

# Development and Evaluation of Nanosuspension Incorporated *in situ* gel of Brimonidine Tartarate for Ocular Drug Delivery

Viresh Hanagandi, Archana Sidagouda Patil\*, Rajashree Shashidhar Masareddy, Panchaxari Mallappa Dandagi, Udaykumar Baburao Bolmal

Department of Pharmaceutics, KLES College of Pharmacy, Constituent Unit of KLE Academy of Higher Education and Research, Belagavi, Karnataka, INDIA.

## ABSTRACT

**Objectives:** The goal of the study was to develop, optimize and *in vitro-ex vivo* investigation of Brimonidine tartrate nanosuspension incorporated *in situ* gel formulation to differentiate with marketed (formulation) eye drops for the efficient treatment of glaucoma. **Materials and Methods:** Nanosuspensions were formulated by solvent evaporation method using probe sonication technique. The effect of the independent variables Tween 80 and Pluronic F68 concentration on Nanosuspension properties were investigated by performing 3<sup>2</sup> factorial design of experiment. Nanosuspensions were characterized by measuring particle diameter and zeta potential, surface morphology, drug entrapment efficiency and then nanosuspensions were incorporated in to *in situ* gel base for *in vitro* release and *ex-vivo* corneal permeability studies and were differentiated with marketed product. **Results and Discussion:** The most excellent nanosuspension formulation system selected via the Design Expert 12 software program was F7 which contains 0.5% of Tween 80 and 3 % of Pluronic F68. An optimized nanosuspension formulation F7 showed an average particle diameter of  $157.4 \pm 0.95$  nm with PDI of 0.379 and a zeta potential of -19.1 mV. It had an average entrapment efficiency of  $85.95 \pm 1.40\%$ . It also showed  $98.36 \pm 0.58\%$  of drug being released *in vitro* when incorporated in to *in situ* gel base over 24 hr. F7 showed significantly greater drug permeation in comparison to the marketed eye drop formulation in *ex vivo* transcorneal permeability studies and was able of retaining its stability for 90 days. **Conclusion:** The developed nanosuspension incorporated *in situ* gel base formulation could be utilized as potential delivery system for long time management of glaucoma with once daily dose.

**Key words:** Brimonidine Tartrate, Ocular bioavailability, Nanosuspension, *In-situ* gel, Increased residence time.

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**Correspondence:**

Dr. Archana S Patil,

Assistant Professor,  
Department of Pharmaceutics,  
KLE College of Pharmacy,  
Constituent Unit of KLE Academy of Higher Education and Research, Belagavi-590010, Karnataka, INDIA.  
Email: archupharma@gmail.com

## INTRODUCTION

The bioavailability of ocular drugs from normal ocular formulations is generally poor due to the pre-corneal loss resulting from the withdraw mechanisms like unproductive absorption, transient residence time in cul-de-sac and respective low permeation of the drugs through corneal membrane (epithelial).<sup>1</sup> A BCS class-II drugs face demanding problems in ophthalmic formulations; in general ocular bioavailability of drugs significantly affects ocular efficacy, which could be improved

by increasing the penetration of drug through cornea, enhancing the saturation solubility of drugs (poorly soluble) and prolonged pre-corneal residence time of drugs.<sup>2,4</sup> The nano-suspension is a colloidal dispersion of drug particles in an aqueous external phase with an average particle size from 10 and 1000 nm.<sup>5,6</sup> The prominent characteristic of nanosuspensions is their potential to enhance saturated solubility and accordingly enhancing dissolution rate of specific drugs.<sup>7-9</sup> Due to this aspect and



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for the other benefits that nanosuspension offers over conventional ocular formulations, including reduced dose size, long-term maintenance of drug release, reduction of systemic drug toxicity, prolonged time of residence of nanoparticles on corneal surface, enhanced concentration of drug in the infected tissue and unsuitability to water-soluble drugs.<sup>10</sup> The ophthalmic delivery of drugs, more than any other means of administration, could take full advantage of the characteristics of nanosuspension stabilized by suitable and potential stabilizers (surfactants/polymers).<sup>11</sup> The system *in situ* gels consists of such a smart polymers which exhibits solution to gel transition when inserted in to eye due to varying pH, temperature (precorneal region) and electrolyte composition in tear fluid. Thus, *in situ* gel incorporated with drug loaded nanosuspension could be highly potential ophthalmic drug delivery with sustained drug delivery pattern.

