Research article

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IN-VITRO AND IN-VIVO EVALUATION OF NANOPARTICLES LOADED TEMPERATURE INDUCED ORAL GEL DRUG DELIVERY SYSTEM OF ACYCLOVIR

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ABSTRACT

The objective of the present study was to prolong the delivery of the active drug in the oral administration using a suitable carrier such as nanoparticles loaded temperature induced oral gel (NLTIOG)which can effectively deliver the drug for an extended duration of time hence not only reduce the systemic side effects but also improve the therapeutic efficacy, patient compliance. Acyclovir remains an antiviral drug used for the treatment of herpes simplex virus infections, with an oral bioavailability of only 10-20%. Nanoparticleloaded temperature induced oral gel drug delivery systems of acyclovir have been designed and optimized. The viscosity of in situ system was found to be in the range (195 to 2579 cps) for the sol, whereas for the gels it was up to (36976 cps). The maximum gel strength and mucoadhesion was found to be up to (151 seconds) and (96762 dynes/cm^2) respectively. The *in vitro* drug release of both the NLTIOGformulations (R6 and R7) released 52.36% and 62.31%respectively at 24^{th} hour. Different techniques, FTIR spectroscopy and differential scanning calorimetry (DSC) were used to estimate the incompatibility. Pharmacokinetic study in rabbits showed significant ($p<0.05$) improvement in bioavailability (six-folds) of the drug from NLTIOG than oral solution.

Keywords: Nanoparticles loaded temperature induced oral gel,Bioavailability,Poloxamer 407, Poloxamer188,HPLC

INTRODUCTION

Acyclovir is used for the treatment of herpes simplex virus, is the extensively used drug for infections such as cutaneous herpes, genital herpes, chicken pox, and varicella zoster. Herpes simplex virus (HSV) is an associate of the family of herpes viridae, a DNA virus. There are two types of herpes simplex virus, viz., HSV type 1 and type 2. HSV type 1 is the herpes virus that is commonly responsible for cold sores of the mouth, thesupposed fever blisters. Herpes simplex virus type 2 is the one that most frequentlycauses genital herpes. [1] The viruscauses painful sores on the genitals in both men and women. Besides, herpes sores afford a way for HIV to go around the body's immune defenses and make it easier to get HIV infection. A recent research found that people with herpes simplex virus had three times the risk of flattering infected withHIV as compared to people without herpes simplex virus. [2] Presently, the treatmentsobtainable for herpes simplex are conventional tablets and topical gel for application on outbreaks. The drugs that are commonly used for herpes simplex are acyclovir, valaclovir and famciclovir. Acyclovir is presently marketed as capsules (200 mg), tablets (200 mg, 400 mg and 800 mg) and topical ointment. [1] Oral acyclovir is commonly used as 200 mg tablets, five times a day. Additionally, long-term administration of acyclovir (6 months or longer) is required in immunocompromised patients with relapsing herpes simplex virus. [2] The currently available conventional therapy is connected with a number of drawbacks for example highly variable absorption and low bioavailability (10-20%) after oral administration. Moreover, with increase in dose, there is decrease in bioavailability. Furthermore, for the reason that the mean plasma half-life of the drug is 2.5 h, five times a day administration is essential. Sequentially to make oral therapy of acyclovir more patient compliant, there is a need of using several approaches like matrix tablets, nanoparticles [4] and polymeric films. [5] Themajordrawback with the therapeutic effectiveness of acyclovir is its absorption which is highly variable

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and dose dependent, thus reducing the bioavailability to 10-20%. [6]Acyclovir is soluble in acidic pH and is mainly absorbed from upper gastrointestinal tract (GIT). [7] There are signs of its active absorption from the duodenum and jejunum regions of GIT. [8]The inherent shortcomings of conventional drug delivery and the potential of nanoparticles as drug delivery systems have offered remarkable scope for researchers in this field and there is a fast movement from concept to reality.Drug loaded nanoparticles may be used for oral administration of easily destroyed by acidic media of stomach or those with low aqueous solubility. [9] These colloidal carriers have the ability to cross the mucosal barrier as such. In addition, they have the potential for enhancing drug bioavailability via particle uptake mechanisms. It was therefore decided to prepare nanoparticle loaded temperature induced oral gel of acyclovir so as to optimize its delivery and overcome its inherent drawbacks.

