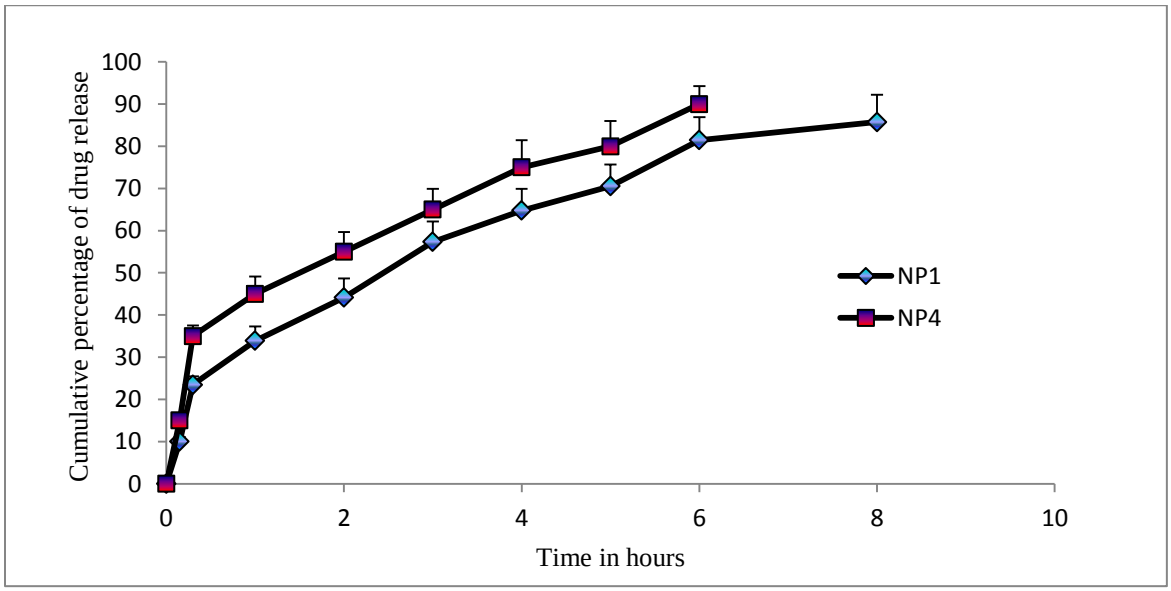

Figure -4a: Showing the drug release of nanoparticle

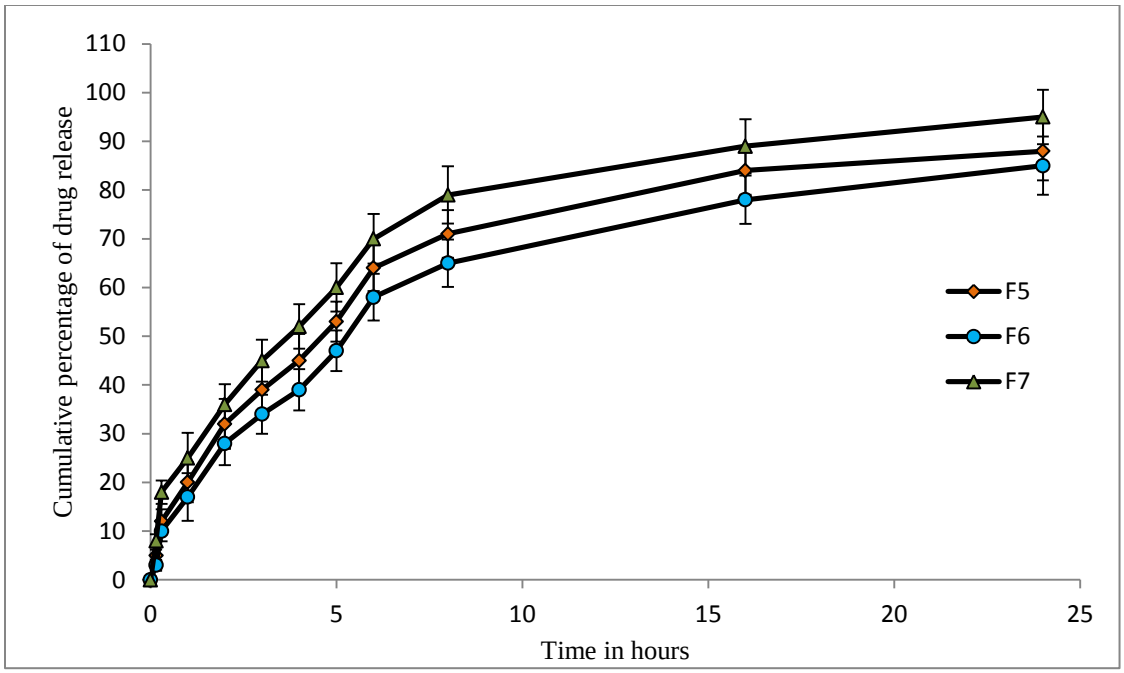

Figure-4b: Showing the drug release of NPLTIG

