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Formulation and Evaluation of Brimonidine Maleate Nanolipid *in Situ* Gel

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ABSTRACT

The main objective of present research work was aimed to formulate and evaluate the nano lipid-based drug delivery system by incorporating a brimonidine maleate drug for ocular therapy. The patient can be improved by preparing nano lipid *in situ* gel as a vehicle by reducing the frequency of administration and increasing the ocular bioavailability. Nanolipids were prepared by film hydration technique and then prepared nanolipids were incorporated into insitu gel by using various polymers like Carbopol 940 and HPMC K15M with different concentration. The various formulations prepared showed excellent and effective results for visual appearance, pH, and gellation study. It was further observed that formulations had entrapment efficiency within the range of 67.20% to 97.3% for brimonidine maleate loaded insitu gel formulations. F1 entrapment efficiency was found to be 97.3% and shown maximum when compared with other formulations. From the drug release data, it was found that F1 (99.0%) shows maximum drug release compare to other formulations.

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INTRODUCTION

Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of the front of the eye for a prolonged time. In recent years, actual observations have been concentrated on the progress of controlled and sustained drug delivery systems (CDDS& SDDS). The involved eye structure limits the access of drug at the site action (Gaudana *et al.*, 2009; Nanjawade *et al.*, 2007).

Various stimuli can form gels theses includes

1. Physical stimuli: Change in temperature, electric fields, light, pressure, sound, and magnetic fields.
2. Chemical stimuli: Change in pH and ion activation from biological fluids.
3. Biological or biochemical stimuli: Change in glucose level.

In-situ Delivery

A new trend of preparing a in situ gel had been proposed in the year 1980s. In situ was a Latin phrase

which translated literally as 'in position'.

In situ gels are low viscosity forming solutions which had been going phase change in the eye (cul-de-sac) due to the presence of polymers.

They may fallow any one of the methods like change in ionic strength or pH change or temperature etc. for phase transition on ocular systems & prolong the drug release.

The conversion of sol to gel increases the ocular time in the eye & they should not have any problem related to the vision of the eye.

Liquid eye drops when instilled into the eye, they will be drained out of the eye, which will lead to drug loss & automatically the bioavailability of the drug will reduce (1 to 10%). Thus all problems can be solved by formulation the medicine in the form of sol to gel type (insitu).

From the above various stimuli only pH, ion activated, and temperature stimuli can be used for designing of ophthalmic drug delivery system (Nanjawade *et al.*, 2007). In this work desired percentage of carbopol 940 and HPMC K-15M was used for the preparation of brimonidine maleate nano lipid in situ gel (Mohan *et al.*, 2009; Lavanya *et al.*, 2014).

MATERIALS AND METHODS

Brimonidine maleate pure drug was purchase from yarrow chem. Product; Mumbai, India and carbopol 940 and HPMC-K-15M were purchased from CDH laboratory India.

Study of interaction of the drug with excipients used in the formula

Infrared spectra of brimonidine maleate were recorded on FTIR spectra photometer. The absorption maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the spectra of in situ gel (Preetha *et al.*, 2010).

Manufacture of nano lipids *in situ* gel

Preparation of nanolipids

Nanolipids were prepared by film hydration technique. The mixture of vesicles forming ingredients like lecithin and cholesterol are dissolved in a volatile organic in a round bottom flask. Rotate the rotary evaporator at 60ºC for 45 minutes. The organic solvent is removed with gentle agitation and evaporating the organic solvent at 60ºC and leaving a thin film of lipid on the wall of the rotary flash evaporator. The aqueous phase containing brimonidine maleate drugs was added slowly with intermittent shaking of the flask at room temperature followed by sonication for 30 minutes. Nanolipid solution cooled was kept in 4-8^{o}C at the freezer.