Brimonidine tartrate (BRT) is an alpha-2 selective adrenergic agonist which is utilized in the treatment of glaucoma. It acts by reducing the production of aqueous humor and enhancing the uveoscleral outflow.<sup>12</sup> In some clinical studies, brimonidine tartrate has shown a reduction in the efficacy of intraocular pressure similar to that of timolol and higher than betaxolol. Literature surveys show that very little studies were conducted on the prolonged-release components of brimonidine tartrate for the treatment of glaucoma. Biodegradable ocular insertion of brimonidine tartrate was recently developed and studied which confirmed the prolonged conduction profile of the insert compared to solution of brimonidine tartrate, but these formulations of ocular inserts lost patient compliance. However, it is clearly necessary to have powerful topical formulations capable of promoting drug penetration and preserving the therapeutic phases with an economic application frequency.<sup>13</sup>

The aim of present study was to prepare and examine Brimonidine Tartrate Nanosuspension incorporated *in situ* gel formulations for ocular delivery. The interaction among two factors i.e., percentage of polymer and surfactant on nanosuspension properties were investigated systematically by applying a 3<sup>2</sup>-full-factorial design. The prepared nanosuspensions were characterized with respect to their particle size, PDI, zeta potential and entrapment efficiency. The nanosuspension incorporated *in-situ* gel formulation was evaluated for *in vitro* drug release, *ex vivo* transcorneal permeability and short-term stability study.

## MATERIALS AND METHODS

### Materials

Brimonidine Tartrate was obtained as a gift sample from Symed Labs Limited, Hyderabad. Pluronic F68, was obtained from Ozone Pharmaceuticals, Mumbai, India. Tween 80, Hydroxypropyl methylcellulose K4M and Benzalkonium chloride was obtained from Sigma-Aldrich chemicals pvt ltd, Bangalore. All other materials used were of pharmacopoeial grade.

### Methods

#### Preparation of Nanosuspension (NS) Formulations by Solvent Evaporation Technique

Accurately weighed quantity of Brimonidine Tartrate and polymer Pluronic F68 was taken and dissolved completely in 5ml of methanol. In another beaker polymers (Tween 80 and Benzalkonium chloride) were added to the water and further sonicated till a clear polymeric solution is obtained. The above drug solution was then slowly injected with a syringe connected to a thin Teflon tube, into 50 mL water containing Tween 80 and Benzalkonium chloride kept at a low temperature in an ice bath. Drug solution was injected with vigorous mixing at stirring velocity of 8000 rpm. The obtained emulsion was then sonicated by using probe-type sonicator for 15 mins. Residues of solvent were left to evaporate off with a slow magnetic stirring of the nanosuspension at room temperature (25- 27°C) for 8-12 hr.

### Factorial Design

In addition to traditional experimentation, factorial design is a recent tool to identify critical parameters of the process and also for the optimization of the concentrations of respective ingredients.<sup>14,15</sup> Thus, different nanosuspension formulations containing Brimonidine Tartrate were prepared by using 3<sup>2</sup> factorial designs.

The independent variables Pluronic F68 percentage (X1) and Tween 80 (X2) percentage were taken in three levels for formulations and nine formulations were prepared using different levels of variables. Dependent variables responses are particle size, zeta potential and entrapment efficiency.

#### Characterization of Nanosuspension Formulations Particle Size Analysis

Particle size of the prepared nano-suspension was determined by Dynamic Light Scattering analyzer.<sup>16</sup> Before analysis, the nanosuspension was diluted

with Millipore water in the ratio of 1:5 and further sonicated for 2 min. Samples were analyzed in triplicates.

### Polydispersity Index

Polydispersity index is also measured using Dynamic light scattering analyzer. The obtained PDI values give an idea about the particle size distribution of nanoparticles. Its value ranges from 0.000-1.000 Mw which demonstrates that lower the value, narrower will be the size distribution of nanoparticles and vice-versa.

### Zeta Potential

Zeta potential provides information about stability of the prepared formulation. It was measured by using zetasizer.

### Percentage Entrapment Efficiency

To know the drug entrapment, 20 ml of freshly prepared nanosuspension was subjected to centrifugation (cold) at ~ 4°C and 15,000 rpm for 30 min (Sigma 3K 30 Centrifuge). 1ml supernatant solution was taken for proper dilution. The diluted solution was analyzed for BRT content using UV-Spectrophotometer and following equation was used to calculate % Entrapment efficiency (% EE).

$$\% EE = \frac{\text{Total amount of drug} - \text{Free dissolved drug}}{\text{Total amount of drug}} \times 100$$

### Scanning Electron Microscopy (SEM)

In order to study the external morphology of Brimonidine Tartrate nanosuspension, SEM was employed and it was performed at SAIF, Cochin. Samples for SEM were prepared by initially diluting with Millipore water and then a drop of dispersion was placed on carbon coated copper grids and stained with phosphotungstic acid. The grid was air dried and was observed at different magnification under SEM.

