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Rationalized Approach for Formulation and Optimization of Ebastine Microemulsion Using Design Expert for Solubility Enhancement

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ABSTRACT

Ebastine is available as an oral antihistamine formula for allergic disorders such as tablets and syrup. Oral ebastine causes unfavorable effects on heart like QT prolongation, severe gastric distress, decreased tear production, resulting in dryness of the ocular surface, which exacerbates ocular discomfort and increasing susceptibility of eye to irritation. To avoid systemic side effects and ocular discomfort, topical ocular therapy could prove to be superior to systemic therapy in treating ocular allergies. Hence, topical formulation was developed to achieve onsite exposure of ebastine for ocular allergies. Moreover, conjunctiva is more accessible to hydrophilic molecules than lipophilic molecules. This creates challenge for a lipophilic molecule such as ebastine for topical ocular development. Successful dissolution of ebastine in o/w microemulsion allows its use in more convenient soluble form. Initially, solubility of drug in various oils, surfactant and cosurfactant was determined, followed by pseudo-ternary phase diagram to find microemulsion area. The D-optimal mixture design was employed for optimization of formulation. The optimized microemulsion formulation was characterized for its transparency, drug content, droplet size, zeta potential, viscosity, isotonicity, osmolarity and surface tension etc. The optimum physicochemical properties were observed to be eye-fitting. Carboxy methyl cellulose and sodium hyaluronate were used as gelling agents at different concentrations to increase residential time at the site of action. In vitro drug release study revealed that ebastine release from microemulsion gel in a sustained manner up to 24 hrs. for the purpose of providing prolonged therapy for ocular allergy. Hence, prepared microemulsion had great potential as an alternative to customary oral formulations of poorly soluble drug.

Keywords: Ebastine, Microemulsion, D-optimal mixture design, Solubility

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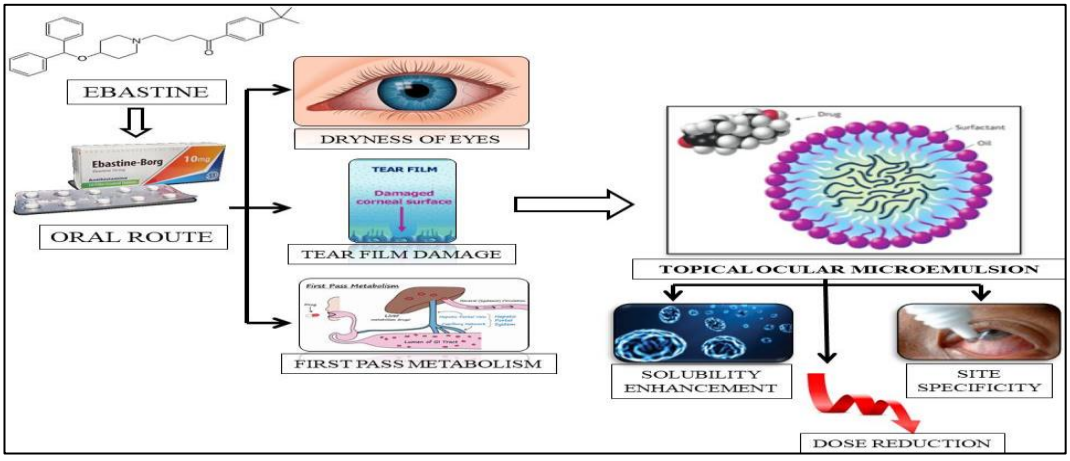
Graphical Abstract



INTRODUCTION

Ocular drug delivery generally involves the delivery of therapeutically active agents into anterior and posterior segments of the eye. Conjunctivitis is a prevalent disease all over the world especially higher rate of infection was found in developing countries. Conjunctivitis may be bacterial, viral or chlamydial, allergic. Allergic conjunctivitis is caused by an allergen-induced inflammatory response in which allergens interact with IgE bound to sensitized mast cells resulting in the clinical ocular allergic expression[1]. A major problem in ocular therapeutics is the attainment of an optimal drug concentration at the site of action. Poor bioavailability of drugs from ocular dosage forms is mainly due to the precorneal loss factors which include tear dynamics, non-productive absorption, transient residence time in the cul-de-sac and relative impermeability of the corneal epithelial membrane[2]. Additionally, most drugs with ocular therapeutic potential have the problem of poor solubility and hence less bioavailability. To overcome it, various technological strategies are reported in the literature including micronization, nanosuspension, polymeric micelles and cyclodextrin based formulation[3].

Among various approaches, microemulsion are promising alternative to enhance the ocular bioavailability of drugs by improved ocular retention, increased corneal drug absorption and reduced systemic side effects and maintain the simplicity and convenience of the dosage form as eye drops[4]. Microemulsions are thermodynamically stable, surfactant-cosurfactant based system, form at low interfacial tension and exhibit high solubilizing potential for hydrophobic drugs. They are good alternative for ophthalmic delivery as it offers the pseudo plastic rheology with increased viscosity after application and increased ocular retention and possibility of releasing drug in sustained and controlled way, increased shelf life, lastly reducing dose and dosing frequency[5].

Ebastine is official in British pharmacopoeia. It is a second-generation H1 receptor antagonist, chemically 1-[4-(1,1-dimethyl ethyl) phenyl]-4-[4-(diphenyl methoxy) piperidin-1-yl] butan-1-one indicated for various allergic manifestations of skin, nasal and ocular site by oral route[6,7,8]. Oral administration of antihistamine leads to dryness of eye which affects physiology of tear film[9]. A successful attempt is made to prepared low dose and low concentration of the surfactant based Ophthalmic ebastine microemulsion formulations employing the concept of design of experiment with goal of solubility enhancement thereby boosting bioavailability due to site specificity as well reduces systemic side effects and hence will enhance the patient compliance.

MATERIALS AND METHODS

Ebastine was procured as a gift sample from, Bal Pharma Pvt. Ltd, Bommasandra, Bangalore, India. Campul MCM EP, Labrasol. Labrafac, Cremophor EL, Lauroglycol FCC was generously supplied by Gattefosse, Saint-Priest, France. Oleic acid, Ethyl oleate, Isopropyl palmitate, Arachis oil, Linseed oil, light liquid paraffin was purchased from Yarrow chemicals Pvt. Ltd Mumbai, India. Propylene glycol, Polyethyleneglycol 400 (PEG-200), Sorbian monooleate (Span-80), Polyoxyethylenesorbitan monooleate (Tween-80), Polyoxyethylenesorbitan monolaurate (Tween-20), Isopropyl alcohol, butanol was purchased form S.D. Fine chemicals, Ahmadabad, India. All other chemicals used in the study were of highest analytical purity grade. The double distilled water is used throughout the study.

