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RESEARCH ARTICLE

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DESIGN OPTIMIZATION AND EVALUATION OPHTHALMIC NANOEMULSION

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Abstract:

Nanoparticulate ophthalmic drug delivery systems enhances the bioavailability of poorly water soluble drugs. It is an isotropic mixture of Oil, Surfactant, Cosurfactant (S_{mix}), water and drug. Nanoemulsion, particularly, oil/water nanoemulsions have been investigated as a potential drug delivery system. Nanoemulsion possesses unique physicochemical properties namely, high solubilizing capacity for various drugs as well as acting as penetration enhancers to facilitate corneal drug delivery. Furthermore, the low surface tension of nanoemulsion would also guarantee a good spreading effect on the cornea and a proper mixing with the precorneal film constituents, thus would possibly improve the contact between the drug and corneal epithelium. Nanoemulsionhave good tissue permeability because of small size of micelles and the presence of surfactant among the components.

Keywords: Olapatadine, Opthalmic Nanoemulsion,

1 Introduction

The word Nanotechnology, arise from the greek word nano meaning drawf, technology means application to the engineering, electronics, physics, material science, medical and manufacturing at a molecular and a submicron level. An early promoter of nanotechnology, Albert franks, defined it as that area of science and technology where dimensions and tolerance are important. (Sharma Jitendra*et al.,* 2011). Nanotechnology based ophthalmic formulations are currently being pursued for both anterior, as well as posterior segment drug delivery. Nanotechnology based systems with an appropriate particle size can be designed to ensure low irritation, adequate bioavailability and ocular tissue compatibility. Several Nanocarriers such as nanoparticles, nanosuspension, nanomicelles, liposomes, and dendrimers have been developed for ocular drug delivery (Campochiaro PA. *et. al.*,2006).

1.1 Advantagesof Nanotechnology based ocular delivery (Rainudevi et al., 2017)

1. Nanocarriers such as nanoparticles have capacity to deliver ocular drug to specific target site.
2. The nanoparticulate nature of drug shows sustained release effect by increasing its residence time in the cul-de-sac. The nanoparticles protect the drug against agents which cause degradation.
3. Nanosuspensionhad a quicker onset of action and enhanced dose proportionality. Nanosuspension also alter the pharmacokinetic parameters, improves the safety and efficacy of the drugs.
4. Nanotechnology can be used in the drug delivery and gene therapy by applying novel self assembled materials and devices of nanoscale size.
5. Increase the residence time of the associated drugs onto the ocular surface and reduce the degradation of labile drug.
6. Nanocarriers improve their interaction with the corneal and conjuctival epithelia and consequently the bioavailability.
7. Increased accurate dosing. To overcome the side effects of pulsed dosingproduced by conventional system.

1.2 Nanoemulsion

Nanoemulsion are oil-in-water (O/W), water-in-oil (W/O) dispersion of two immiscible liquids stabilized using an appropriate surfactant. The mean droplet diameter attained is usually less than 500nm. Small droplet size gives them a clear or hazy appearance which differs from milky white color associated with coarse emulsion. The word nanoemulsion is sometimes used interchangeably with submicron emulsion or mini emulsion; however it should not be confused with microemulsion. Nanoemulsion despite having the same droplet size range as microemulsion, differ tremendously in structural aspects and long term thermodynamic stability (McClements D.J. *et al*, 2012).

Nanoemulsions can be rendered into several dosage forms like liquids, creams, sprays, gels, aerosol, foams; and can be administered by equally varying routes like topical, oral, intravenous, intranasal, pulmonary and ocular. They possess higher solubilization capacity than simple miceller dispersion, greater kinetic stability than coarse emulsion and found use in cosmetic and pesticide industry as aqueous base for organic deliverables. Their long-term physical stability is a direct consequence of small droplet size, which impairs conventional destabilization phenomena like creaming, sedimentation and coalescence. Often Brownian motion is strong enough to offset gravity or viscosity induced kinetic stability. In parenteral form, nanoemulsion have been used to solubilize and protect drugs against harsh environmental factors (oxidation, pH, hydrolysis), to target specific organs by exploiting enhanced permeability and retention effect, and to evade reticuloendothelial system.

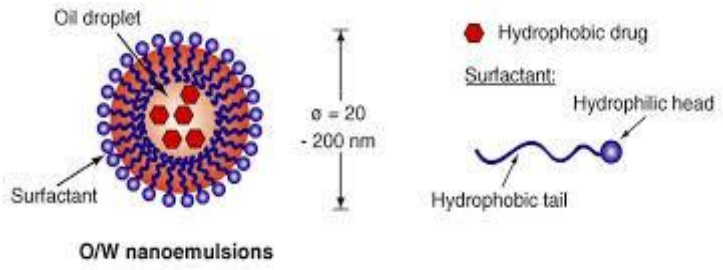


Figure: Diagram of Nanoemulsion

2 Material and Methods

2.1 Materials

Olopatadine HCl was kindly supplied by Indoco Remedies Pvt. Ltd (Mumbai, India). Tween 60 was acquired from Research Lab. Fine Chemical Industries (Mumbai, India), Tween 20, Tween 80, Tween 40. Benzalkonium chloride, Sodium chloride, Potassium chloride Research Lab. Fine

Chemical Industries (Mumbai, India), Soyabean casein diagest media, Fluid thioglycolate media HiMedia laboratories Pvt. Ltd, (Mumbai, India), PEG400, HPMC K4M, Olive oil Research Lab. Fine Chemical Industries (Mumbai, India).

2.2 Solubility studies

Olopatadine HCl drug powder was added excess in different oils, surfactant and co-surfactant in a centrifuge tube, followed by sonication for 4 h. The samples were centrifuged at 5000 rpm for 30 min after which the supernatant was removed. The supernatant analyzed by UV- visible spectrophotometer. The oil, surfactant and co-surfactant with maximum solubility of Olopatadine HCl was selected for formulation of nanoemulsion.