### Preparation of *in-situ* Gel Base

To obtain Pluronic gel base 1% HPMC K4M was dissolved in distilled with stirring and then Pluronic F68 10% was added. The resulting solution was kept in refrigerator at 4°C for about 24 hr and subjected for terminal sterilization in autoclave. Calculated amount of each Brimonidine Tartrate nanosuspensions were incorporated in to *in-situ* gel formulation and used for the *in vitro* release and *ex vivo* transcorneal permeability studies.

### Physicochemical Characterization of Gel Base Clarity

The clarity of gel base earlier than and after gelling was investigated by visual inspection under white and black background.

### Viscosity

The viscosity of gel base was determined by using Brookfield CAP 2000+ Viscometer. The gel base sample was taken and settled for 1min later the viscosity was determined using spindle number 1 at 100 rpm (shear speed=1333) at a temperature of 25 ± 2°C.

### Gelling Capacity

The gelling capacity of the gel base was tested by pouring 1 ml of it in glass test tube containing 10 ml of fresh simulated tear fluid with a temperature equal to 37±2°C. The time taken for the solution to gel transition was noted down and the period of time for which the gel doesn't dissolve in the buffer was noted.

### *In-vitro* Drug Release

All of the Brimonidine Tartrate nanosuspension formulation equivalent to 1mg of drug were incorporated into the prepared gel base before performing their release studies. The *in-vitro* drug release of Brimonidine Tartrate was carried out by using the dialysis bag diffusion technique. An accurately weighed amount of Nanosuspension incorporated *in-situ* gel formulation was filled in to the dialysis bag. The bag was sealed, then suspended initially for 1hr in beakers containing 100ml of artificial tear fluid. Aliquots of 5ml of the sample were collected at pre-determined intervals from the compartment which was replaced with fresh artificial tear fluid. The drug release was determined spectrophotometrically after dilution at 248nm.<sup>17</sup>

### *Ex-vivo* Trans Corneal Permeability Study

*Ex-vivo* trans corneal permeation studies were performed by keeping the Brimonidine Tartrate nanosuspension incorporated *in-situ* gel formulation (optimized formulation F7)/ marketed eye drop on a freshly excised goat cornea.<sup>18</sup> From the butcher's shop fresh, whole goat eyeballs were obtained, chilled in to the normal saline (4°C) and transported to experimental laboratory. The cornea was carefully excised along with 2 to 4 mm of surrounding sclera tissue and then with the help of normal saline solution it was made free from protein. The excised cornea was then fixed in between the clamped donor and receptor compartments with its epithelial

surface facing the donor compartment in Franz diffusion cell.

The corneal area which is available for a drug to diffuse was 0.50 cm<sup>2</sup>. The 10 ml fresh artificial tear fluid (pH 7.4) filled in receptor compartment and air bubbles were expelled from it. The aliquot of 1ml of the prepared nanosuspension incorporated *in situ* gel formulation/ marketed formulation was kept on the cornea and the donor cell opening was sealed with a glass cover slip; the receptor fluid was constantly kept stirring at 37°C. The study was performed for about for 8 hr and samples were withdrawn (from the receptor) and analyzed for Brimonidine Tartrate content using UV/Visible spectrophotometer at 248 nm.

### Short Term Stability Studies

Short term stability study was performed on the optimized formulation. The formulation was subjected to different condition of temperature and relative humidity that is 25°C/ 60% RH and 4°C / 65% RH for a period of 3 month in Humidity Control Oven. Samples were withdrawn at the interval of 0, 30, 60 and 90 days and were evaluated for particle size and % CDR.<sup>19</sup>

## RESULTS AND DISCUSSION

### Preparation of Nanosuspension Formulation

To exploit a vision of the formulation of Nanosuspension, two stabilizers such as Pluronic F68 (macromolecular polymer) and a Tween 80 (surfactant), at concentration levels unique in their kind. Factorial design with two independent variable factors; quantity of polymer (X<sub>1</sub>) and surfactant quantity (X<sub>2</sub>); at three levels have been considered.