Screening of Microemulsion Components

The solubility of ebastine was determined in various oils, surfactants and cosurfactants. Drug powder was added in excess to each of the oils, surfactants and cosurfactants, thereafter subjected to vortexing. After vortexing, the samples were kept for 24 h at ambient temperature for attaining equilibrium. The equilibrated samples were then centrifuged at 3000 rpm for 20 min to remove the undissolved drug[10]. The aliquots of supernatant were filtered through 0.45 µm membrane filters and the solubility of Ebastine was determined by analyzing the filtrate spectrophotometrically (Shimadzu 1800, Japan) after dilution with methanol at 252 nm. Appropriately diluted solutions of oils in methanol were taken as blank.

Construction of Pseudo-ternary phase Diagrams

In order to find out the concentration range of components for the existing range of microemulsion, pseudo ternary phase diagrams were constructed using aqua- titration method at ambient temperature (25°C). Pseudo-ternary phase diagrams were constructed by Prosim software [11]. Campul MCM EP selected as the oil phase. The blend of Labrasol with Tween 80 and blend of Propylene glycol with glycerol were selected as surfactant and co surfactant, respectively. Double distilled water was used as an aqueous phase. Various phase diagrams were prepared with weight ratios of surfactant to cosurfactant individual and blend. For each phase diagram at a specific surfactant/cosurfactant weight ratio, the ratios of oil to the mixture of surfactant and cosurfactant were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. The mixtures of oil, surfactant and cosurfactant at certain weight ratios were diluted with water drop wise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsion, crude emulsions or gels. No experimental attempt was made to distinguish between oil-in-water, water in-oil or bicontinuous type microemulsion. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after tilted to an angle of 90°.

Three phase diagrams were obtained for three different S_{mix} individual ratios 1:1, 2:1, 3:1.The comparatively maximum microemulsion area was obtained in 2:1 S_{mix} ratio. The selected S_{mix} ratio was further studied by S_{mix} blend, 2(1:1):1, 2(1:1): 1(1:1). The S_{mix} blend ratio which produced broader microemulsion region was selected for formulation optimization. This attempt was made to keep the surfactant concentration as low as possible in the ophthalmic formulation to avoid any associated toxicity.

Optimization of Microemulsion by D-Optimal Mixture Design

D-optimal mixture design (Design-Expert 7.0.0 (Stat-Ease Inc., Minneapolis, USA) was selected because the generalized variance of the estimates of the coefficients is minimized. The software selected a set of candidate points as a base design included factorial points (high and low level from the constraints on each factor, centers of edges, constraint plane centroids, axial checkpoint, and an overall center point).

It is commonly used to reveal main effects and interaction effects between the independent variables of the experiment[12]. Moreover, the numbers of trials required are less. Twelve runs were carried out to optimize microemulsion formulation. Different design constraints, i.e. A (amount of oil), B (amount of Smix), and C (amount of water) were taken at high and low levels. The sum of A, B, and C were kept fixed at 100%. The effect of these

formulation variables was studied on the % Transmittance, globule size and viscosity. Validity of experimental design was confirmed by plotting a standard error of design graph. The probability value (α) for determination of statistical significance was set at 0.05, which indicated that a "hypothesis" theory would be rejected if their corresponding p-values were ≤ 0.05[13]. Models were selected on the basis of sequential comparison and lack of fit test. Significance of the models was further confirmed by statistical analysis. Response surface, contour plot, residual plot and overlay plots were constructed for the response variables.

Preparation of Drug Loaded Microemulsion

The D-optimal design suggested different combinations of oil, S_{mix}, and water. The suggested quantity of oil and S_{mix} was mixed using a magnetic stirrer to produce the oily phase, at this stage the Ebastine was dissolved in the oily phase. Finally aqueous phase was added drop wise to obtain drug loaded microemulsion formulation.

Preparation of Drug Loaded Microemulsion Gel

The optimized microemulsion has very low viscosity, which may restrict its topical application. To overcome this, gelling agents were incorporated into formulation. The ocular delivery improved by adding mucoadhesive polymer in formulation. The weight ratio of CMC (1%) and SH (1.5%) was found satisfactory based on proper gel formation. The former polymer used in commercial ocular formulations, as it has desirable mucoadhesive and a high retention time on the ocular surface and the latter one exhibit excellent viscoelastic, lubricating and water retention properties. The literature reveled that, this combination benefited with high viscosity under low friction conditions (between blinking) which stabilizes the tear film and low viscosity under high friction conditions (during the blinking) which reduces discomfort in animal as well as humans[14].

A specified amount of drug consisting of the chosen oil and Smix was magnetically stirred until the drug completely dissolved; microemulsion was prepared by adding aqueous phase. 1% CMC and 1.5% SH polymers dispersion was formed by suspending in water. The polymer dispersion kept for overnight to form viscous gel matrix. Prepared microemulsion and polymer dispersion was mixed in 1:1 v/w ratio [15]. Smooth viscous, transparent gel was formed.

Characterization and Evaluation of Microemulsion

Drug Excipient Compatibility

Fourier-transform Infrared Spectroscopy (FTIR) Study

IR spectroscopy was conducted using an FTIR spectrophotometer and the spectrum was recorded in the wavelength region of 4000– 400 cm−1[16, 17, 18]. The procedure consisted of dispersing the samples in KBr, thus avoiding solid transition possibly inducing by extended grinding. The spectrums were scan at a resolution of 0.15 cm−1 and scan speed 20 scan/sec. The Infra-Red spectra's of pure Ebastine and optimized formulation were obtained on Fourier Transform Infrared Spectrophotometer in order to detect the existence of a possible interaction between drug and excipients.

Measurement of pH

For optimized formulation, pH was measured using pH meter which was previously calibrated using standard buffers of pH 4 and pH 7 as per the established procedure[16, 17, 18].

Measurement of Refractive Index

After administration of eye drops, possible impairments of vision or discomfort to the patient is detected by refractive index measurements. Refractive index proved the transparency of formulation. The refractive index of the system was measured by Abbe Refractometer (RICO, Model RSR-1) by placing one drop of the formulation on the slide in triplicate and compared it with water[16, 17, 18].

Measurement of Osmolarity

Evaluation of osmolarity using an osmometer is of vital importance for physiological acceptance of the formulation by ocular tissues[16, 17, 18]. Osmolarity of optimized formulation measured using Osmometer (Advanced Instruments Inc., USA; Model 3250).

Measurement of Surface Tension

Surface tension determination ensures the uniform spreading of the formulation on the corneal surface[16, 17, 18]. Tensiometer (Kruss Tensiometer; Dimensions: 19.900 mm*0.200mm*10.00mm, Model K12PSS) was employed for the determination of surface tension.