The concept of mucosal adhesives or mucoadhesives was introduced into the controlled drug delivery arena in the early 1980s. [10] Mucoadhesives are synthetic or natural polymers which interact with the mucus layer covering the mucosal epithelial surface and mucin molecules constituting a major part of the mucus. They localize the formulation at a particular region of the body, thereby improving bioavailability of the drugs with low bioavailability. The increased contact time and localization of the drug due to applying nanoparticles of acyclovir which are made mucoadhesive thus enhances its delivery. Possible added advantage of this approach would be increase in bioavailability as well as reduction in frequency of administration.

For the present investigation, nanoparticles loaded temperature induced oral in situ gel of acyclovir have beendeveloped and optimized.

MATERIALS AND METHODS

Materials:

Acyclovir was a gift sample from Ranbaxy (M) SdnBhd(Sungai Petani, Malaysia); Ethylcellulose and poloxamer 407 procured from Sigma Aldrich USA. Poloxamer 188 was purchased from Merck (Germany). Carbopol 940 and polyvinyl alcohol procured from SD Essex (UK). The experimental work was performed by using HPLC water. All other chemicals were of analytical grade.

Table-1: Composition& Physiochemical parameters of acyclovir loaded nanoparticles

Composition								
Ingredients	AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8
Acyclovir **:** EC	1	1	1	1	1	1	1	1
	0.5	0.5	0.5	0.5	0.5	0.5	1	2
Poloxamer407 (mg)	500	500	500	500	500	500	500	500
Methyl alcohol (ml)	10	-	-	-	-	-	-	-
DCM (ml)	-	10	-	-	-	10	10	10
Acetone (ml)		-	10	-	-	-	-	-
DMSO (ml)	-	-	-	10	-	-	-	-
Diethyl ether (ml)	-	-	-	-	10	-	-	-
Deionized water (ml)	100	100	100	100	100	100	100	100
Physiochemical parameters								
Particle size (nm)*	245	165	250	270	300	169	173	180
Zeta potential (mV)	-40.23	-45.75	-49.61	-41.84	-40.61	-42.33	-41.51	-45.64
Entrapment efficiency* (%)	80.4±1.1	73.2±0.5	78.1±1.5	68.6±0.1	78.1±1.3	80.8±0.2	82.0±1.0	84.2±0.7

*Mean± SD, n=3.

Table-2: Composition & Characteristics of NLTIOG Formulations

Polymers (in 80mL)	R1	R2	R3	R4	R5	R6	R7
Poloxamer 188 (g)	50	54	58	58	58	58	58
Poloxamer 407 (g)	-	-	-	4	4	4	4
Carbopol 940 (g)	-	-	-	-	0.2	0.3	0.9
Gelation Temperature (°C)*	40	35.5	32.5	24	26.8	32.4	34.3
Gelation Time (s)	36	24	22	18	22	25	27
Gelation Capacity	***	***	***	***	***	***	***
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear

*Mean± SD, n=3.

Table-3: Comparative pharmacokinetic parameters of acyclovir following the administration of oral solution and nanoparticles loaded temperature induced oral gel (NLTIOG) in rabbits.