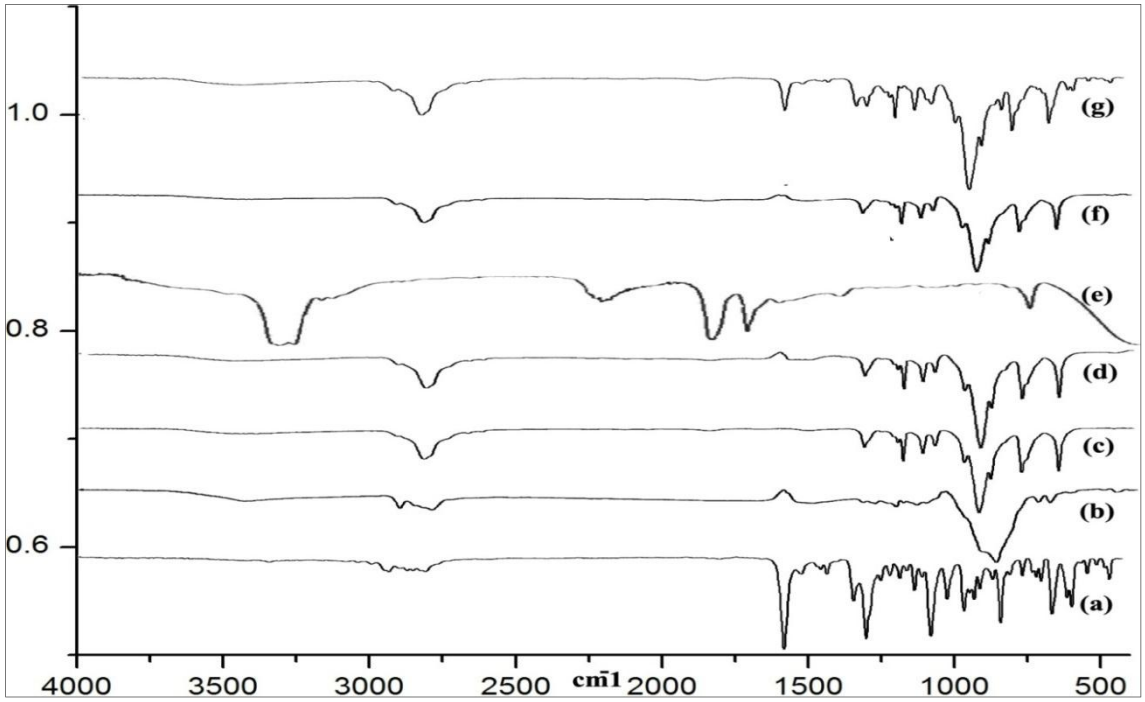

Figure-5: FTIR spectra (a) Loratidine (b) Ethyl Cellulose (c) P 407 (d) P 188 (e) C 940 (f) EC+P 188+P 407+C 940 (g) Loratidine + EC+P 188+P 407+C 940