Droplet size, Zeta potential and Viscosity measurement

The droplet size of the microemulsion was determined by photon correlation spectroscopy (which analyzes the fluctuations in light scattering due to the Brownian motion of the particles) using a Malvern zeta sizer (Nano ZS, Malvern instruments, UK), Zeta sizer able to measure sizes between 10 and 5000 nm. The measurements were performed at 25°C at a 90° angle [19]. Each size value reported was the average of at least three independent measurements. Samples were suitably diluted with double distilled filtered water to avoid multi-scattering phenomena and then placed in quartz cuvettes. The real and imaginary refractive indexes were set at 1.59 and 0.0, respectively. Zeta Potential was determined by Zeta sizer (Malvern instruments UK) using clear disposable zeta cell and filed strength of 20 V/cm was employed. The electrophoretic mobility was converted into to the zeta potential[19]. The viscosity of microemulsion was determined by Ostwald Type Capillary viscometer at room temperature[20].

Drug Content

0.5 ml optimized microemulsion formulation (1% w/v) containing drug equivalent to 5 mg was extracted with methanol followed by further appropriate dilution with methanol and the drug content was determined using UV spectrophotometer (Shimadzu 1800, Japan) at 252 nm in the formulation.

Transmission Electron Microscopy

To study the microstructures of microemulsion, transmission electron microscopy is the most important technique as it directly produces high-resolution images. It can capture any co-existent structure and microstructural transitions[21]. The morphology of formulation was performed using (Technai-20, Phillips, Holland, Electron source: LaB6, Tungsten Filament) A drop of sample was placed onto a carbon coated grid on a single tilt sample holder to form a thin liquid film. The excess solution was removed followed by negative staining with 1% phophotungstic acid. The sample was examined and simultaneously photographed at an accelerating voltage with point resolution 0.27nm and magnification up to 25x to 7, 50,000x.

In Vitro Drug Release Study

The optimized microemulsion and microemulsion gel formulation was evaluated for drug release. The in vitro drug release study was carried out using the dialysis bag method. (Molecular weight 12–14 kDa) [22 23]. Methanolic Phosphate-buffered saline pH 7.4 was used as release medium. The system was maintained at 32 ± 0.5^{0}C to mimic conditions eye surface temperature with continuous stirring on magnetic stirrer at 150 rpm. The samples were collected periodically until 24hr. Ebastine content in the receptor chamber was determined by spectrophotometricaly. Sink conditions were maintained in the receptor compartment during in vitro release studies. Each sample analysis was performed in triplicate.

Sterility Testing

Sterility test was performed to examine the growth of bacteria or fungus. The optimized microemulsion formulation was sterilized using membrane filtration unit by passing the formulation through 0.22 µm membrane filter under aseptic conditions[24]. The media used to detect aerobic and anaerobic bacteria is fluid thioglycollate media and soyabean casein digest media is used to detect fungal organisms. For positive control aerobic bacteria *Staphylococcus aureus* and fungal organism *Candida albicans* were inoculated into fluid thioglycollate media and soyabean casein digest media respectively. The optimized formulation was incubated in an incubator at 37±1^0 C for a period of 14 days using both the medias. The gelling agent incorporated into microemulsion system in aseptic cabinet in between burners to avoid further possible contamination.

Accelerated Stability Tests by Centrifugation Stress Test

Stress stability study of the microemulsion sample was carried out by subjecting it to centrifugation. The formulation was centrifuged at 9,000 rpm for 20 min by Centrifuge (Make Remi) and examined for phase separation[25].

RESULTS AND DISCUSSION

Preliminary Screening of Microemulsion Components by Solubility Study

Amongst various oils tested, Ebastine showed low solubility in all oils except for Campul MCM EP (28.5 ± 0.2 mg/ ml). Campul MCM EP is a mono-diglyceride of medium chain fatty acids (mainly caprylic and capric). After selection of Campul MCM EP as the oil phase, the goal was to identify the surfactant which shows the highest solubilization capacity for the drug. Ebastine shown maximum solubility in Labrasol (23.1 ± 0.3mg/ml) followed by Tween 80 (19.8 ± 0.1mg/ ml). Therefore, blend of Labrasol and tween 80 was selected as the surfactant for microemulsion formulation. Amongst cosurfactant tested, maximum solubility in propylene glycol (18.2 ± 0.1mg/ml) followed by alcohols. Amongst various S mix blends, propylene glycol, isopropyl alcohol and ethanol forms transparent system with 98.21%T, 99.01%T, and 99.17% T respectively compared to other tested cosurfactants with selected surfactant. Due to ocular compatibility and volatility issue, selection of any alcohol as microemulsion component was prohibited. Therefore, blend of propylene glycol and glycerol was selected as the co-surfactant for microemulsion formulation. Moreover, glycerol will help in maintaining osmolarity of formulation. Figure 1 exhibited comparative account for solubility of drug in various components of microemulsion formulation. Various cosurfactants were screened for solubility as well miscibility with a surfactant. The toxicity of nonionic surfactants is generally lesser than ionic surfactants, besides they have lower critical micelle concentration (CMC) and offer better in vivo stability of o/w microemulsion dosage forms. Therefore the screening of surfactant was done from amongst the nonionic surfactants only.

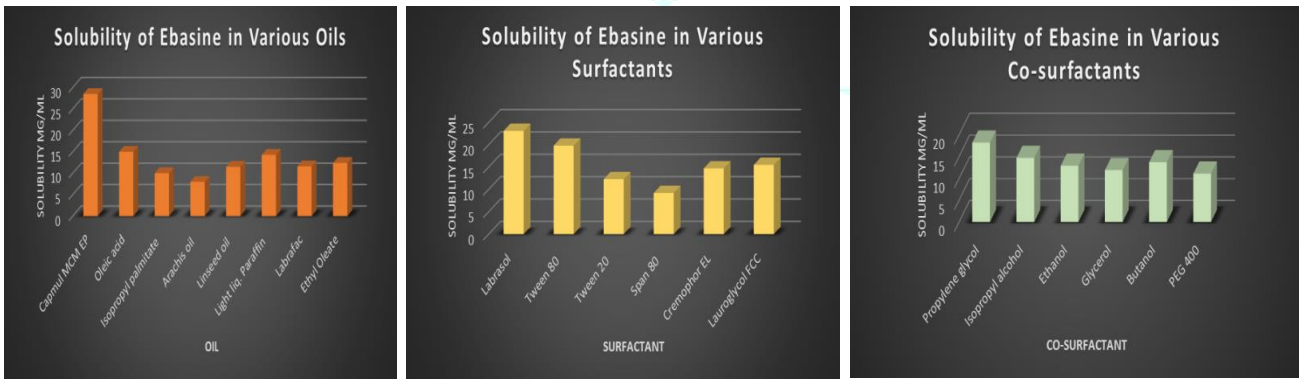


Figure 1: Solubility profile of drug in various Oils, Surfactants and Co surfactants