2.3 Construction of pseudo-ternary phase diagram

The pseudo-ternsry phase diagram of oil, surfactant, co-surfactant and water were constructed using a water titration method to obtain the concentration of components.

Five different Smix ratios (3:1, 2:1, 1:1, 1:2, and 1:3) were taken for construction of pseudo-ternary phase diagram. It has played an important role in the selection of oils/surfactants/co-surfactants ratios for preparation of stable nanoemulsionsystem.In this total 45 batches was taken for construction of pseudo-ternary phase diagram. The pseudoternary phase diagram was constructed using software CHEMIX Version 4.0

2.4 Formulation of Nanoemulsion

This step involved preparation of mixture of the oil (Olive oil) and Olopatadine HCl by solubilisingOlopatadine HCl into Olive oil using bath sonicator. The second step was addition of surfactant and co-surfactant (Tween®80) and PEG400 to distilled water under vigorous stirring using high speed homogenizer. The third step involved addition of oil phase in a drop wise manner to the aqueous phase under vigorous stirring to form a pre-emulsion. Formulation of nanoemulsion was done by introducing pre-emulsion to high speed homogenizer. The speed was increased from 200 to 600 rpm. Each speed was maintained for 20 homogenization cycles. Aliquots were withdrawn after each 20 cycles for estimation of globule size.

2.5 Experimental Design:

A Box-Behnkenexperimental design (BBD) was applied for the optimization of nanoemulsion formulation using State-Ease (Design-Expert 11) software. The statistical data was representing the effect of formulation variables such as oil phase Olive oil (A), S_{mix}1:1 ratio of Tween80: PEG400 (B) and distilled water (C) on responses. The software suggested quantities of oil, Smix and water for the preparation of nanoemulsion were found 4-9%, 39-40% and 51-66%, respectively. Regression polynomials were calculated for the individual dependent variables and the 3D surface graph were obtained for each individual dependent variable. Mathematicals models were generated for each individual dependent variable or response (R). X1, X2, and X3 are the main effects which represent the average result of the changing one factor at a time from its low to high value. The values of the coefficients X1, X2 and X3 are related to effect these variable on the responses. A positive sign of coefficient indicate an antagonistic effect upon the response. Using the ANOVA provision available in the software, the polynomial equations involving the main effects and interaction factors were based on determined based on estimination of various statistical parameters. The software constructed formula of a Quadratic model was given in Eq. (1).

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + B_{33}C^2 \quad (1)$$

Where the responses were represented by Y, the intercept was prepresented by b0, the regression coefficients were represented by b1 to b33, and the independent variables were represented by A, B, and C.

2.6 Physicochemical Characterization

A. Appearance

Olopatadine HCl nanoemulsion was visually observed for cracking, creaming and other physical instability.

B. Measurement of %Transmittance

The % T of nanoemulsion was mainly use to determine clarity and transparency of formulation.

C. Globule size and Polydispersivity index

The globule size and polydispersity index (PDI) of the optimized nanoemulsion was investigated by photon cross correlation spectroscopy (PCCS). The analysis was performed at a scattering angle of 90º and temperature of 25ºC. The PDI was calculated using the following formula.

$$PDI = \frac{X90 - X10}{X50} \quad \text{.....eq 2.5}$$

Where, X_{50} is mean globule size, X_{90} and X_{10} are the size of nanoglobules below 10% and 90% respectively of the sample population.

D. Zeta Potential

Zeta potential of the Olopatadine HCl nanoemulsion was determined using Zeta meter (Delsa Nano C, USA). Olopatadine HCl nanoemulsion was added to sample cuvette and placed into sample holder unit and measurement was carried out using software. Before each experiment the cuvettes were thoroughly washed and rinsed with the sample formulation.

E. FTIR

The FTIR spectral measurements were carried out at ambient temperature on FTIR spectrophotometer. The sample of nanoemulsion, physical mixture of Olopatadine HCl and formulation was subjected to FTIR studies.

F. Refractive index

The refractive index of the optimized Olopatadine HCl nanoemulsion (1.326 +0.12) was evaluated by using Abbe's refractometer.

G. Viscosity study

Viscosity was evaluated by using small volume adapter of Brookfield Viscometer. (Oswal s Scintee PES/MCOP) Viscosity measured at pH 4 (non physiological pH) and pH raised to 7.4 (physiological pH) with angular viscosity increased gradually from 5 to 100 rpm (Shrividya, Cardoza, & Amin, 2001)

H. pH

The developed formulations were evaluated for pH by preparing 1% aqueous solution of prepared gel using calibrated Equip- Tronics digital pH meter model EQ- 610.The pH of the formulations was found in the range of 6-7 indicating safe in chronic treatment of eye infection (Abou*et al.* 2014).

I. ***Determination of Isotonicity***

Tonicity refers to the osmotic pressure exerted by salts in aqueous solutions. Eye can tolerate solutions equivalent to the range of 0.5% to 1.8% sodium chloride. Isotonicity of optimized formulation (F16) was determined by using digital osmometer (Osmomat 030/050 Terminal).

J. Determination of Drug content

The Olopatadine HCl content (%w/v) of optimized formulation was analysed by validated UV method. The assay value is well within acceptable limit (95-105%) of labeled claim.

K. Scanning electron microscopy (SEM)

SEM gives a three-dimensional image of the globules. The samples are examined at suitable accelerating voltage, usually 20 kV, at different magnifications. A good analysis of surface morphology of disperse phase in the formulation is obtained through SEM. Image analysis software, (e.g., Leica Imaging systems, Cambridge, UK), may be employed to obtain an automatic analysis result of the shape and sur- face morphology (Barea M. J. *et al.*, 2010).