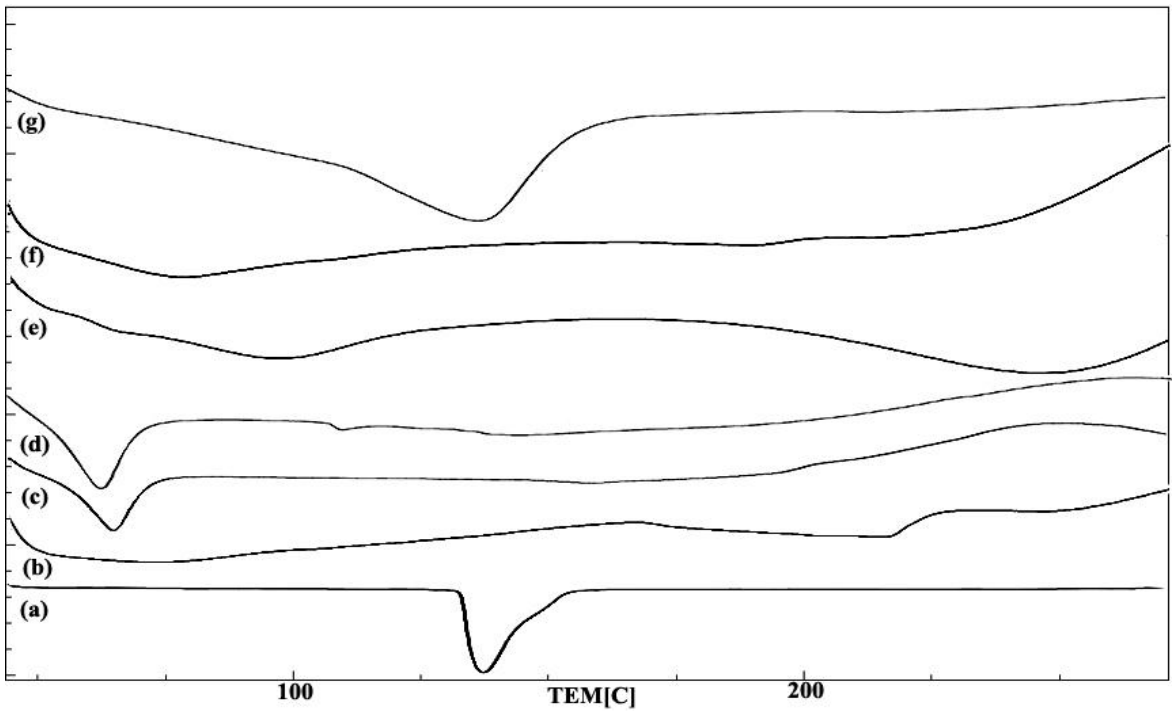

Figure-6: DSC spectra (a) Loratidine (b) Ethyl Cellulose (c) P 407 (d) P 188 (e) C 940 (f) EC+P 188+P 407+C 940 (g) Loratidine + EC+P 188+P 407+C 940

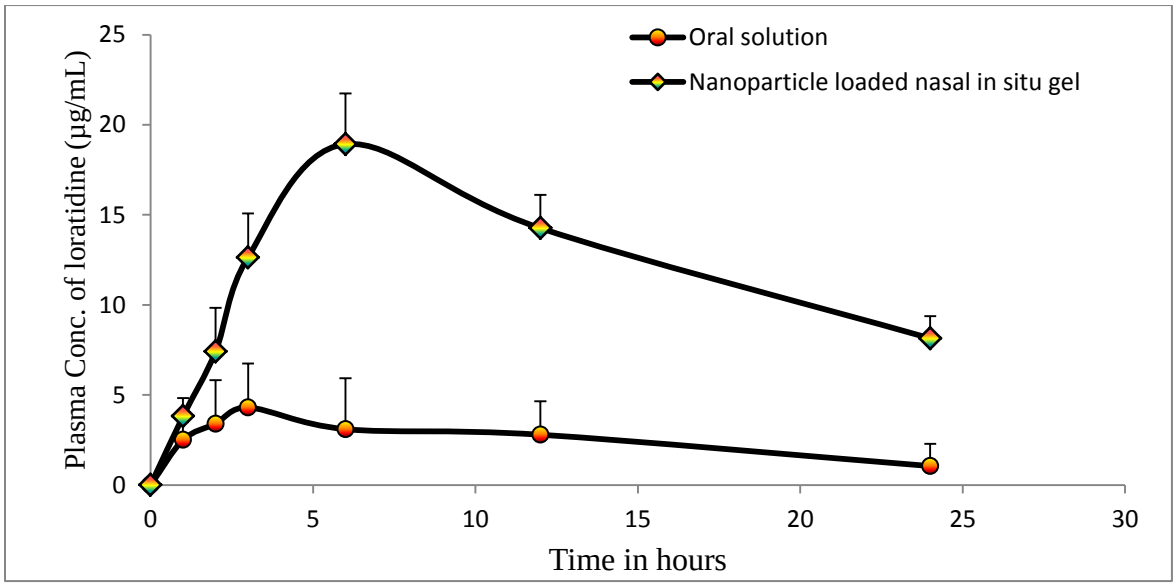

Figure-7: Mean plasma concentration–time profiles following administration of oral solutions and intranasal nanoparticle loaded in situ gel of loratidine in rabbits. Values are expressed as the mean of three measurements.