Construction of Pseudo-ternary Phase Diagram

Initially, based on the results of maximum solubility, various pseudo ternary phase diagrams were constructed employing Campul MCM EP (Oil), Labrasol & Tween 80 (surfactants blend) and propylene glycol & glycerol (co-surfactant blend) for identifying the maximal region for formation of the thermo-dynamically stable microemulsion. Figure 2A and 2B illustrated pseudo ternary phase diagram for Smix individual system and Smix blend system respectively. Among the various combinations of individual and blend of surfactants and cosurfactants (i.e. 1:1, 2:1, 3:1) explored. The maximal region for microemulsion was observed at the ratio of 2:1 Smix blend system as compared to same ratio with Smix individual system. An o/w microemulsion region was found towards the water-rich apex of the phase diagram. As the surfactant concentration was increased in the S mix ratio, a higher microemulsion region was observed. The probable reasons are a reduction of the interfacial tension by surfactant and increased the fluidity of the interface by cosurfactant.

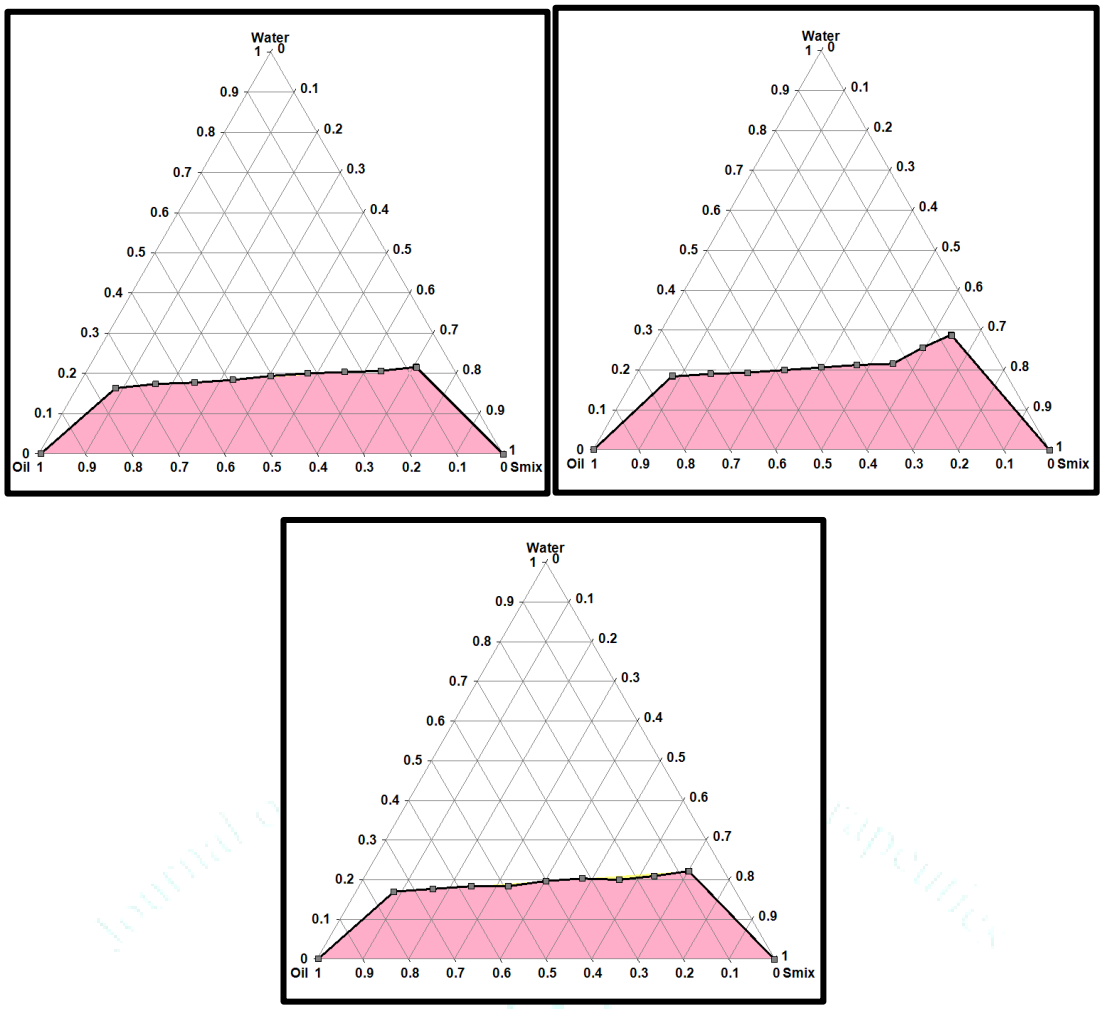


Figure 2 (A): Pseudo Ternary diagrams of Oil: Smix individual system (1:1), (2:1), (3:1)

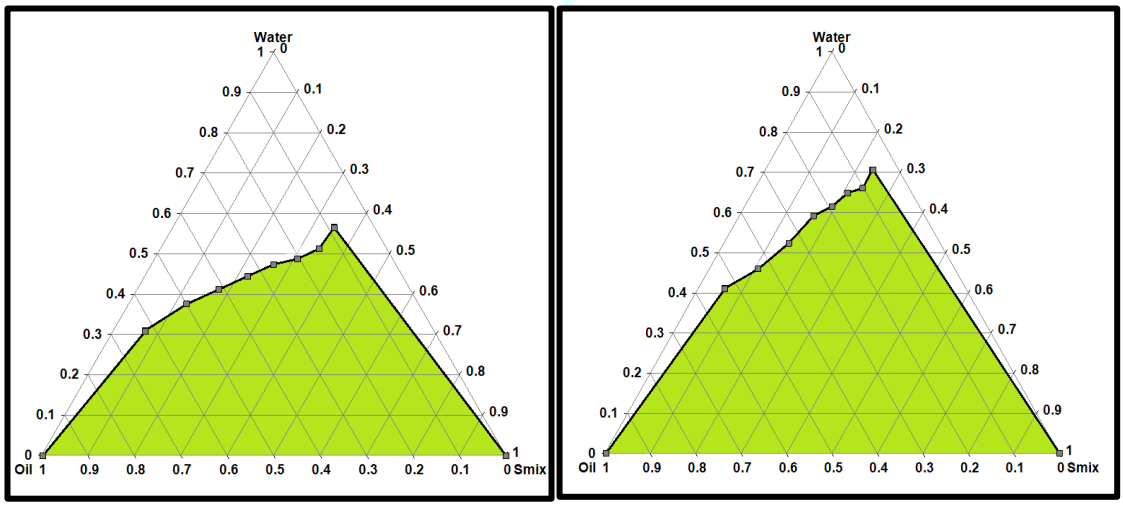


Figure 2 (B): Pseudo Ternary diagrams of Oil: Smix blend system 2(1:1):1, 2(1:1): 1(1:1).