L. X-ray powder diffraction

X-Ray powder diffraction patterns were obtained at room temperature using a diffractometer (D8 Advance, Bruker, Germany), with Cu Anode and Dermic X-ray tube, operated at a current of 40 mA and voltage of 40 kV. The Lynux Eye Detector, Ni beta Filter and zero background and PMMA sample holder were used. The samples of Olopatadine HCl was analyzed in the 2θ angle range of 5°- 60°and the process parameters used were set as scan step size of 0.1002 (2θ) & scan step time of 125 s. X-ray powder diffraction is a rapid analytical technique primarily used for phase

identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

M. Sterility testing

Sterility test was carried out with the help of direct inoculation method **(I.P., 2018)**. The optimized formulation (F16) was evaluated for the sterility study by using fluid thioglycolate medium (FTG) and soyabein casein digest medium (SCDM).

N. Culture Media

Fluid thiglycolate medium was used as culture media for bacteria while soyabein casein digest media used for fungi. Media were prepared and sterilized in test tubes by autoclaving at 121°C at 15 lb/inch guage pressure for 20 minutes.

O. Test Sample Preparation

2 ml of the optimized formulation Batch (F16) were taken for the sterility test and inoculated into fluid thioglycolate medium and 2 ml into soyabein casein digest medium.

P. Positive and Negative Control

Pseudomonas aeruginosa microbial suspension in media act as positive control.Sterile media without test sample act as negative control. (Gupta *et al*., 2010)

Q. Incubation

The inoculated culture media for bacteria and fungi were incubated at 30° C-35°C and 20° C-25°C respectively in BOD incubator for 14 days.

R. Preservative Efficacy Studies

Preservative efficacy study of optimized formulation (F16) was performed by challenging formulation with *Pseudomonas aeruginosa* and *Staphylococcus aureus*(I.P., 2018). The serial dilution method was used to adjust the colony count to about 1×10^5 to 1×10^6 with sterile saline solution, 0.1 ml microbial suspension mix with 20 ml formulation. Inoculated containers were incubated at 20°C to 25°C. The viable count was determined by plate count method at 7, 14, 21 and 28 day.

S. In vitro drug diffusion study (Lu et al., 2015)

In vitro Olopatadine HCl diffusion studies for, Olopatadine HCl nanoemulsionwere performed by the dialysis bag diffusion technique (Lu *et al*., 2015). All samples equivalent to 1 mg (1 ml liquid nanoemulsion) were placed in different dialysis bags (Dialysis membrane 110, HIMEDIA; MWCO 12,000-14,000 Daltons). The dialysis bags were securely tied with a thermo-resistant thread and placed in glass beaker containing 100 ml of water and 0.2 M phosphate buffer pH 7.4. The entire system was maintained at 37 ± 0.5°C with continuous magnetic stirring at 100 rpm. Samples were withdrawn from the receptor compartment at predetermined time intervals (60, 120, 180, 240, 300, 360, 420 and 480 mins) and replaced by fresh medium. The amount of Olopatadine HCl diffused across the membrane was determined by UV spectrophotometric analysis at 300 nm. The cumulative percentage of drug diffused verses time was estimated graphically.

T. Ex vivo corneal drug permeation study (Prajapati et al. 2013)

Corneal permeability study was performed using goat cornea obtained from sacrificed goat. The cornea was thoroughly washed and each having length of 5-5.5 mm.Cornea was filled with 1 ml of the Olopatadine HCl nanoemulsion . The tissue was placed in an organ bath with continuous aeration at 37°C. Around 35 ml of phosphate buffer pH 7.4 was filled in the receptor compartment (organ tube). At specified time intervals (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 mins), samples were withdrawn from the receptor compartment. Fresh buffer was used to replenish the receptor compartment. The samples were analyzed spectrophotometrically at 300 nm for the content of Olopatadine HCl. Permeability coefficient (P) was calculated as slope x 1/ $A.C_D$ where Concentration of donor solution (C_D = 4 mg/ ml), Surface area of goat cornea [$A = 2 (\pi r^2) + (2\pi r)$ x h= 10.85 cm^2], radius (r) and height (h) of corneal segment was used for experiment. Flux (J) = slope/A (Lalla*et al*., 2002).

U. Accelerated stability study of nanoemulsion

Stability studies for the optimized batch F16 was carried out to determine the effect of presence of formulation additives on the stability of the drug and also to determine the physical stability of the

formulation under accelerated storage conditions.The optimized batch F16 was subjected to elevated temperature and humidity conditions of 25±1°C/ 60%RH, 30±1°C/ 65%RH and 40 ± 2°C/ 75 ± 5 % RH. Samples were withdrawn at the end of 0, 30, 60 and 90 days and evaluated for physical appearance, drug content, zeta potential and PDI.

3. Results and Discussion

3.1 Physicochemical Characterization of Nanoemulsion

Olopatadine HCl nanoemulsion was found to be stable. There is no cracking, creaming and other physical instability appear. Olopatadine HCl is BCS class I drug having, molecular weight (373.877 g/mol). By using (Design Expert 11) software composition of optimized formulation was determined, where optimised formulation was composed of Olive oil (Oil), Tween 80 (Surfactant), PEG400 (Co-surfactant) and distilled water (Aqueous phase).

3.2 Particle Size and PDI

The PDI of the formulation have effect on the uniformity and stability of the formulation. Lesser PDI, more uniformity of the formulation is considered. The PDI of nanoemulsion formulation was obtained within range (0.3) that shows significant uniform distribution of particles. The nanoemulsion system demonstrated good transparency with particle size in the nanometer range. Particle size is one of the most important factor for intranasal delivery of drug to the brain, since particle with smaller size (>100) will have large surface area which increase rate of drug absorption. The particle size of nanoemulsion was found to be 292.1nm.