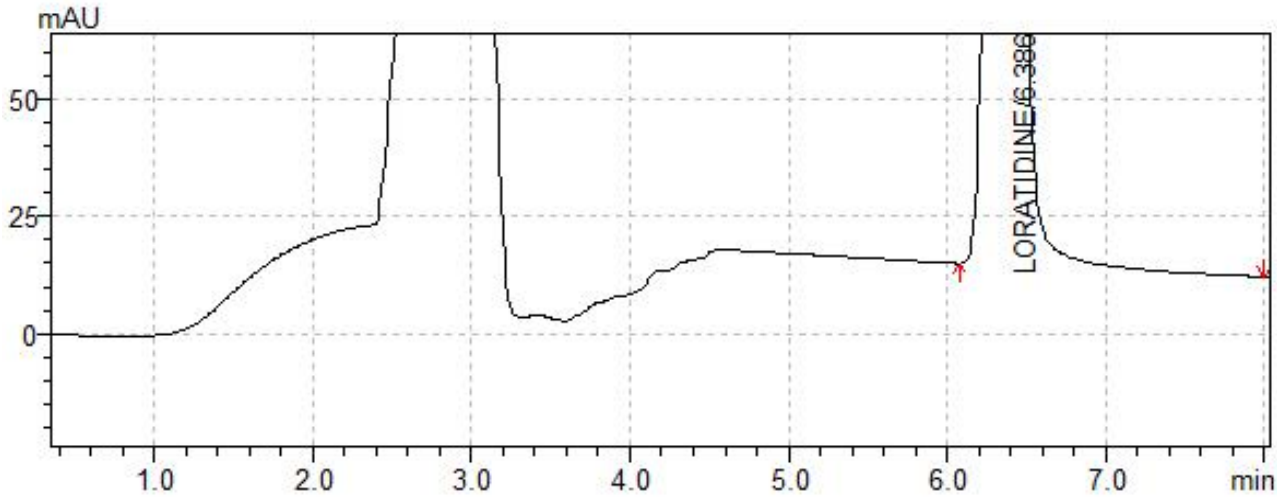

Figure-8: Typical chromatogram of plasma drug sample

has no change of effect after polymerizations (Figure 5).

The DSC curve of loratidine displayed a sharp endothermic peak at 137.50°C (ΔH-9.35 kJ/g) due to melting of loratidine. The thermogram of poloxamer 188 revealed a broad endothermic peak at 58.10°C (ΔH-6.09 kJ/g). The thermogram of poloxamer 407 exhibited a broad endothermic peak at 60.86°C (ΔH-7.90 kJ/g). The physical mixture of ethyl cellulose, P407, P188 and carbopol 940 endothermic peak at 76.46°C (ΔH-1.18 kJ/g). The physical mixture of loratidine, ethyl cellulose, P407, P188 and carbopol 940 endothermic peak at 141.27°C (ΔH844.98 J/g). The drug and polymers peak shift slightly towards higher temperatures was the result of compatibility drug and polymers (Figure 6).

To assess the in-vivo performance of NPLTIG, pharmacokinetic studies were performed in New Zealand white rabbits. From the plasma concentration profile (Figure 7 and 8), apparently, both intranasal NPLTIG formulations revealed better bioavailability for loratidine than oral solution. In both these formulations, maximum plasma concentration (Cmax) was significantly ($p<0.05$) higher than oral solution. However, no change was observed in Tmax values of the formulations. Dose normalized $AUC_{0-\infty}$ values (Table 5) for loratidine were significantly higher ($p<0.05$) in NPLTIG formulations than oral solution. The bioavailability of loratidine from NPLTIG formulation containing P188/ P407/C940 was 6.19-folds higher than oral solution.

CONCLUSION

Nanoparticle loaded intranasal thermosensitive *in situ* gel could prove to be useful alternatives to oral formulations. The NPLTIG showed six-fold increase in bioavailability of the drug than oral solution. The NPLTIG of loratidine were prepared with P188/P407 and carbopol 940 showed drug release up to 24 hours. These NPLTIG are liquid at room temperature and undergo gelation when in contact with body temperature (37 ºC). All the optimized formulae found to have less gelation time, excellent gelation capacity and mucoadhesive force with physiological temperature.

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