Optimization of Ebastine Microemulsion using D-Optimal Mixture Design

D-optimal mixture experimental design was applied in the present study. Campul MCM EP (X1), S_{mix} (X2), and water (X3) were chosen as formulation variables and Globule size (nm) (Y1), Viscosity (cp) (Y2) and Transmittance (%) (Y3) were selected as response variables. The data obtained from globule size (response Y1), viscosity (response Y2), and transmittance (response Y3) was analyzed using Design Expert® Software. The polynomial equations comprise the coefficients for intercept, main first-order effects, interaction term. The value of the coefficients exhibits the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while negative term indicates an antagonistic effect on the response. The data summarized in Table 1. After generating the polynomial equations through MLRA (Multiple linear regression analysis) relating the

dependent and independent variables, mixture components were optimized for the responses. The values of all the responses were fitted to models viz linear, quadratic, special cubic and cubic model where the best fit model was found to be cubic model for all the responses as compared to other models (Table 2). R^2 values were reported resemble to unity indicating the high predictive ability of Response Surface Methodology (RSM) of underlying study. Further, the higher values (>4) of "Adequate Precision" indicate adequate signal.

Figure 3A, 3B, 4A, 4B and 5A, 5B shows contour, 3D response curve for dependent variables viz globule size (nm), viscosity (cps) and transmittance (%) respectively. It can be observed from the response variables plots of Globule size that as the concentration of oil increases, globule size also increases while the concentration of Smix increase then globule size decreases. It can be observed from the response variables plots of viscosity that as the concentration of Smix increases and decrease in amount of water, viscosity increases. It can be observed from the response variables plots of transmittance that as the concentration of oil increases % transmittance decreases and Smix increases % transmittance increases. Further, linear correlation was found analogous for actual response and predicated response (Figure 3C, 4C, 5C). The reliability of these response surfaces was also confirmed by the corresponding residual plot between the experimental run and the internally studentized residuals for all response variables, as shown in Figure 3D, 4D, 5D. The vertical distribution of the internally studenized residuals was in line from top to bottom under the completely randomized run. These findings revealed that all points fall within a confidence interval of 95 %.

Table 1: Coefficient of Cubic equation for each independent variable

Coefficient	Globule size (nm)	Transmittance (%)	Viscosity (cps)
A(Oil)	+12332.37	-17.42	+319.48
B(S mix)	-183.07	+96.87	+0.16
C(Water)	+395.14	+98.00	-9.28
AB	-21162.35	+222.24	-528.76
AC	-20873.16	+188.96	-549.36
BC	+267.36	+9.88	+29.37
ABC	+19659.51	-211.96	+512.18
AB (A-B)	-11469.5	+122.26	-338.12
AC (A-C)	-9475.61	+65.83	-274.17
BC(B-C)	+991.69	+12.02	+23.55

Table 2: Summary of regression analysis for all response

Model	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	Remark
Response 1 Globule size(nm)					
Linear	41.60808297	0.837278282	0.8011179	0.715878822	
Quadratic	32.84677275	0.932394153	0.876055946	0.63422757	
Special Cubic	14.55714352	0.988934528	0.975655962	0.936588798	
Cubic	1.573384878	0.999948293	0.999715613	0.881835238	Suggested
Response 2 Viscosity (cps)					
Linear	0.356569823	0.965192595	0.957457616	0.941721624	
Quadratic	0.364390049	0.975766049	0.955571089	0.933770144	
Special Cubic	0.392523532	0.976566273	0.948445801	0.904700682	
Cubic	0.321333363	0.993718236	0.9654503	0.954526667	Suggested
Response 3 Transmittance (%)					
Linear	0.438370154	0.65729547	0.581138908	0.327795929	
Quadratic	0.476286834	0.730298239	0.505546771	-0.520492351	
Special Cubic	0.459194362	0.791090361	0.540398794	-0.908508779	
Cubic	0.024307431	0.999765845	0.998712147	0.464988912	Suggested