3.3 Analysis of Zeta Potential

Zeta potential measurement is an important surface characterization technique which provides information regarding surface charge of nanopaticulate systems. The magnitude of zeta potential gives an indication of the potential stability of colloidal system. It is generally believed that a higher value of zeta potential thereby maintains homogeneity of droplet size. However, some scientific report also suggest that lower value of zeta potential has been observed to agument uptake of nano droplet as well as exerts its stability and efficiency. This justification is important since it has observed negative value of zeta potential in formulation. This might be influenced by used non-ionic and zwitterions surfactants. Zeta potential of optimized nanoemulsion formulation was found to be -16.23.

3.4 Refractive index

The refractive index of the optimized Olopatadine HCl nanoemulsion (1.326 +0.12) was found to be similar to placebo and closer to that of olive (1.329+0.15) although these optical values were found to be directly proportional to the oil content of the formulation. This proved isotropic nature of prepared formulation.

3.5 Measurement of Viscosity

Rheological properties of optimized nanoemulsionis of much scientific importance since it is well known that viscosity increase with increase in concentration of oil owing to interfacial tension with water. It is observed that aoptimized formulation exhibited a minimum viscosity of 7.74+0.17 cps. Such a low viscosity value of prepared formulation is certainly advantageous from patient compliance and manufacturing point of view.

3.6 pH

The normal physiological pH of goat cornea is 7.2-7.4; however the cornea can tolerate the solution within pH range of 6-7; more deviation from this pH range may persist the irritation to cornea. pH was well within the physiological pH range and significant indicating the prepared formulation will not cause any irritation.

3.7 Isotonicity study

Isotonicity is important characteristics of ophthalmic formulation to avoid any tissue damage and irritation to the eye. Optimised batch was subjected for tonicity evaluation. Tonicity was found to be 295±1.28 mOsmol Kg^{-1} which is within the acceptable limit (290-310 mOsmol/kg).

3.8 Determination of Drug content

The Olopatadine HCl content (%w/v) of optimized formulation was analysed by validated UV method. The drug content was found to be in range 99.89+0.25. The assay value is well within acceptable limit (95-105%) of labeled claim.

3.9 Liquid Scanning electron microscopy

The surface morphology of nanosizeddroplets was determined by liquid SEM study. The Results of SEM images for optimized nanoemulsion formulation were shown Spherical shaped borders are observed. The SEM analysis was observed nanometricnanoemulsion particle with microscopic investigation.

3.10 X-Ray powder diffraction (XRPD) study

XRPD analysis can determine the solid-form structure and crystal-packing relationship among individual molecules (Ganapuram*et al.,* 2013). The XRPD of nanoemulsion is shown in fig 3.16. The major sharp diffractions were seen at 21.93º, 22.33º, 26.34º and 27.24º corresponding to 328, 318, 226 and 237 peak intensities. Numerous diffractions of Olopatadine HCl observed at 2θ of fingerprint region confirm the presence of crystalline Olopatadine HCl.

3.11 Sterility Testing as per I.P 2018

It is essential that ophthalmic formulation must be sterilised. Optimised formulation was subjected for sterility testing. There was no turbidity observed after 14 days of incubation at specified condition. However considerable turbidity were observed in all media incubated as positive control.

3.12 Preservative Efficacy study as per I.P 2018

To check effectiveness of added antimicrobial preservative, preservative efficacy study were conducted. Observations are shown in Table 3.8 No microbial growth was observed at the end of 28 days of inoculation Fig. 3.18. Results clearly indicate that added concentration of benzalkonium chloride is significant to inhibit microbial growth till 28 days after opening of container.

3.13 In vitro drug Release

The drug release behaviour was studied in simulated tear Fluid solution. Incorporation of drug in nanoemulsion based system improved drug solubilization and *in vitro* release, as compared to drug suspension.

3.14 Ex vivo permeation study

Drug candidate must possess adequate permeability to deliver successfully through ophthalmic route. Physicochemical properties of drug plays key role in permeability. Goat cornea was used for *ex vivo* transcorneal permeability study of Olopatadine HCl nanoemulsion. Cumulative percent drug permeated for Olopatadine HCl in nanoemulsion (Batch F16) was calculated at 37°C ± 2°C.*Ex vivo* transcorneal cumulative % drug release. Cumulative % drug release through goat cornea was found to be 99.66 ± 1.84% (Table 3.14), which is less as compared to *in vitro* drug release profile. This may be happened due to biological membrane contained epithelium, stroma and endothelium having hydrophilic- lipophilic in nature, which act as barrier for permeation of drug molecule and dialysis membrane acts as mechanical barrieras explained by Sawant et al. (2016).

3.15 Accelerated Stability study

Optimized formulation was subjected for stability testing. It was found that formulation remained stable at various conditions of temperature and relative humidity used as per ICH guidelines.