Formulation of nano lipid in-situ gel

The batch which provided maximum entrapment efficiency was chosen to prepare nano lipid in situ gel. To avoid lump formation and to allow the hydration, an appropriate quantity of Carbopol 940 and HPMC K 15M have been sprinkled over nano lipid dispersion under the constant agitation with a glass rod. Benzalkonium chloride (as preservative) and sodium chloride (to make gel formulations isotonic with tear fluid) were added to the gel batches in sufficient quantity (Table 1) (Ramachandra *et al.*, 2012).

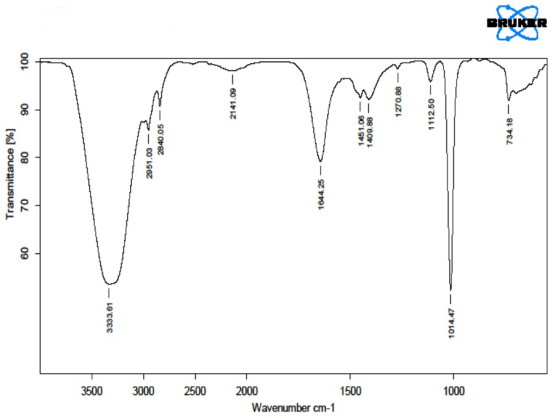

Figure 1: FTIR spectra of Brimonidine maleate.

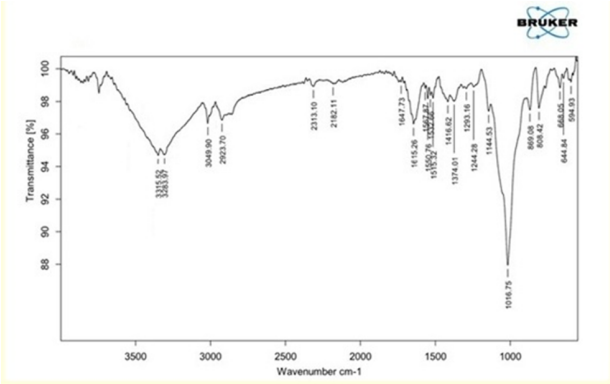

Figure 2: FTIR spectra of formulated Brimonidine maleate in-situ gel

Drug entrapment efficiency

The unentrapped drug was separated by ultracentrifugation method from the nano lipid formulation where the nano lipid dispersion was centrifuged at 14000 rpm for about 90 min. The resulting solution eliminated the transparent supernatant. The pH 7.4 phosphate buffer is diluted and analyzed according to the UV spectrophotometric methods.

Calculate the entrapment efficiency by using the fol-

Table 1: Formulation of Brimonidine maleate loaded nanolipid insitu gels

Ingredients	F1	F2	F3	F4	F5	F6
Brimonidine Maleate % w/v	0.05	0.05	0.05	0.05	0.05	0.05
Lecithin % w/v	0.05	0.05	0.1	0.05	0.15	0.2
Cholesterol % w/v	0.05	0.1	0.05	0.15	0.1	0.05
Methanol % w/v	7.5	7.5	7.5	7.5	7.5	7.5
Water % w/v	10	10	10	10	10	10
HPMC % w/v	0.2	0.2	0.4	0.4	0.3	0.2
Carbopol % w/v	0.2	0.4	0.2	0.4	0.2	0.3
EDTA % w/v	0.1	0.1	0.1	0.1	0.1	0.1
Benzolkonium chloride % v/v	0.01	0.01	0.01	0.01	0.01	0.01
Sodium chloride % w/v	0.9	0.9	0.9	0.9	0.9	0.9
Phosphate buffer % v/v	100	100	100	100	100	100

Table 2: FTIR Spectra Data of Brimonidine maleate

IR (KBr) cm^{-1}Peaks
3333.61 (**NH**$_{str}$),
2951.03 (**Ar-CH**$_{str}$),
2840.05 (**OH**$_{str}$),
1644.25 (**C=N**),
1014.47 (**C-O-C**$_{str}$)