S.No	Parameters	Oral Solution (20 mg/kg)	NLTIOG (10 mg/kg)
1	Cmax (µg/mL)	0.98	1.92
2	Tmax (h)	1	4
3	T1/2 (h)	2.79	27.30
4	AUC (µg/ml*h)	2.94	25.47
5	AUMC (µg/ml*h^2)	12.86	2098.04
6	MRT (h)	3.84	38.68
7	Clearances ((µg/ml)/h)	5.98	0.18

Preparation of acyclovir *nanoparticles:*

Preparation of acyclovir nanoparticles was prepared by emulsion solvent diffusion technique [11]. Drug and polymer (1:0.5, 1:1, and 1:2) were dissolved in different solvent like methyl alcohol,DCM,Acetone,DMSO and diethyl ether 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 of poloxamer 407 (0.5% 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 acyclovir nanoparticles were recovered by centrifugation at 5,000 rpm for 10 min followed by washing twice with distilled water and washed nanoparticles were subjected to freeze drying.

Preparation of nanoparticles loaded temperature induced oral gel:

Preparations of nanoparticles loaded temperature induced oral gel were prepared by the cold method [12]. Specified amount of poloxamer 188 (P188), poloxamer 407 (P407) and carbopol 940 (C940) were stirred in the calculated amount of cold distilled water (Table 2). The dispersions was cooled to 4°C by keeping it in a refrigerator for overnight. Equivalent to 100 mg of nanoparticles (equivalent to 100 mg of acyclovir) 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.

Evaluation of nanoparticles loaded temperatureinduced oral gel:

The prepared acyclovir nanoparticle formulations were evaluated by particle size, zeta potential and entrapment efficacy. All the parameters were evaluated by tripilicate [13].

Gelation temperature:

The different formulations of NLTIOG 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 [14].

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 [15].

Determination of mucoadhesive force:

The experimental technique used for determining the bioadhesive force has been derived from a previously published method [16,17]. The mucoadhesive force of the formulations was determined as follows; a section of sheep intestinal 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). NLTIOG was added onto the mucosa of first vial. Before applying the gel, thermostaticallycontrol the system to attain equilibrium temperature. 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/cm^2), 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 [18].

In vitro studies:

Typically, equivalent amount of free drug and nanoparticleswas dispersed in 80 mL of potassium phosphatebuffer, pH 6.8 (corresponds to the pH of human small intestine)for 24 h at 37 ºC. 5ml of samples wastaken at time intervals of 1, 2, 4, 4,6, 8, 12, 18 and 24 hours. After each time of taking the sample, equalamount of fresh buffer has been added to maintain the sinkconditions. 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 ACN 5mm, ammonium acetate (pH 5.5) (60:40, v/v), and detection was made at 290 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.Released acyclovir from nanoparticles was determined by HPLC at 290 nm.

In vivo absorption study:

A total of six female New Zealand white rabbits (n=3/ group), weighing 2.4±0.2kg were used for the study. The rabbits housed individually in stainless steel cages, fed with commercial rabbit diet, green leaves vegetables and provided drinking water *ad libitum*. The rabbits were fasted for 12 h before drug administration and 2 hr after drug administration, but had free access to water. All the animals were conscious throughout the duration of experiment.The single-dose parallel study design was used to measure the pharmacokinetic variations between standard acyclovir solution and NLTIOG.

The rabbits were divided into two different groups of three animals in each group. The animals in group I were treated with standard acyclovir solution (20 mg/kg) and group II were treated with NLTIOG 10 mg/kg. The blood samples were (approximately 200-300 µL) were collected from the ear vain at 1, 2, 4, 6, 8, 12, 18 and 24 h after drug administration [19].

Blood samples were collected into 1mL sample collection tubes containing 4% w/v sodium citrate solution as an anti-coagulant. Plasma samples were obtained by centrifuging blood samples in a centrifuge at 3000 rpm for 20 min. The samples were stored at -80ºC until further analysis.