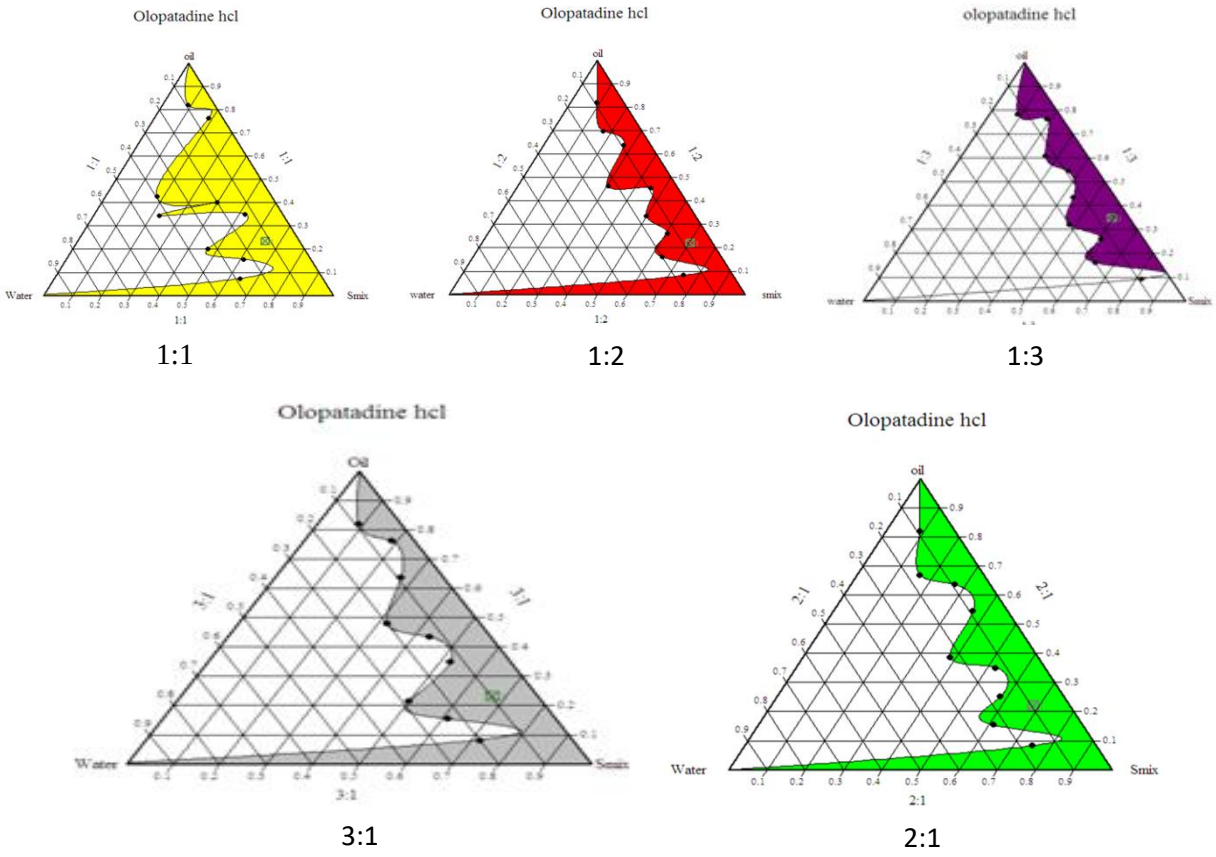
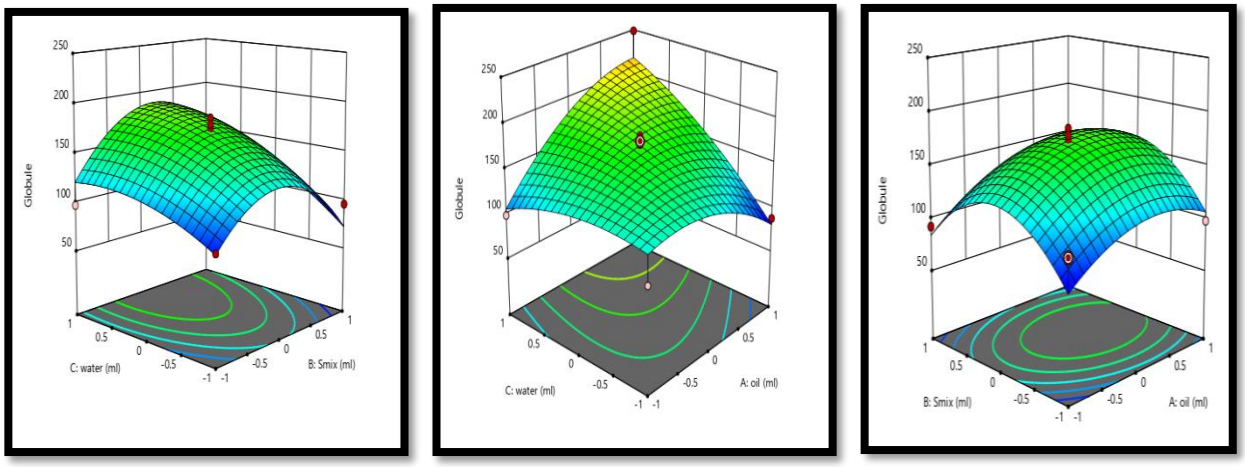
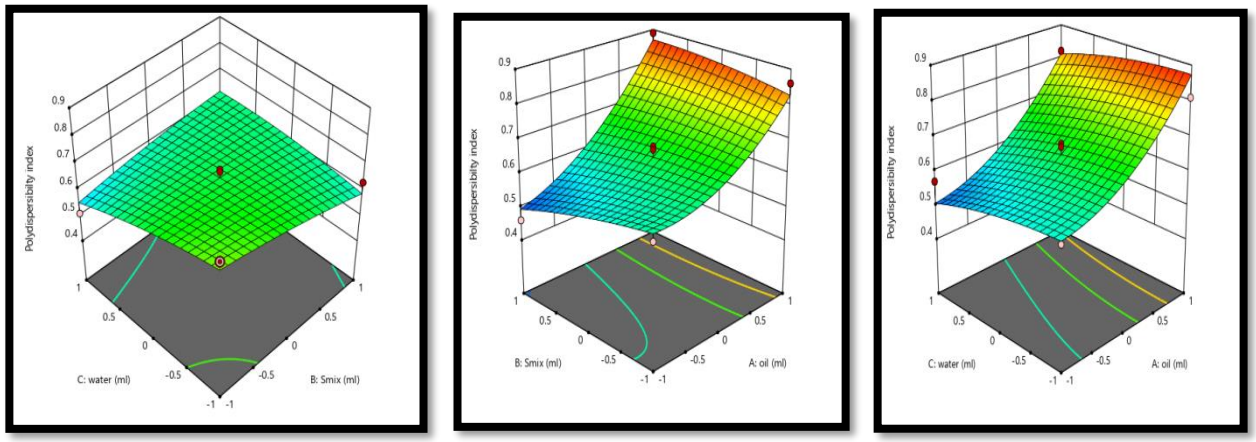


Figure: 1 Pseudoternary phase diagrams of Tween80: PEG400 (Smix) 1:1, 1:2, 1:3, 3:1, 2:1.