Pluronic F68 has been claimed to be the best stabilizer for insoluble drugs which was attributed to its smaller molecular weight than that of other Pluronics that

results in low kinetic constrains within the adsorption path and faster diffusion.

Tween 80, a small molecular surfactant that forms a thin adsorption phase and its on-site presence leads to instantaneous formation of nanosized nanospheres dispersed homogeneously attributing to high drug loading ability.

The aim at here was not just formulation optimization but also to study the effect of selected excipients alone or in combination on various NS properties like particle size, zeta potential and entrapment efficiency with the same accuracy of the percentage of drug released over time 24hrs time. Excipients concentrations were taken on preliminary studies. Nine batches were prepared by using the assumed values of variables X<sub>1</sub> and X<sub>2</sub> at three levels as shown in Table 1.

### Evaluation of Formulated Nanosuspension

#### Particle size and Zeta Potential

All nine BRT NS formulations were evaluated for their PDI, particle diameter as well as for zeta potential (Table 2). The mean size of particles of all formulations were observed to be less than 289 nm which was appropriate for ophthalmic use, PDI turned into lower than 0.53 and zeta potential values were enough to maintain stable nanosuspensions.

#### Entrapment Efficiency

The determined entrapment efficiency (EE) of BRT with NS is listed in Table 2 which was varied in the range from 54.04% to 85.85%. It was noticed that, the EE was enhanced with higher quantities of Pluronic F68. The F7 formulation with 3% of Pluronic F68 shows highest EE while F6 formulation with 2% of polymer shows least EE. On the contrary, increase in concentration of Tween 80 reduces the EE this is

**Table 1: Formulation design of Brimonidine Tartrate Nanosuspension using solvent evaporation method.**

Formulation number	Brimonidine Tartrate (mg)	Pluronic F68 {X <sub>1</sub> } (%)	Tween 80 {X <sub>2</sub> } (%)	Benzalkonium chloride (%)	Methanol (ml)	Water (ml)
F1	10	1%	0.5%	0.1%	5	50
F2	10	1%	1%	0.1%	5	50
F3	10	1%	1.5%	0.1%	5	50
F4	10	2%	0.5%	0.1%	5	50
F5	10	2%	1%	0.1%	5	50
F6	10	2%	1.5%	0.1%	5	50
F7	10	3%	0.5%	0.1%	5	50
F8	10	3%	1%	0.1%	5	50
F9	10	3%	1.5%	0.1%	5	50

**Table 2: Experimental Condition, Design and Responses of 3<sup>2</sup> Factorial Design for Preparation of Brimonidine Tartrate Nanosuspension.**

Formula code	Pluronic F68 {X1} (%)	Tween 80 {X2} (%)	Particle Diameter (nm)	PDI	Zeta Potential mV	Entrapment efficiency (%)	Release percentage of (%)
F1	1%	0.5%	283.3±0.57	0.494±0.035	-18.8	75.68	85.83±0.70
F2	1%	1%	233.3±2.08	0.446±0.004	-18.2	67.09	80.20±0.90
F3	1%	1.5%	221.3±1.52	0.270±0.008	-18.3	61.84	75.68±0.74
F4	2%	0.5%	179.9±1.05	0.533±0.048	-18.6	79.65	82.16±1.12
F5	2%	1%	269.7±1.36	0.296±0.012	-17.2	78.22	89.08±1.28
F6	2%	1.5%	291.3±1.57	0.312±0.022	-13.4	54.05	89.85±1.62
F7	3%	0.5%	157.4±0.95	0.379±0.076	-19.1	85.85	99.16±0.18
F8	3%	1%	262.6±1.52	0.260±0.011	-14.6	70.91	87.62±0.80
F9	3%	1.5%	289.9±1.00	0.522±0.032	-15	63.4	86.63±1.06

PDI- Polydispersity Index

Data are expressed as Mean ±S.D. (n=3)

**Table 3a: ANOVA Table for Response Particle Size.**

Source	Sum of Squares	d <sub>f</sub>	Mean Square	F-value	p-value	
<b>Model</b>	15144.59	3	5048.20	6.27	0.0379	Significant
A-Pluronic F68	127.88	1	127.88	0.1589	0.7066	
B-Tween 80	5442.08	1	5442.08	6.76	0.0482	
AB	9574.62	1	9574.62	11.90	0.0183	
<b>Residual</b>	4023.57	5	804.71			
<b>Cor Total</b>	19168.16	8				

