Formulation and Evaluation of Brimonidine Maleate Nanolipid in Situ Gel

Mohd Azharuddin¹, Theivendren Panner Selvam², Maya Sharma³, Jayesh Dwivedi⁴

¹Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India
²Department of Pharmaceutical Chemistry, Swamy Vivekananda College of Pharmacy, Elayampalayam, Namakkal, Tamil Nadu, India
³Department of Pharmaceutical Chemistry, Pacific College of Pharmacy, PAHER University, Udaipur, Rajasthan, India
⁴Pacific College of Pharmacy, PAHER University, Udaipur, Rajasthan, India

Article History:
Received on: 05 Aug 2020
Revised on: 06 Sep 2020
Accepted on: 12 Sep 2020

Keywords:
Brimonidine maleate, in situ gel, entrapment efficiency, in-vitro release, gellation study

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 in situ 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 in situ 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.

*Corresponding Author
Name: Mohd Azharuddin
Phone: +919886447725
Email: azhartzcop@gmail.com

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v11i4.3827

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; Nanjwade et al., 2007) Various stimuli can form gels these 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 follow 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 60°C for 45 minutes. The organic solvent is removed with gentle agitation and evaporating the organic solvent at 60°C 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°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)

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 60°C for 45 minutes. The organic solvent is removed with gentle agitation and evaporating the organic solvent at 60°C 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°C at the freezer.
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 |
| Benzalkonium 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       | (NH₃str) |
| 2951.03       | (Ar-CH₃str) |
| 2840.05       | (OHstr) |
| 1644.25       | (C=N) |
| 1014.47       | (C-O-Cstr) |

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

| IR (KBr) cm⁻¹ | Peaks |
|---------------|-------|
| 3323.90       | (NH₃str) |
| 2913.73       | (Ar-CH₃str) |
| 2846.64       | (OHstr) |
| 1694.73       | (C=N) |
| 1024.71       | (C-O-Cstr) |

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 | ±0.579 | 6.98   | ±0.438 | ±0.749 | ±0.205 |
| 1        | 38.04  | ±0.975 | 17.42  | ±0.445 | ±1.972 | 21.13  | ±0.707 | ±0.735 | ±0.459 |
| 2        | 50.16  | ±3.061 | 38.48  | ±1.626 | ±0.784 | 38.42  | ±0.748 | ±1.445 | ±3.48  |
| 4        | 64.08  | ±1.555 | 50.23  | ±0.318 | ±1.870 | 53.82  | ±0.537 | ±1.715 | ±5.13  |
| 6        | 81.45  | ±2.764 | 68.46  | ±2.142 | ±0.459 | 67.07  | ±2.057 | ±0.700 | ±1.484 |
| 10       | 99.0   | ±2.121 | 83.53  | ±1.180 | ±1.778 | 84.73  | ±2.220 | ±1.343 | ±0.671 |

Table 9: Stability Data of Optimized Formulation

| Drug content | 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 |
| F6 27°C±2°C  | 96.36±0.517  | 94.99±1.029  | 93.21±0.988  | 91.09±0.727  |
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 °C. 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 in situ 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 Nano lipid 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 248 nm. 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±2°C and 27±2°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.

**CONCLUSIONS**

We had formulated different formulation of nanoparticle drug delivery system they are nano lipid incorporate in situ gels with drug Brimonidine Maleate for ocular therapy. The formulated nanoparticles are characterized and evaluated. In nano lipid in situ 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. F6 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.

**ACKNOWLEDGEMENT**

Authors are thankful to the management and principal of TVM college of Pharmacy, Bellary, Karnataka for providing the necessary facilities to carry out the research work.

**Conflict of interest**

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

**Funding support**

The authors declare that they have no funding support for this study.

**REFERENCES**

Gaudana, R., Jwala, J., Boddu, S. H. S., Mitra, A. K. 2009. Recent Perspectives in Ocular Drug Delivery. Pharmaceutical Research, 26(5):1197–1216.

Kumar, K. S., Bhowmik, D., Paswan, S., Srivastava,
S. 2012. Recent challenges and advances in ophthalmic drug delivery system. *The Pharma Innovation*, 1(4):1–31.

Lavanya, B., Indira, S., Srinivas, P. 2014. Formulation and Evaluation of ocular niosomal in situ gels of linezolid. *International Journal of Pharmaceutical Sciences and Research*, 5(4):1367–1375.

Mohan, E. C., Kandukuri, J. M., Allenki, V. 2009. Preparation and Evaluation of in-situ-gels for ocular drug delivery. *J Pharm Res*, 2(6):1089–1094.

Moorthi, C., Krishnan, K., Manavalan, R., Kathiresan, K. 2012. Preparation and characterization of curcumin–piperine dual drug loaded nanoparticles. *Asian Pacific Journal of Tropical Biomedicine*, 2(11):841–848.

Nagalakshmi, S., Ramaswamy, R., Shanmuganathan, S. 2014. Formulation and Evaluation of stimuli sensitive pH triggered in-situ gelling system of fluconazole in ocular drug delivery. *International Journal of Pharmaceutical Sciences and Research*, 5(4):1339–1344.

Nagesh, C., Patil, M., Chandrashekhara, S., Sutar, R. 2012. A novel in situ gel for sustained ophthalmic delivery of ciprofloxacin hydrochloride and dexamethasone–Design and characterization. *Der Pharmacia Lettre*, 4(3):821–827.

Nanjawade, B. K., Manvi, F. V., Manjappa, A. S. 2007. RETRACTED: In situ-forming hydrogels for sustained ophthalmic drug delivery. *Journal of Controlled Release*, 122(2):119–134.

Nayak, N. S., Sogali, B. S., Thakur, R. S. 2012. Formulation and Evaluation of pH triggered in situ ophthalmic gel of Moxifloxacin hydrochloride. *Int J Pharm Pharm Sci*, 4(2):452–59.

Nayak, S., Srinivasa, U. 2017a. Design and Evaluation of ion activated in situ gel of moxifloxacin hydrochloride and Ketorolac tromethamine combination using carboxymethylated tamarind kernel powder. *Saudi J Med Pharm Sci*, 3(1):1–8.

Nayak, S., Srinivasa, U. 2017b. Design and Evaluation of pH triggered in situ ophthalmic gel of moxifloxacin hydrochloride and ketorolac tromethamine combination. *Int J Current Res*, 9(5):50444–50451.

OECD 2012. The Guideline O.E.C.D 405 for the Testing of Chemicals: Acute Eye Irritation.

Preetha, J. P., Karthika, K., Rekha, N. R., Elshafie, K. 2010. Formulation and Evaluation of in situ ophthalmic gels of Diclofenac sodium. *Journal of Chemical and Pharmaceutical Research*, 2(3):528–535.

Ramachandra, L. U., Vikas, D. G., Gadhave, M. V., Jadhav, S. L., Gaikwad, D. D. 2012. Design and development of pH-triggered in situ gelling system of Ciprofloxacin. *Int. Res. J Pharmacy*, 3(5):418–422.

Shashank, N. N., Srinivasa, U., Shwetha, S. K., Shabaraya, A. R. 2015. UV spectroscopic analysis of Moxifloxacin hydrochloride in Simulated tear fluid. *Int J Universal Pharm and Bio Sci*, 4(5):11–15.