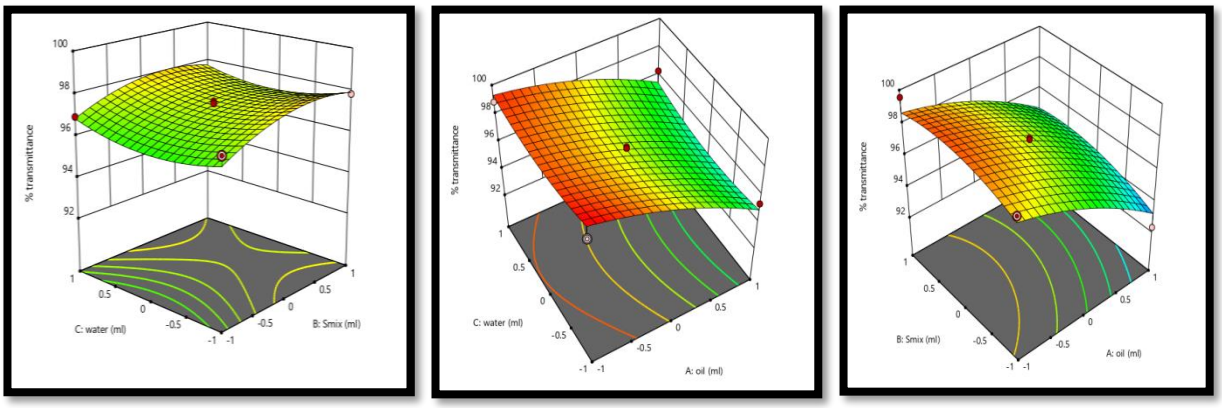
(a) **(b)** **(c)**

Figure: 2(I) Response Surface plot three- dimensional showing effect of **a)** Oil(A) and Smix(B), **b)** Oil(A) and Water(C), **c)** Smix (B) and Water (C) on Globule Size (Y_1).



(a) **(b)** **(c)**

Figure:2(II) Response Surface plot three- dimensional showing effect of **a)** Oil(A) and Smix(B), **b)** Oil(A) and Water(C), **c)** Smix (B) and Water (C) on PDI (Y_3).



(a) **(b)** **(c)**

Figure: 2(III) Response Surface plot three- dimensional showing effect of **a)** Oil(A) and Smix(B), **b)** Oil(A) and Water(C), **c)** Smix (B) and Water (C) on % Transmittance (Y_3).

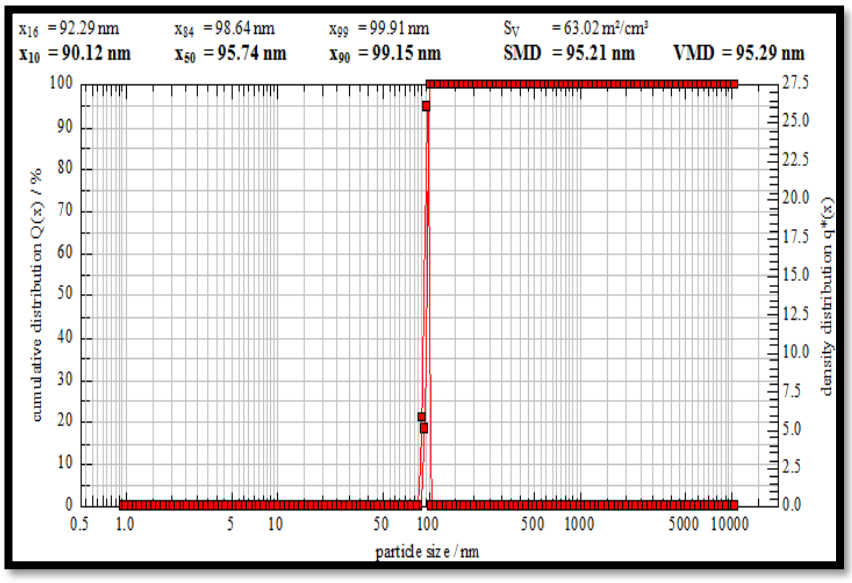


Figure: 3 Particle size of Olopatadine HCl Nanoemulsion.

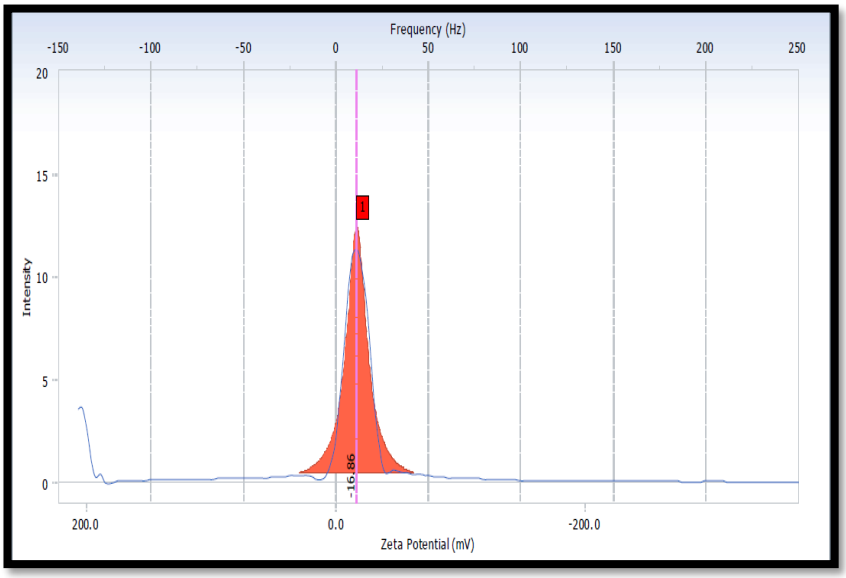


Figure: 4 Zeta Potential of Olopatadine HCl Nanoemulsion.