**Table 3b: ANOVA Table for Response Entrapment Efficiency.**

Source	Sum of Squares	d <sub>f</sub>	Mean Square	F-value	P-value	
<b>Model</b>	678.70	2	339.35	15.15	0.0045	Significant
A-Pluronic F68	40.30	1	40.30	1.80	0.2283	
B-Tween 80	638.40	1	638.40	28.51	0.0018	
<b>Residual</b>	134.35	6	22.39			
<b>Cor Total</b>	813.05	8				

**Table 3c: ANOVA Table for Response Zeta Potential.**

Source	Sum of Squares	d <sub>f</sub>	Mean Square	F-value	P-value	
<b>Model</b>	23.27	2	11.63	5.44	0.0449	Significant
A-Pluronic F68	7.26	1	7.26	3.40	0.1150	
B-Tween 80	16.01	1	16.01	7.49	0.0339	
<b>Residual</b>	12.83	6	2.14			
<b>Cor Total</b>	36.10	8				

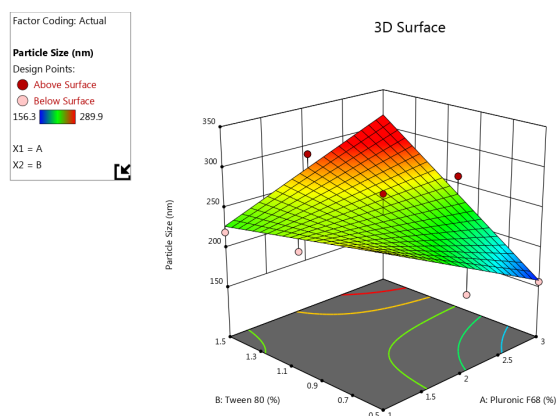
because of an increased concentration of tween 80 above its CMC (critical micelle concentration).

### Factorial Design

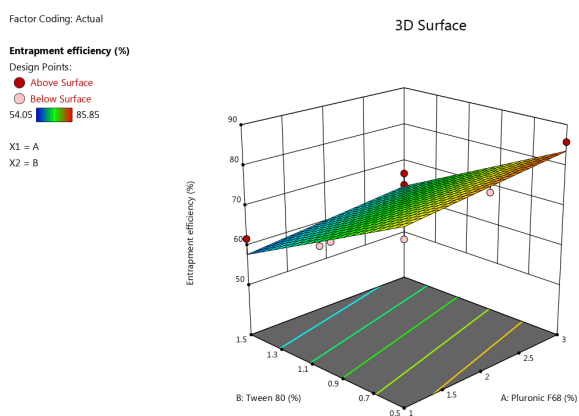
The findings of ANOVA for factorial design are represented in Table 3. 3a, 3b and 3c. As per ANOVA

findings, the particle size was observed to be affected by the interactions among the X1 and X2 significantly ( $p < 0.01$ ) as represented in surface plot of particle size in response to the determined factors; polymer quantity and surfactant quantity (Figure 1a, 1b and 1c). While the EE as well as zeta potential were significantly

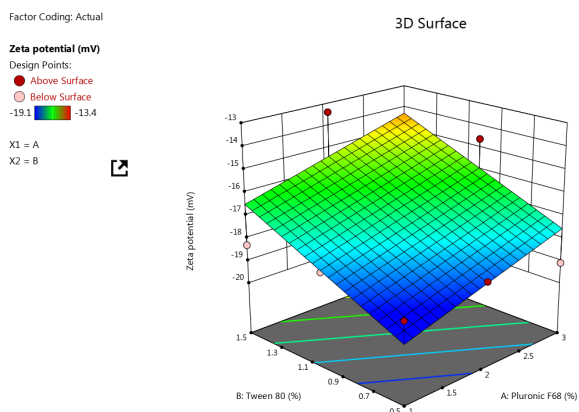




**Figure 1a: 3D Graph Representing the Influence of Pluronic F68 and Tween 80 on Particle Size.**



**Figure 1b: 3D Graph Representing the Influence of Pluronic F68 and Tween 80 on Entrapment Efficiency.**



**Figure 1c: 3D Graph Representing the Influence of Pluronic F68 and Tween 80 on Zeta Potential.**

affected by the concentration of surfactant ( $p < 0.01$ ). The polymer concentration reflected very little effect on the zeta potential and EE. There was no any significant interaction among the two factors as suggested by the

Design Expert 12 software, i.e. the quantity of polymer effect on the zeta potential and EE is independent on surfactant concentration.