Table 3: FTIR spectra data of formulated Brimonidine maleate in-situ gel

IR (KBr) cm^{-1} Peaks
3323.90 (**NH**$_{str}$),
2913.73 (**Ar-CH**$_{str}$),
2846.64 (**OH**$_{str}$),
1694.73 (**C=N**),
1024.71 (**C-O-C**$_{str}$)

Table 4: Entrapment Efficiency of Brimonidine maleate loaded nano lipid insitu gels

Formulation	Entrapment Efficiency %
F1	97.3 ±1.909
F2	87.00 ±1.121
F3	69.00 ±0.707
F4	77.56 ±0.459
F5	67.20 ±1.050
F6	89.63 ±0.940

Table 5: Drug Content Estimation of Brimonidine maleate loaded nano lipid insitu gels

S.NO	Formulations	Drug content %
1	F1	87.03±0.906
2	F2	75.83±1.552
3	F3	65.69±0.254
4	F4	87.79±1.449
5	F5	80.00±0.828
6	F6	96.36±0.933

Table 6: Visual Appearance and pH of Brimonidine maleate nano lipid insitu gel formulations

S.NO	Formulations	Visual appearance	pH
1	F1	Cloudy	5.9±0.070
2	F2	Clear	5.1±0.141
3	F3	Clear	6.2±0.749
4	F4	Cloudy	4.9±0.021
5	F5	Clear	6.1±0.728
6	F6	Clear	7.1±0.145

Table 7: Gelling Capacity of Brimonidine maleate nano lipid insitu gel formulations

S.NO	Formulations	Gellation capacity
1	F1	++
2	F2	++
3	F3	+ +
4	F4	+++
5	F5	++
6	F6	+++

Table 8: Cumulative percentage drug release profile of Brimonidine maleate nano lipid in situ gel formulations

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
0.5	8.53 ±0.021	5.25 ± 0.678	6.98 ± 0.579	17.97 ± 0.438	15.45 ± 0.749	12.87 ± 0.205
1	38.04 ± 0.975	17.42 ± 0.445	21.13 ± 1.972	40.21 ± 0.707	39.65 ± 0.735	29.7 ± 0.459
2	50.16 ± 3.061	40.32 ± 3.457	38.48 ± 1.626	52.05 ± 0.784	41.97 ± 2.305	34.8 ± 1.445
4	64.08 ±1.555	50.23 ± 0.318	53.82 ±1.870	66.55 ± 0.537	56.98 ± 0.459	51.3 ± 1.715
6	81.45 ± 2.764	68.46 ± 2.142	67.07 ± 0.459	84.59 ± 2.057	79.8 ± 0.700	69.9 ± 1.484
10	99 .0 ± 2.121	83.53 ± 1.180	85.46 ± 1.778	84.73 ± 2.220	83.6 ± 1.343	79.8 ± 0.671

Table 9: Stability Data of Optimized Formulation

		Drug content			
	Storage Conditions	Initial	1 month	2 months	3 months
F6	4°C±2°C	96.36±1.517	95.21±1.745	94.17±1.921	92.11±1.879
	27°C±2°C	96.36±0.517	94.99±1.029	93.21±0.988	91.09±0.727

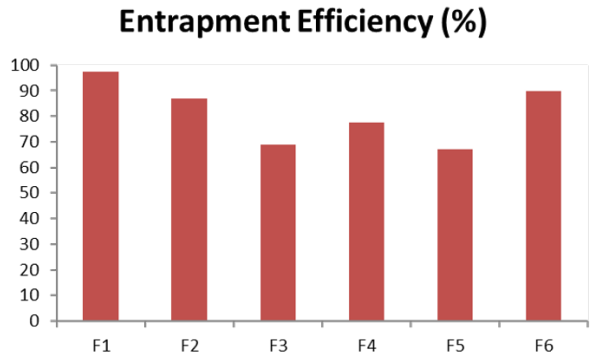

Figure 3: Comparative entrapment efficiencies of Brimonidine maleate loaded nano lipid in-situ gels