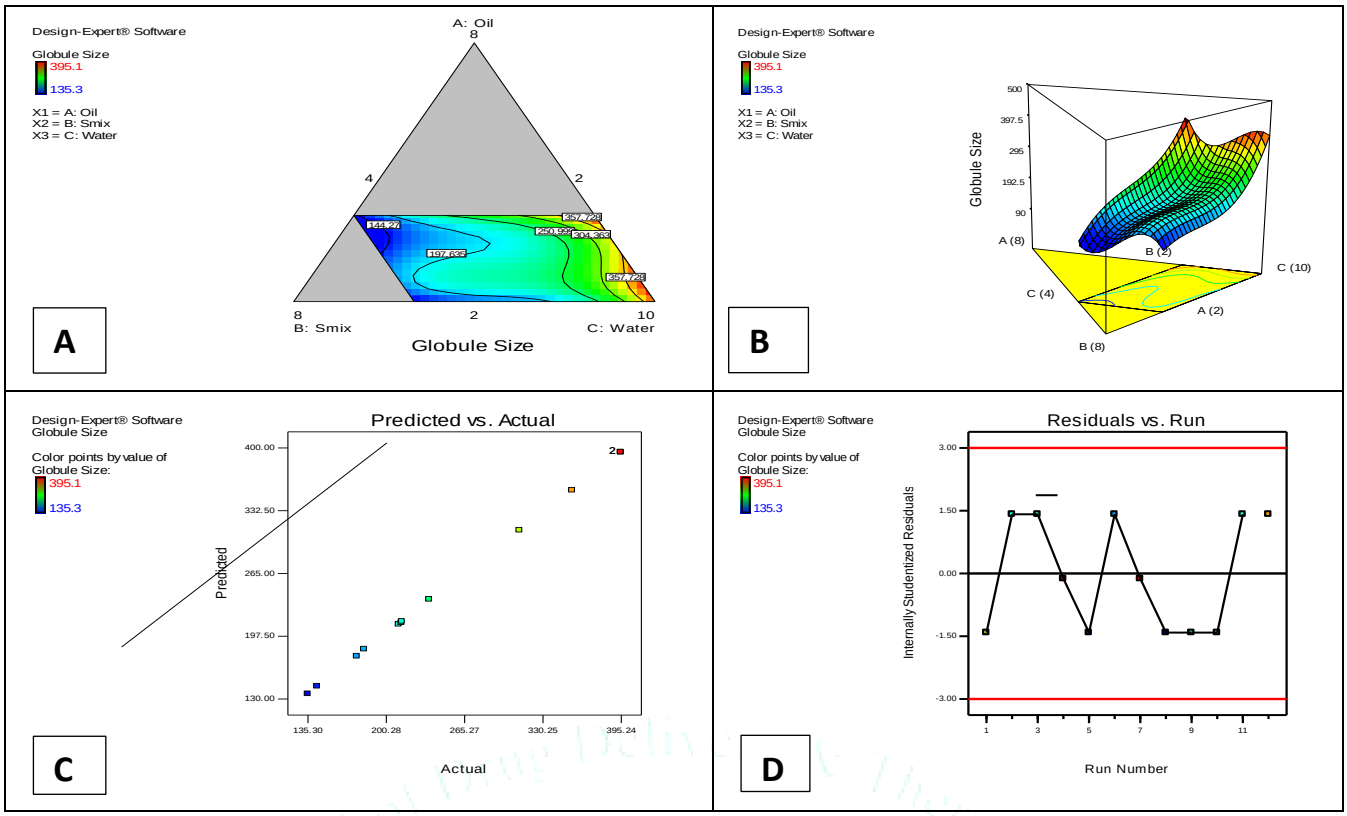


Figure 3: Response variable Globule size (Y1)

A. Contour plot **B.** Surface response curve **C.** Predicted Vs. Actual response and **D.** Residual Vs. run

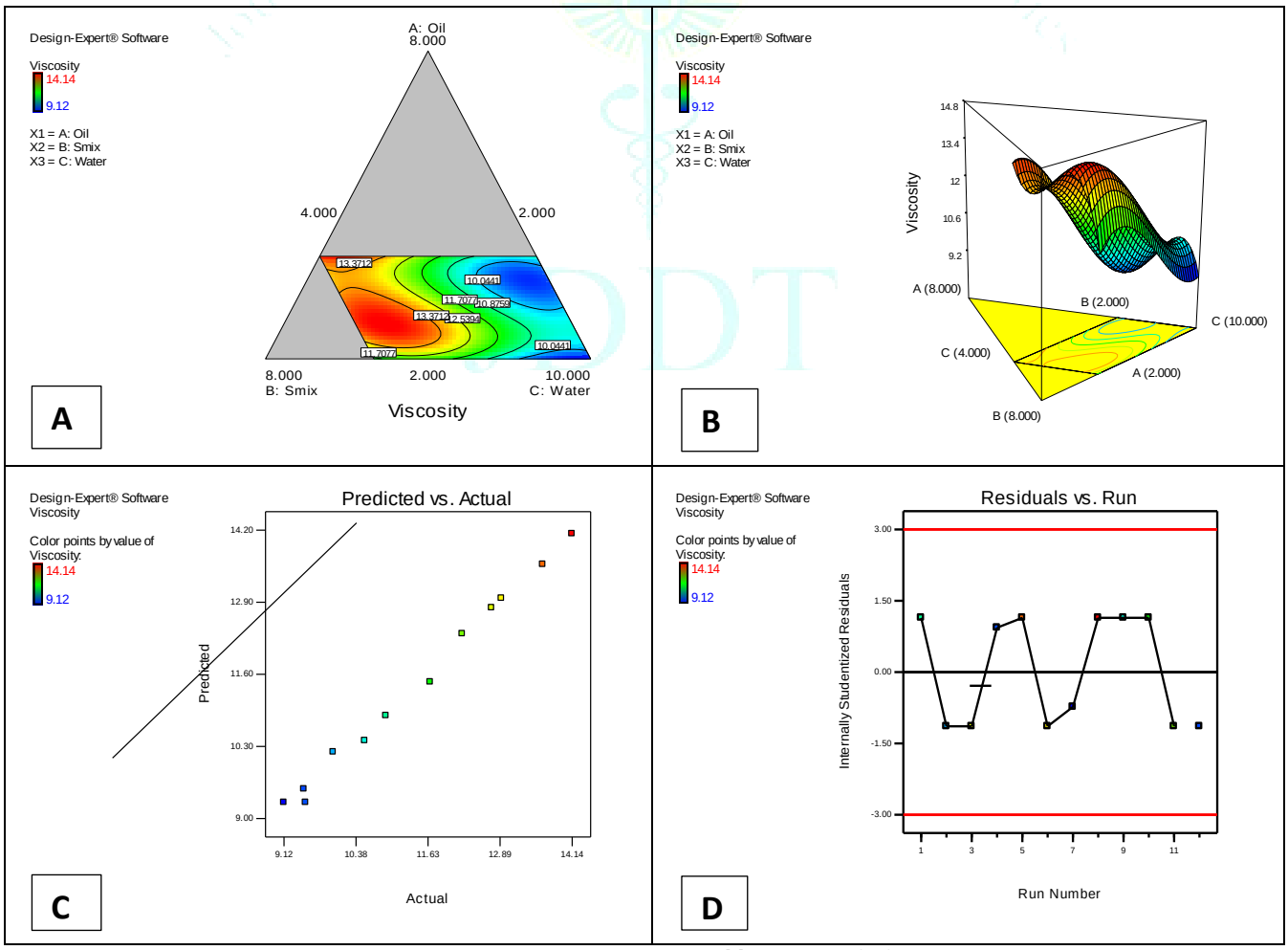


Figure 4: Response variable Viscosity (Y2)