### Optimization of Formulation

By applying  $3^2$  factorial design, the process was optimized with respect to particle size, EE and zeta potential (response variables). Formulation F7 was selected as an optimized formulation as it greatly fitted in the given criteria for optimization of formulation. Formulation F7 was found to possess less particle size and achieved higher entrapment efficiency and high zeta potential.

### Scanning Electron Microscope

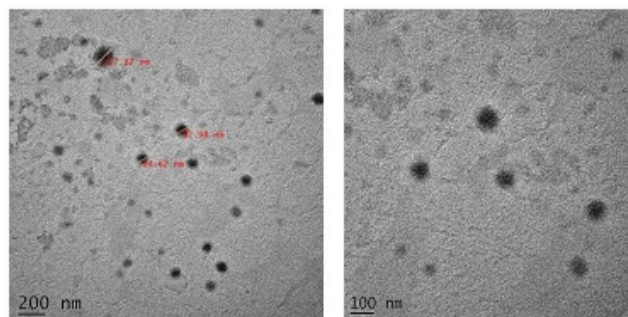
SEM images of the optimized formulation F7 were found to be in nano range as shown in Figure 2. The particles were found to be in nano range, spherical in shape and evenly distributed.

### Preparation of Brimonidine Tartrate in Pluronic *in situ* Gel Base

To reach the goal of enhancing the effectiveness of Brimonidine Tartrate in the nanosuspension formulations, a suitable dosage form selection plays a very important role. Therefore, an *in situ* gel was formulated using Pluronic to prolong the contact time of Brimonidine Tartrate nanosuspension formulation inside the eye which results in sustained release of drug that ultimately enhances drug bioavailability. Prepared gel base was evaluated for its physicochemical properties. Prepared all Brimonidine Tartrate nanosuspension formulations were incorporated into the solution of gel before performing their release studies.

### Physicochemical Characterization of Gel Base

The pluronic gel base was physicochemically evaluated by measuring clarity, viscosity, gelling capacity. The gel found clear before and after gelling. The viscosity of gel base was found to be  $4056 \pm 123$  mPas at physiological pH. In case the gel viscosity is high, it reduces drug

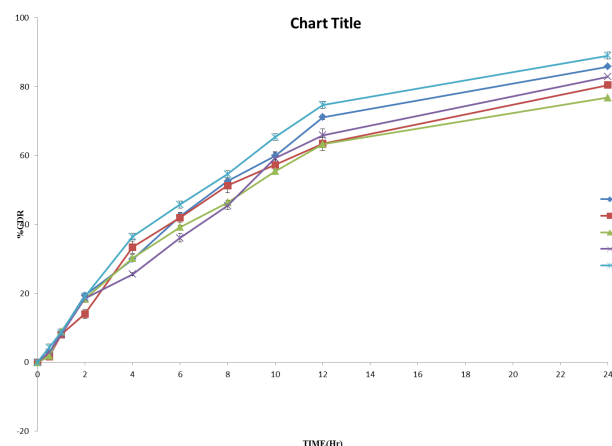


**Figure 2: SEM Images of Optimized Formulation F7.**

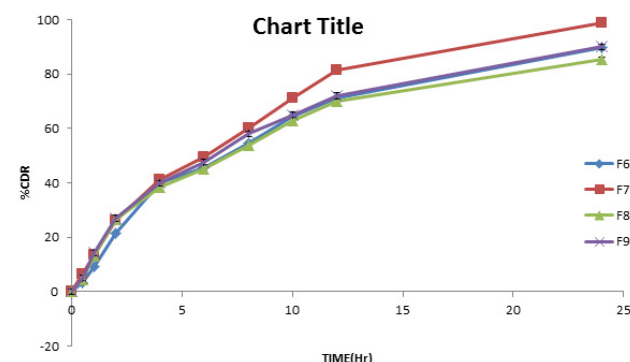
release from the formulation but leads to discomfort in patients. If the viscosity is low it leads low corneal residence time of formulation. Thus, optimum viscosity is needed for effective ophthalmic drug delivery and as per literature gels should have a viscosity of about 50-5000 mPas to achieve desired drug release from the *in-situ* gel formulations. Gelling capacity of *in situ* gel base was formed immediately after it becomes accessible to the simulated gastric fluid also, the gels formed should remain stable for extended period of time. This demonstrates that the *in-situ* gel base will get gelled immediately in tear fluid.