For analysis of acyclovir 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

The particle size of prepared nanoparticles were analysed by scanning electron microscopy analysis. The particle sizes of acyclovir nanoparticles were found to be 165 to 300 nm (Table 1). All the prepared acyclovir nanoparticles were appeared spherical in shape (Figure 1). Zeta potential of acyclovir nanoparticles were -40.23 mV to -49.61mV (Table 1). Entrapment efficiency is the important parameter to optimize the nanoparticle formulations. In the acyclovir nanoparticle preparation, ethyl cellulose nanoparticle showed 82.0% and 84.2% (Table 1). The prepared nanoparticle AB8 was higher than AB7 formulation code, due to over coating of (increased polymer concentration).

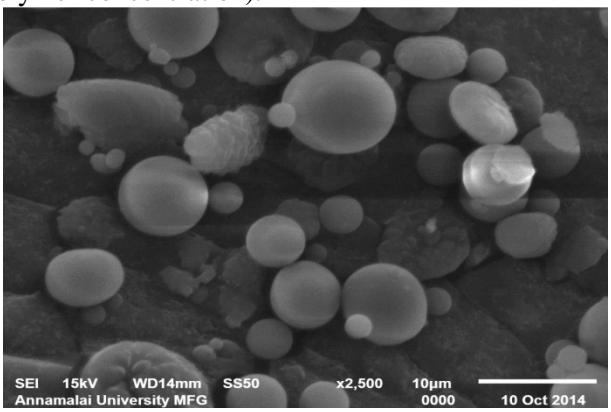

Figure-1: SEM image of acyclovir nanoparticles

Sevenformulations of nanoparticles loaded temperature induced oral gel were prepared with various ratios of poloxamer 188, poloxamer 407 and carbopol940 as shown in table 2.

Gelation temperature of NLTIOGof acyclovir decreased with increase in concentration of poloxamer 188 from 40ºC to 24ºC for a concentration of 50g to 58g (R1 to R4).Gelation temperature of the R4 formula which contains 58gof poloxamer 188, 4g ofpoloxamer 407was found to be 24ºC and to have a viscosity of 34980 cps was taken for further studies. However the optimized concentration of poloxamer188 (58g) and poloxamer 407 (4g)showed a viscosity of 34980 cps and a gelation temperature of 24ºC which is less than body temperature which is not suitable for maintaining a 'Sol' form at room temperature. So, carbopol 940 was added in increasing concentration (0.2g to 0.9g) which led to the increase in gelation temperature from 32.4ºC (R6), 34.3ºC (R7) and a viscosity of 35976 cps, 37271 cps respectively (Figure 2).

Gelation time was defined as the time when the elasticity modulus became higher than the viscosity modulus. The gelation time of NLTIOGP188/ P407/C940 (58/4/0.3) was observed at 25s, but it took longer time for P188 (50g) which began to show the viscoelastic property of a gel at 36s. At 40 °C, the formulation with 58g of P188 gelled within 18s.The optimized concentration of carbopol was found to be 0.3g and 0.9g for R6 and R7 and further addition led to an increase of gelation temperature more than body temperature. So, the formulae R6 and R7 were considered to be optimized formulae.

The gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out from the site of administration. The gel strength of formulation R6 and R7 (147, 151 sec) exhibited good gel strength, which may due to increase in concentration of poloxamer188 andpoloxamer 407 (Figure 3).

The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The spreadability of optimized formulations R6 and R7 showed 13.34, and11.9 g.cm/sec(Figure4).

The mucoadhesive force is an important physicochemical parameter of oral mucoadhesive system. The mucoadhesive force was significantly increased from 60758.97 dynes/cm^2 to 96762.76 dynes/cm^2 for the formula R6 and R7 which consists of 0.3gand 0.9g of carbopol 940, as the concentration of mucoadhesive polymer (carbopol 940) increased. This also proved that carbopol has better mucoadhesive property than poloxamer combination (Figure5).

The in vitro permeation studies conducted through membranefrom the nanoparticle formulationAB7 and AB8 released 92.7% and 81.0% at the end of 24thhour (Figure 6). Whereas the percentage CDR (NLTIOG) through membrane at the end of 24th hour for formulation R6 and R7 was found to be 52.36% and 62.31%respectively (Figure 7). The release of drug from the nanoparticles loaded temperature induced oral gelR6 and R7 was found to be less which may be due to presence of P188/ P407/C940, which undergoes slow swelling and helps in slower drug release.