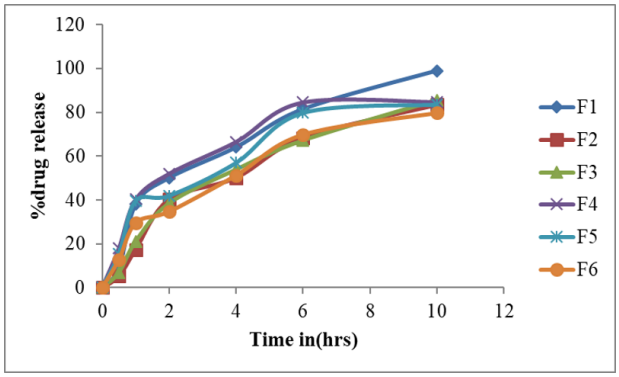

Figure 4: In-vitro drug releases of Brimonidine maleate nano lipid in-situ gels formulations

lowing equation (Nagesh *et al.*, 2012).

The amount of Brimonidine Maleate encapsulated in the Nanolipid was determined by using.

E E = Total drug conc – free drug conc / total drug conc x 100

Where EE is entrapment efficiency (%)

Estimation of drug content

Nanolipid suspension equivalent to 50mg was taken into a standard volumetric flask. Then were lysed with100ml of propane-1-ol by shaking. Then 0.1ml of this solution is diluted to 10ml with phosphate buffer 7.4. The absorbance was measured at 248nm for brimonidine maleate and calculates drug content from the calibration curve (Nagalakshmi *et al.*, 2014; Moorthi *et al.*, 2012).

Visual appearance and pH

For the nature of some particular matter, visual appearance and clarification were observed. The acidic or alkaline composition of the corneal membrane is expected to cause discomfort.

For this reason, there was a wireless electrode pH meter. By bringing the electrode near the surface of the formulation and allowing it to be balanced for 60sec, pH has been noted (OECD, 2012).

***In vitro* gellation study**

The gelling capacity was determined by placing a drop of the polymer solution in a vial containing 2 ml of freshly prepared simulated tear fluid (STF) equilibrated at 37 ºC. After that, the visual assessment of the gel formation was done, and the time required for gelation and dissolution of the gel formed was noted (Kumar *et al.*, 2012; Nayak *et al.*, 2012).

***In vitro* drug release of nano lipid *in situ* gel**

A 37°C phosphate buffer (pH 7.4) was used to test *in vitro* release studies for brimonidine maleate insitu gel. Brimonidine maleate containing nano lipid in situ gel (5 ml) was carefully weighed and transferred into the membrane of dialysis. Gently move the gel down to the membrane gel surface and in contact with the membrane. In the reservoir tank, phosphate buffer (1 ml, pH 7,4) was used to wet the gel; the dialysis membrane was only immersed in the phosphate buffer that served as a receiving enclosure. At 37°C, the receiving section (100 rpm, Remi, India) was removed magnetically. Samples of (1 ml) were removed periodically from the reception area. A spectrophotometer of 248nm (Shimadzu1800) was used to calculate the amount of brimonidine maleate released by the nano lipid in situ gel. Following through sample withdrawal, the reception bay was filled with a quantity equal to the phosphate buffer (Shashank *et al.*, 2015; Nayak and Srinivasa, 2017a).

Accelerated stability studies

For a short-term, accelerated stability test at 4ºC±2ºC and 27ºC±2ºC engineered nano lipid dispersion that had higher trapping efficiency was put in vials and screened with aluminium foil as amended by the international harmonization guidelines conference. Every 90 days product content samples were analyzed (Nayak and Srinivasa, 2017b).

RESULTS AND DISCUSSION

Study of interaction of the drug with excipients used in the formulation

The FTIR spectrum studies of brimonidine maleate pure drug and drugs loaded nano lipid gel were analyzed. The primary functional group's peaks of brimonidine maleate present in loaded nano lipid gel were intact and were present (Table 3 & Figures 1 and 2).