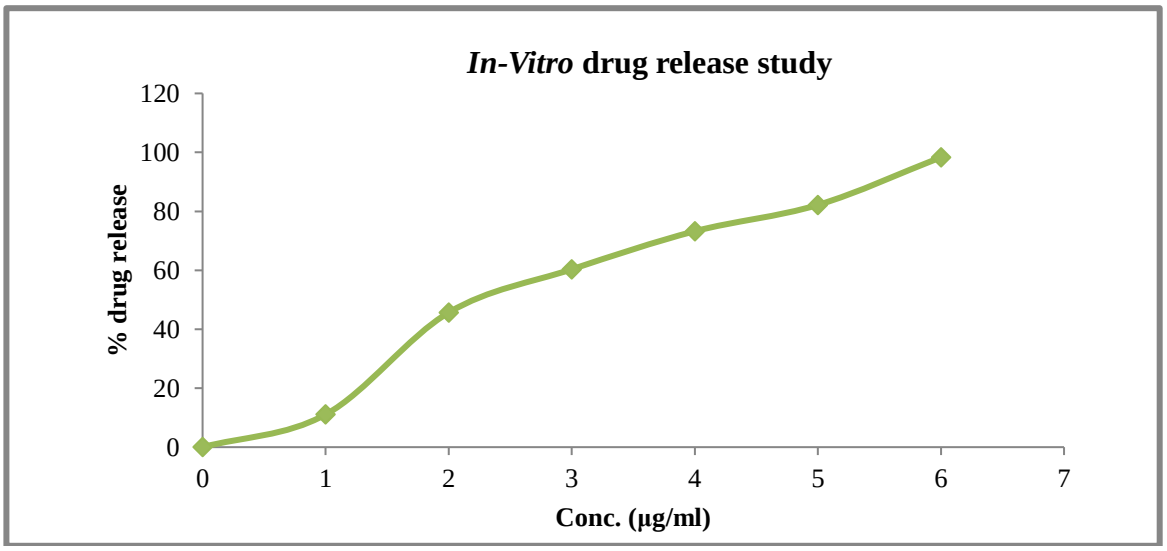


Figure: 5 In-vitro drug release study of nanoemulsion.

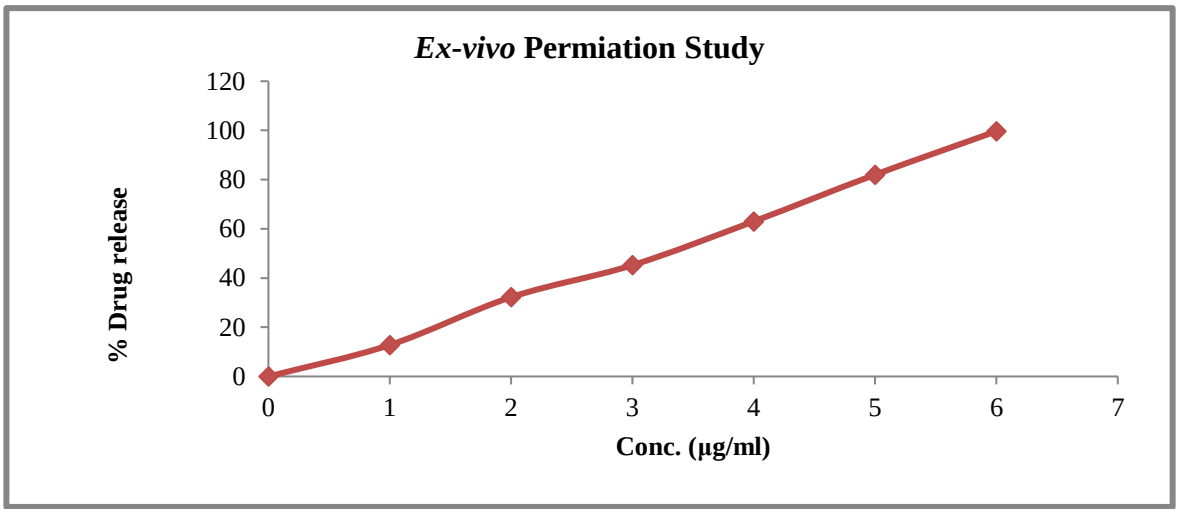


Figure: 6 Ex vivo percent drug release study of nanoemulsion.

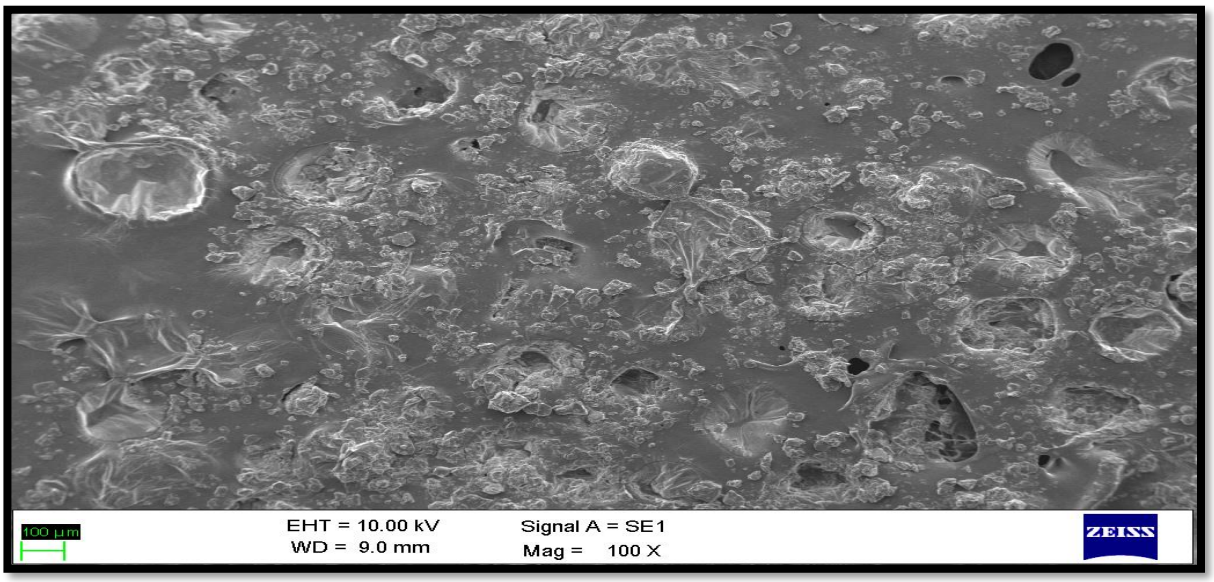


Figure: 7 SEM analysis of nanoemulsion.