A. Contour plot B. Surface response curve C. Predicted Vs. Actual response and D. Residual Vs. run

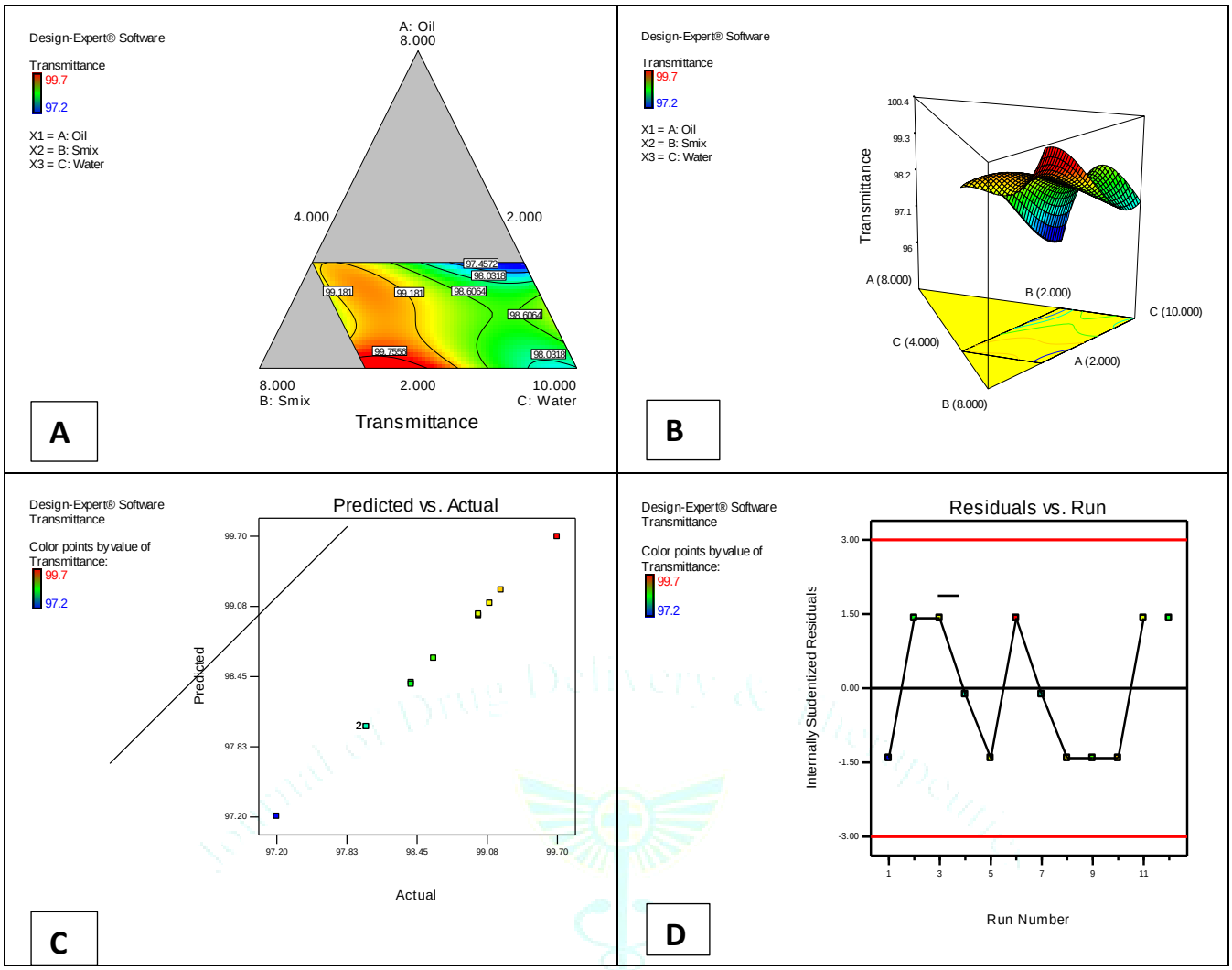


Figure 5: Response variable Transmittance (Y3)

A. Contour plot **B.** Surface response curve **C.** Predicted Vs. Actual response and **D.** Residual Vs. run

Experimental Validation of Design Space

Experimental validation of DoE trials for formulation variables was undertaken by formulation and characterization of microemulsion formulation at the check point batch suggested by the software. Figure 6 shows the overlay plot displaying the design space and optimized parameters as check point suggested by DoE software to obtain the desired responses. The observed values were comparable with the predicted values establishing the reliability of the optimization procedure as shown in Table 3. Calculated percentage prediction error was found to be less than 5 percent, confirming the validity of D- optimal mixture design for microemulsion formulation optimization.

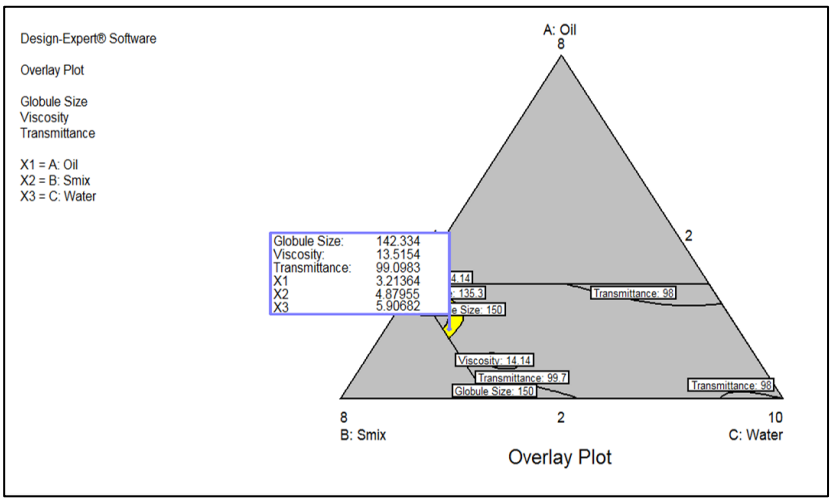


Figure 6: Overlay plot

Table 3: Checkpoint analysis of optimized formulation

Parameters	Predicted value	Experimental value	% Error
Globule Size (nm)	143.33	142 ± 0.16	0.92
Viscosity(cps)	13.51	13.19± 0.121	2.36
Transmittance (%)	99.09	99.79± 0.134	0.70

Error (%) = (predicted value – experimental value)/ predicted value × 100.

Data expressed were of mean ±SEM (n=3)

Characterization and Evaluation of Microemulsion

Drug–Excipient Compatibility

FTIR studies were carried out for pure drug alone and optimized formulation. The all characteristic peaks of pure ebastine were found in the optimized formation, suggested that there is no interaction between drug and excipients as shown in Figure 7A and 7B.