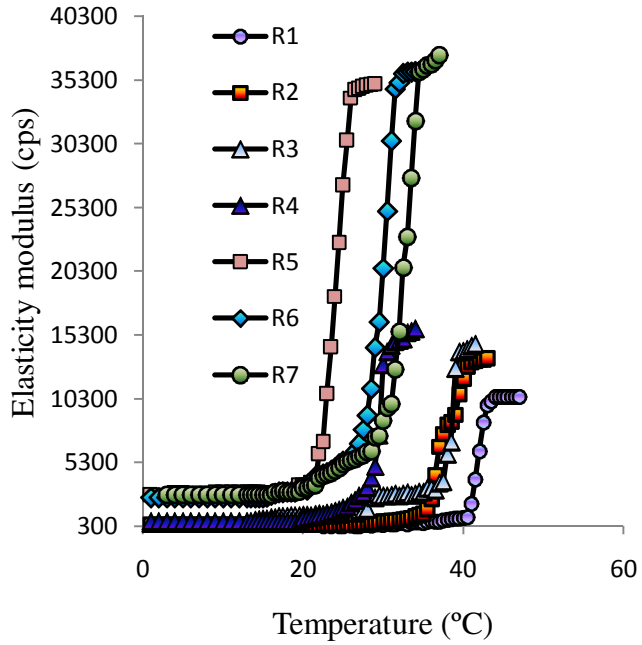

Figure-2: Temperature-dependentchanges of the elasticity modulus

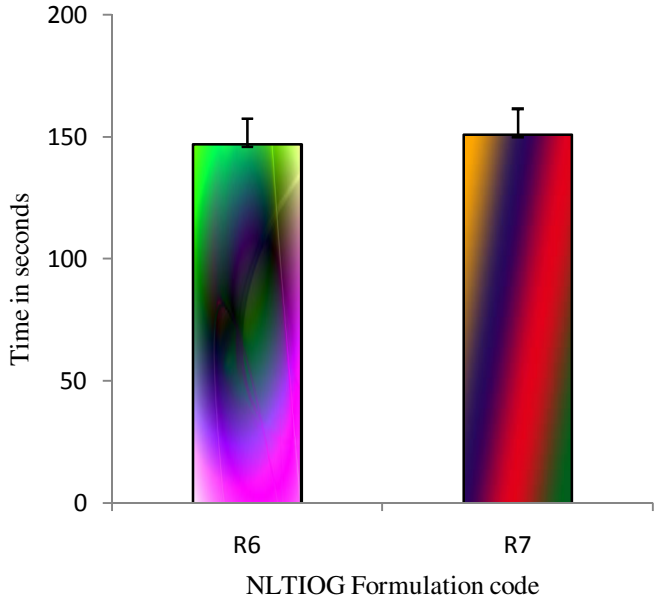

Figure-3: Gel strength of NLTIOG

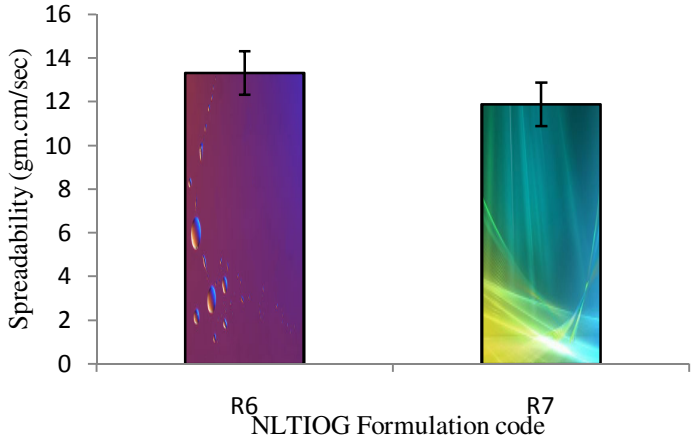

Figure-4:Spreadability of NLTIOG

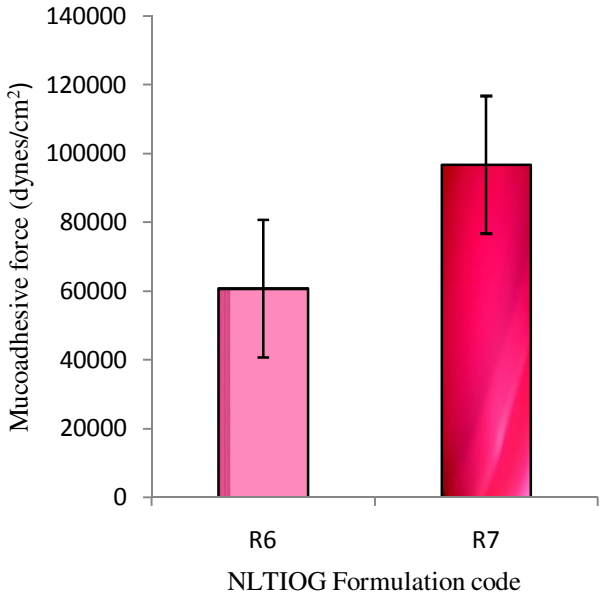

Figure-5: MucoadhesiveForceof NLTIOG

FT-IR spectrum of acyclovir and polymer combination formed the polymer active with no disturbance in the functional group; therefore a polymerized active constituent has no change of effect (Figure 8).

The DSC curve of acyclovir 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 9).