### **In vitro Release Study**

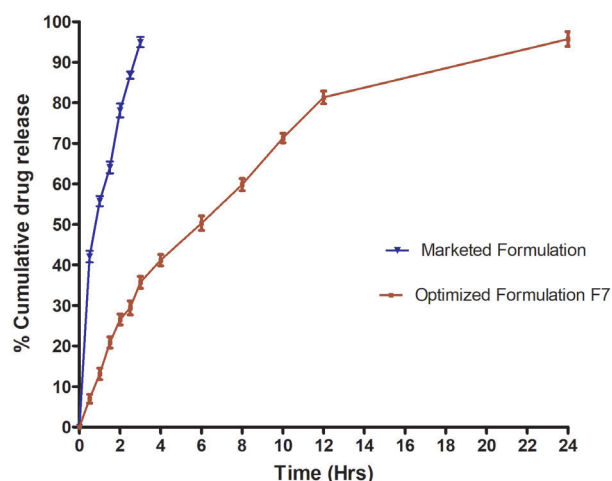
The *in vitro* release studies of drug from ocular systems represents an important parameter for predicting bioavailability of drug from various formulations. The drug release profile of nanosuspension incorporated *in-situ* gel formulation is as represented in Table 2 and Figure 3a, Figure 3b and Figure 3c. The release of drug



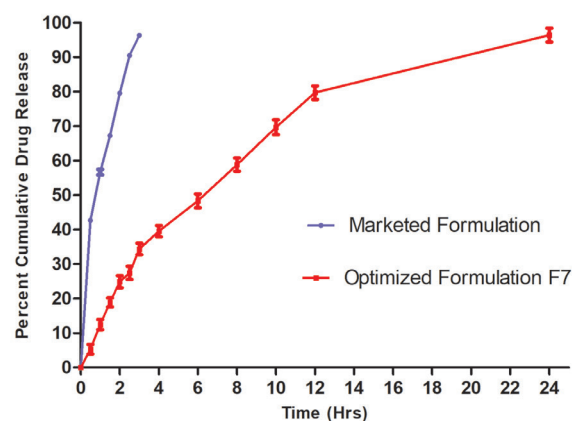
**Figure 3a: *In vitro* Release Profile of BRT from NS Incorporated *in-situ* Gel Formulations F1-F5.**



**Figure 3b: *In vitro* Release Profile of BRT from NS Incorporated *in-situ* gel Formulations F6-F9.**



**Figure 3c: *In vitro* Release Profile of BRT from Optimized NS Incorporated *in-situ* gel Formulation and Marketed formulation.**



**Figure 4: Comparative *ex-vivo* Transcorneal Permeability of Optimized Formulation F7 with Marketed Formulation.**

from marketed eye drop formulations was 96% within 3 hrs whereas, all NS incorporated *in-situ* gel formulations showed ~30 % drug release in 3 hrs and sustained release of drug for about 24 hr. The optimized F7 formulation exhibited maximum drug release in 24 hr. From the drug release observations, it can be concluded that NS incorporated *in situ* gel formulations show sustained drug release for about 24hr that could enhance the corneal permeation and bioavailability of drug. Thus, frequency of drug administration can be reduced but as eye drops are liquid solutions of drugs they need frequent administration due to faster drug release in shortest time period.

### Ex-vivo Transcorneal Permeability Study

The result indicated that Nanosuspension incorporated *in-situ* gel formulation system significantly increase the drug penetration rate across the cornea. Brimonidine Tartrate nanosuspension incorporated optimized *in situ* gel formulation F7 showed a significantly greater drug permeation capacity as compared to commercial marketed eye drop. Brimonidine Tartrate from marketed formulation permeated 94.65% in 3 hr whereas Brimonidine Tartrate nanosuspension incorporated optimized *in situ* gel formulation F7 permeated 36.26% in 3 hrs (Figure 4). Prepared *in-situ* gel formulation showed a significantly greater drug permeation capacity as compared to commercial marketed eye drops in a sustained manner. This enhanced permeation of Brimonidine Tartrate across the cornea can be attributed to the nanoparticles agglomeration in the conjunctival sac and phase transition of *in-situ* gel thus, forming depot from which the drug is slowly delivered to the precorneal area.