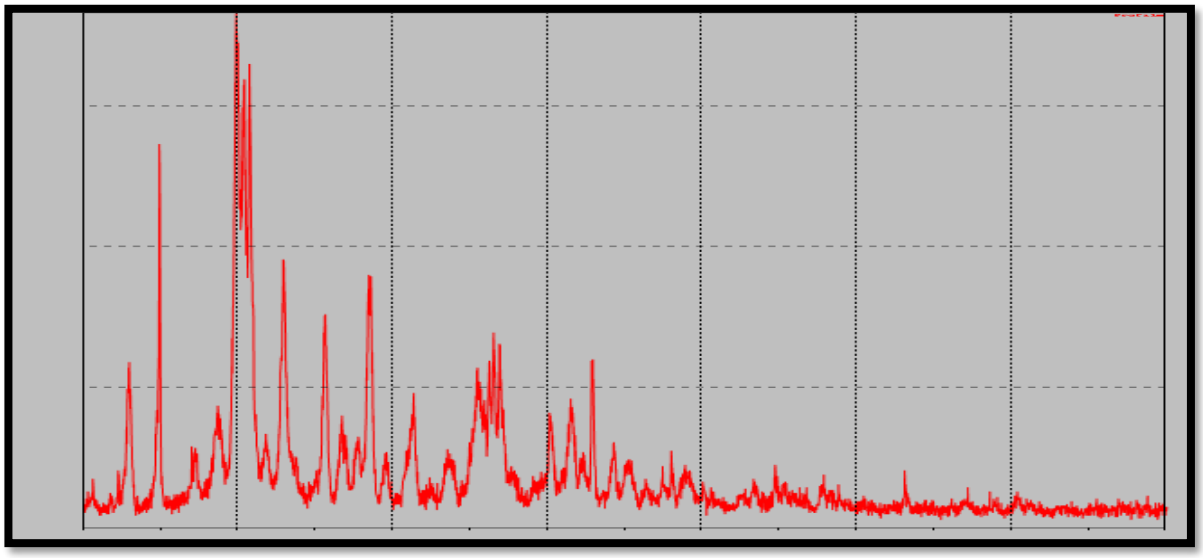


Figure: 8 XRPD of nanoemulsion.

Table: I Optimized Composition of Nanoemulsion.

Ingredient's	Quantity
Olopatadine HCl	5mg
Olive oil	0.4ml
Tween 80	1.5ml
PEG 400	0.5ml
Benzalkonium chloride	0.01% v/v
HPMC K4M	0.05% w/v
Distilled water	q.s.

Table: II The Experimental Design layout developed for 17 Batches Composition of Olopatadine HCl Nanoemulsion.

Batch	Oil	Smix	Water	Particle size(nm)	Polydispersity index	% Transmittance
1	0	-1	-1	203.12	0.76	97.08
2	0	0	0	49.77	0.32	97.47
3	0	0	0	157.38	0.61	97.58
4	-1	0	-1	98.87	0.58	98.95
5	0	-1	1	149.25	0.51	96.92
6	0	1	-1	134.48	0.63	97.83
7	1	-1	0	230.12	0.86	92.31
8	-1	0	1	95.47	0.57	98.86
9	0	1	1	149.37	0.59	97.29
10	-1	1	0	292.1	0.46	99.57
11	1	1	0	387.44	0.89	94.35
12	0	0	0	176.31	0.68	96.97
13	1	0	-1	223.98	0.81	95.43
14	1	0	1	248.59	0.82	96.34
15	0	0	0	184.66	0.67	97.47
16	0	0	0	178.24	0.64	97.47
17	-1	-1	0	110.12	0.59	98.31

Table: III Data analysis for accelerated stability studies of nanoemulsion formulation

Time (month)	Temperature/ % RH	Parameter			
		Appearance	% Drug content	Ph	Globule size(nm)
1	30±1°C/ 65 ± 5	No change	99.6 ± 0.05	7.3	167.56
	40 ±2ºC/ 75 ± 5	No change	99.3 ± 0.51	7.3	173.10
2	30±1°C/ 65 ± 5	No change	98.9 ± 0.52	7.2	209.51
	40 ±2ºC/ 75 ± 5	No change	99.2 ± 0.47	7.4	178.56
3	30±1°C/ 65 ± 5	No change	99.6 ± 0.36	7.3	251.88
	40 ±2ºC/ 75 ± 5	No change	99.2 ± 0.46	7.4	204.90

4. Conclusion

Formulation of nanoemulsion is promising approach for enhancement in therapeutic efficacy. The preformulation studies confirmed that the procured Olopatadine HCL was pure. *In vitro and Ex vivo* permeation studies of Olopatadine HCL across isolated goat cornea showed that there is 3.3 folds increase in flux of nanoemulsion. This shows improved permeability of nanoemulsion across the corneal membrane. Stability studies showed that the optimized Olopatadine HCL nanoemulsion was stable at room temperature (25ºC) and refrigeration temperature (4°C) for 3 months. Thus it can be concluded that ocular administration of Olopatadine HCL nanoemulsion can be a promising approach for enhancing the permeability and improving the therapeutic efficacy of Olopatadine HCL.

5. References

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