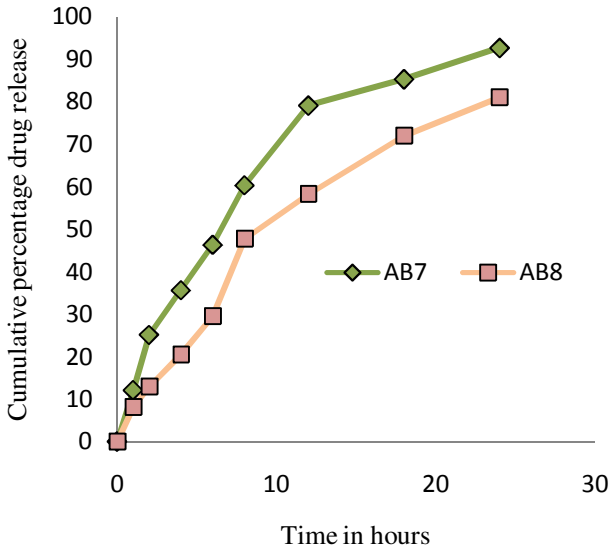

Figure-6: Showing the drug release ofnanoparticles

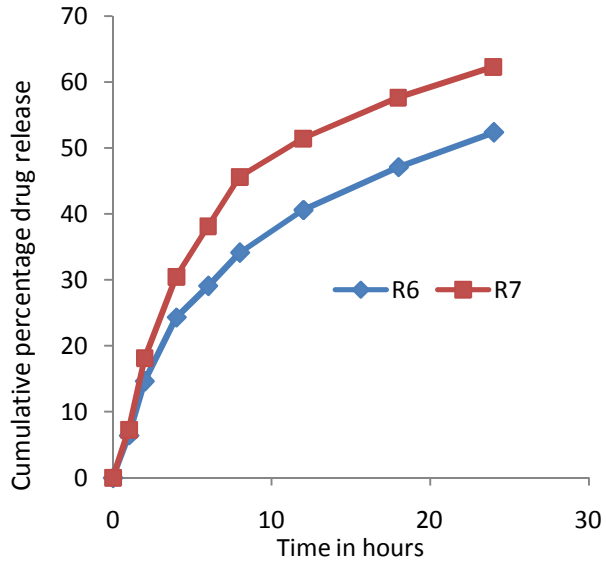

Figure-7: Showing the drug release of NLTIOG

To assess the in-vivo performance of NLTIOG, pharmacokinetic studies were performed in New Zealand white rabbits. From the plasma concentration profile (Figure 10 and 11), apparently, both intranasal NLTIOG formulations revealed better bioavailability for acyclovir 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 AUC0-∞ values (Table 3) for acyclovir were significantly higher ($p<0.05$) in NLTIOG formulations than oral solution. The bioavailability of acyclovir from NLTIOG formulation containing P188/ P407/C940 was 8.66-folds higher than oral solution (Table 3).

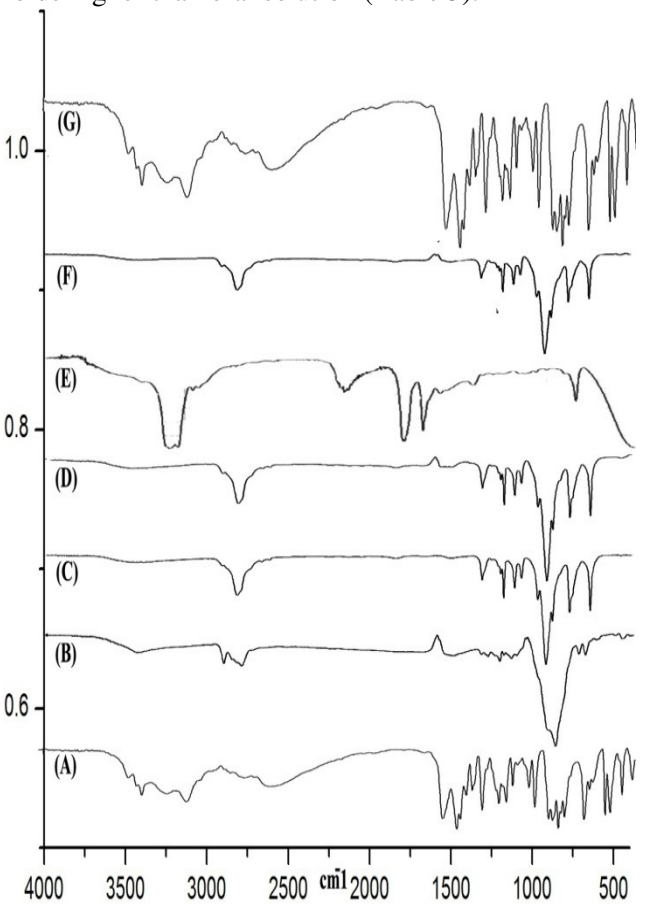

Figure-8: FTIR spectra (A) Acyclovir (B) Ethyl Cellulose (C) P 188 (D) P 407 (E)C 940 (F) EC+ P407+P 188+C 940 (G) AV+ EC+ P 407 + P 188+C 940