### Short Term Stability Studies

Stability studies were performed on the nanosuspension incorporated *in-situ* gel optimized formulation F7 as per ICH Guidelines for a period of 3 months. Physical appearance of F7 changed slightly when samples were stored at room temperature  $25\pm 2^{\circ}\text{C}$ / RH  $65\pm 5\%$  for 3 months and a thin layer of sediment was observed. However, it disappeared immediately with slight shaking. No change in the physical appearance was observed when *in situ* gel formulations were stored in refrigerator at  $4\pm 2^{\circ}\text{C}$ /RH  $65\pm 5\%$  for 3 months. By comparing this data with initial data it was observed that there was no significant change in particle size and *in vitro* drug release for the formulation stored at  $4\pm 2^{\circ}\text{C}$ / RH  $65\pm 5\%$  to that stored at room temperature. Thus, formulation stored at  $4\pm 2^{\circ}\text{C}$ / RH  $65\pm 5\%$  showed better stability as compared to the formulation stored at  $25\pm 2^{\circ}\text{C}$ / RH  $65\pm 5\%$ .

### CONCLUSION

The study demonstrated a combined effect of concentration of two independent variables; surfactant and polymer as an attempt to produce optimized nanosuspension formulation (F7) as a promising ocular delivery system to enhance the absorption and ocular bioavailability of Brimonidine Tartrate. Applied  $3^2$  factorial design optimized various factors to produce spherical and stable nanosuspension with smaller particle size, lower PDI and higher EE. The sustained or prolonged release BTR in Pluronic *in situ*

gel base drug delivery was developed successfully and investigated for physicochemical characters of gel base and *in vitro-ex vivo* permeation bioavailability study. The experimental data developed suggested that, optimized formulation possess better viscosity and gelling ability at physiological conditions. Brimonidine Tartrate nanosuspension incorporated *in-situ* gel formulation exhibited prolonged drug release relative to the marketed eye drop. *Ex-vivo* permeation bioavailability study was carried out using cornea of goat and it shows improved transcorneal permeation in comparison to the marketed eye drop formulation which decreases frequency of dosing. The drug was released in the controlled manner for 24 hrs through membrane. The nanosuspension incorporated optimized *in situ* gel formulation F7 retained its stability for 3 months.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### ABBREVIATIONS

**BRT:** Brimonidine Tartrate; **NS:** Nano suspension; **SEM:** Scanning Electron Microscope; **ML:** Milli Liter; **°C:** Degree Centigrade; **µg:** Microgram; **Rpm:** Revolutions per minute; **µm:** Micrometer; **nm:** Nanometer.

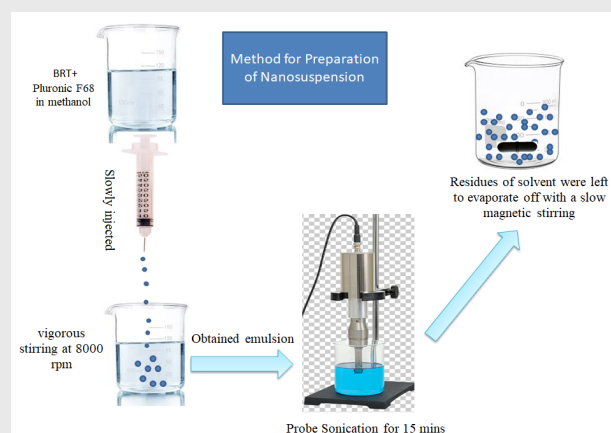
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## PICTORIAL ABSTRACT



## About Authors



**Dr. Archana Sidagouda Patil**, Is an Assistant Professor, Department of pharmaceuticals, KLE College of Pharmacy, Constituent Unit of K.L.E Academy of Higher Education and Research, Belagavi. She is working in the area of targeted drug delivery systems viz, pH and temperature responsive co-polymeric nanoparticles, pulsatile drug delivery systems as well as synthesis and characterization of graft.

## SUMMARY

- A total nine formulations with varying polymer Pluronic F68 and surfactant and Tween 80
- By applying  $3^2$  full factorial design optimized various factors to produce spherical and stable nanosuspension with smaller particle size, lower PDI and high EE.
- The SEM results showed particles were in nano range, spherical in shape and evenly distributed.
- In situ* gel base was prepared by using HPMC K4M and Pluronic F68 and evaluated for its physicochemical properties.
- The prepared *in-situ* gel base was used to incorporate all nanosuspension formulations before *in-vitro* drug release study as to attain the sustained drug release of nanoformulations.
- Brimonidine Tartrate nanosuspension incorporated *in-situ* gel formulation resulted sustained drug release as compared to marketed eye drop formulation.
- Ex-vivo* drug permeation study was carried out using cornea of goat and it shows improved transcorneal permeation in comparison to the marketed eye drop formulation which decreases frequency of dosing.

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