This proves the fact that there was no potential interaction of the drug with the excipients used in

the formulation. This indicates the stable nature of drugs in all formulations.

Percentage drug entrapment efficiency of Nanolipid *in situ* formulations

The nature of lipids played a significant role in drug entrapment efficiency. The entrapment efficiency of the system was calculated as a ratio of the amount of drug entrapped by the system to the amount of drug taken, expressed in percentage. The entrapment efficiency was within the range of 67.20% to 97.3% for Brimonidine maleate loaded insitu gel formulations. F1 entrapment efficiency was found to be 97.3% and shown maximum when compared with other formulations (Table 4 & Figure 3).

Percentage drug content of Nanolipid *in situ* formulations

Brimonidine maleate loaded insitu gel formulations were analyzed for drug content spectrophotometrically at 248nm. Brimonidine maleate loaded insitu gel formulations exhibited relatively uniform drug content. The drug content was between 65.69% and 96.36% for all formulations, as shown in [Table 5]. The F6 formulation showed maximum drug content of 96.36%.

Visual appearance and pH of Nanolipid *insitu* formulations

For the nature of some particular matter, visual appearance and clarity were observed. In the corneal membrane, an acidic or alkaline solution induces irritation. The pH of nanoparticle insitu gel was detected by using digital pH meter. Nanolipid in-situ gel pH range lies between 4.9-7.1 pH (Table 6). Nanolipid in-situ gel shows maximum pH 7.1 for F6 Brimonidine maleate loaded insitu gel formulations the pH of the reported formulations was non-irritable to the eye. This reflects the gel is not harmful to the eye surface.

The gelling capacity of Nanolipid *in situ* formulations

The gelling capacity was determined by freshly prepared simulated tear fluid (STF). Gelation study revealed that the formulations F1 & F3 gels slowly and dissolves rapidly within 1hr. F2 & F5 showed immediate gelation and remained for a few hours. Formulations F4 & F6 exhibited immediate gelation, which remains for 2-4 hours. As shown in (Table 7).

***In-vitro* drug release of Nanolipid *in situ* formulations**

The drug release studies of nanolipids with Brimonidine maleate was performed for 10hrs in pH 7.4 buffer. The *in-vitro* drug release of nanolipids was within the range of 79.8% to 99.0% for Brimonidine maleate loaded insitu gel formulations. From the drug release data, it was found that F1 (99.0%) shows maximum drug release compare to other formulations (Table 8 & Figure 4).

Stability studies of Nanolipid *in situ* formulations

Stability studies of optimized BF6 Brimonidine maleate insitu Gel were conducted for three months at $4\pm2^{\circ}C$ and $27\pm2^{\circ}C$. For precipitation, the formulations were visually tested. The drug content was measured for three months every 30 days. The physical appearance of the solution was found to be unchanged. The drug quality of these formulations was analyzed, and there were small variations between them at different temperatures, as shown in (Table 9). During the whole study, nano lipid in situ formulations maintained good stability.

CONCLUSION

We had formulated different formulation of nanoparticle drug delivery system they are nano lipid incorporate *in suit* gels with drug Brimonidine Maleate for ocular therapy. The formulated nanoparticles are characterized and evaluated. In nano lipid *in suit* gel formulations of Brimonidine Maleate can able to overcome precorneal and nasolacrimal drainage disadvantages. The formulations showed excellent drug loading capacity. The formulation was stable, nonirritant and release drugs in sustain manner form the gel formulation. It was finally concluded from the above work that formulation F1 has a maximum entrapment efficiency of 97.30% and drug content of about 96.36% for formulation F6. F1 formulation containing HPMC K-15M and Carbopol 940 about 0.2% w/v and 0.4% w/v respectively showed the drug release of about 99% for 10 hrs.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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