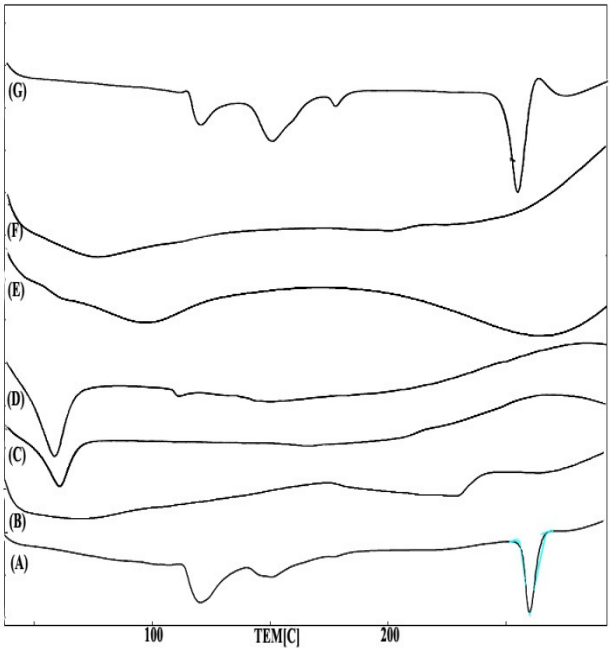

Figure-9: DSC spectra (A) Acyclovir (B) Ethyl Cellulose (C) P 407 (D) P 188 (E) C 940 (F) EC+P 188+P 407+C 940 (G) Acyclovir + EC+P 188+P 407+C 940

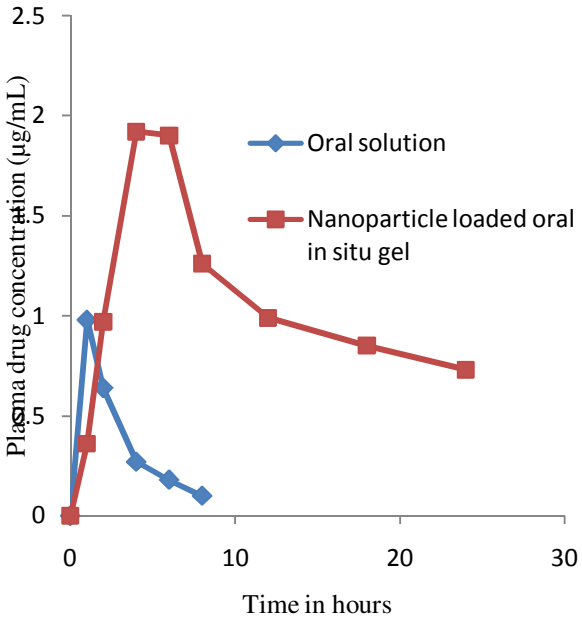

Figure-10: Mean plasma concentration–time profiles following administration of oral solutions and nanoparticle loaded temperature induced oral gel of acyclovir in rabbits. Values are expressed as the mean of three measurements

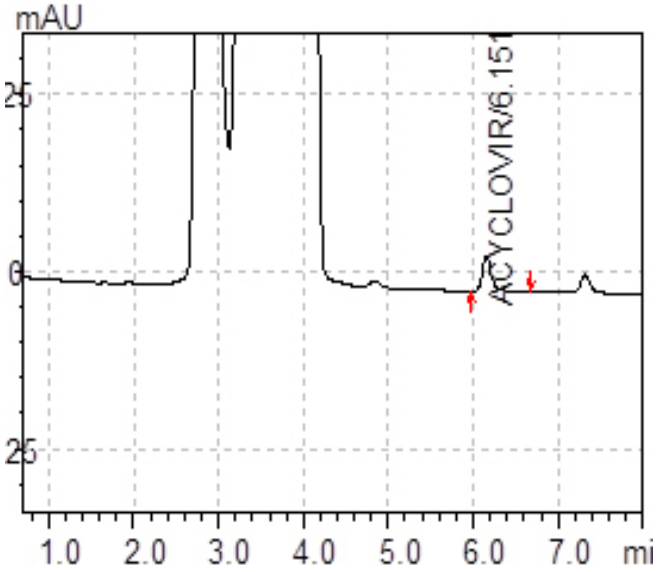

Figure-11: Typical chromatogram of plasma drug sample

CONCLUSION

NLTIOGcould demonstrate to be useful substitutes to oral formulations. The NLTIOG showed six-fold increase in bioavailability of the drug than oral solution. The NLTIOG of acyclovir were prepared with P188/P407 and carbopol 940 showed drug release up to 62.31% release for 24 hours. These NLTIOGare liquid at room temperature and undergo gelation when in contact with body temperature. All the optimized formulae found to have less gelation time, excellent spreadability and gelation capacity with human physiological temperature.

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