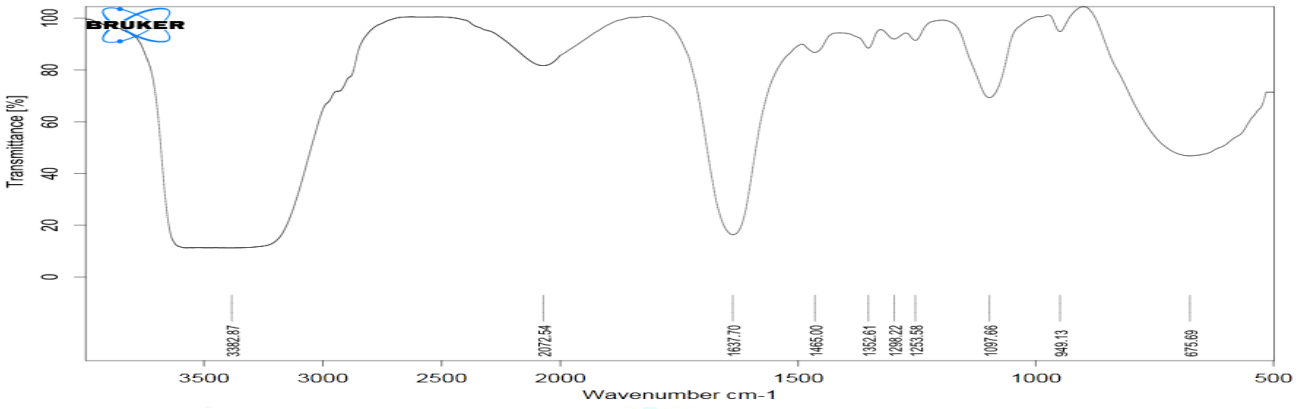


Figure 7 (A): FT-IR spectra of Pure Ebastine

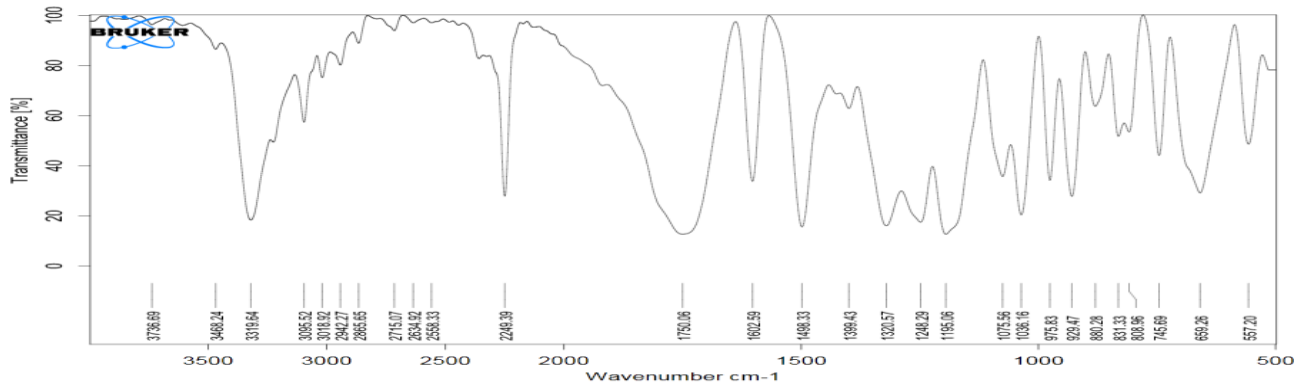


Figure 7 (B): FT-IR spectra of optimized formulation

Measurement of pH

Without much discomfort, the eye can tolerate pH of 6.5-8.0. The pH value of the developed microemulsion is 6.9 ± 0.12, which can be easily buffered by tear fluid (pH 7.2-7.4); consequently, it is adequate to apply to the eye without causing irritation, reflex tear and rapid tear blinking[21].

Measurement of Refractive Index

Refractive index measurements detect possible impairment of vision or discomfort to the patient after administration of eye drops. Refractive index of tear fluid is 1.340 to 1.360. It is recommended that eye drops should have refractive index values not higher than 1.476[4]. The optimized formulation had refractive index values ranging from 1.369 ±0.04 which is resemble to the recommended values.

Measurement of Osmolarity

In formulating ophthalmic preparations consideration of isotonicity is of prime concern. The osmolarity of human tear film after prolonged eye closure is 288-293 mOsm/L and as eye is open, it progressively rises up to 302-318 mOsm/L[21]. An Osmolarity of optimized formulation was found to be 291 ± 0.301mOsm/L indicating appropriateness for ocular application. The glycerol used in said formulation performed dual role of imparting osmolarity to formulation and act as a cosurfactant also.

Measurement of Surface Tension

Ophthalmic formulation had the surface tension range at the surface to air interface of 34.3-70.9 mN/m. Formulations indicated for treatment red eye had surface tensions below normal tear[26]. The surface tension of the optimized microemulsion formulation was found to be 34.75 ±0.13 mN/m. Low microemulsion surface tension ensures good

spreading effect on the conjunctive, cornea and mixing with precorneal film components, thereby improving contact between the drug and the conjunctival tissue.

Droplet size, Zeta potential and Viscosity measurement

The droplet size of prepared microemulsion formulation was found to be 142± 0.16 nm as shown in the Figure 8. The particle size that human eyes can tolerate is about 10 micrometer[21], indicating suitability of developed formulation for ocular use. The Polydispersity Index (PdI) was found to be well below 1.0 which confirms that the optimized microemulsion remains stable upon dilution. Zeta potential of prepared microemulsion formulations was found to be -22.6 ± 0.39 mV as shown in the Figure 9 indicating that dilution does not have a significant impact on the microemulsion zeta potential. The Viscosity of optimized formulation was found to be 13.19 ± 0.121cps. The residential capacity of formulation at physiological site (eye) can be increased by adding gelling agent.

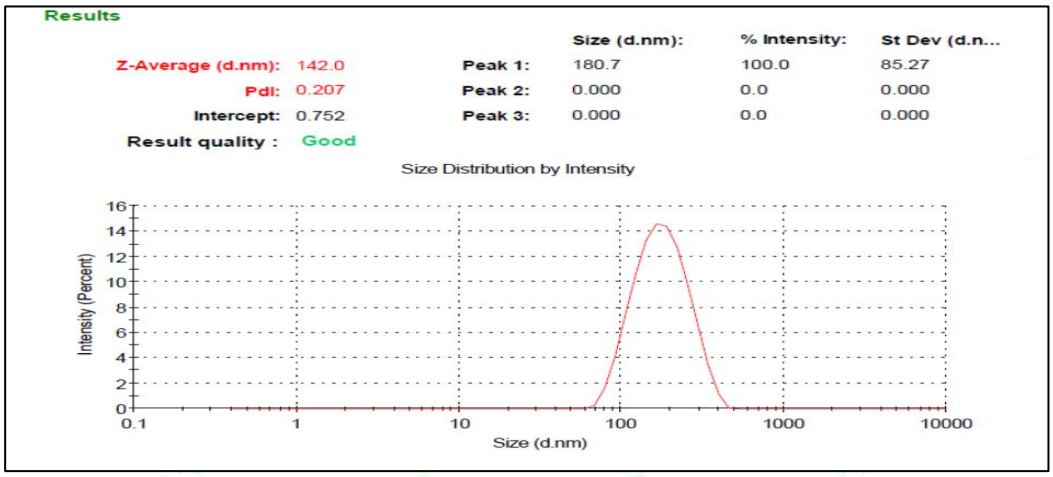


Figure 8: Globule size measurement of optimized formulation

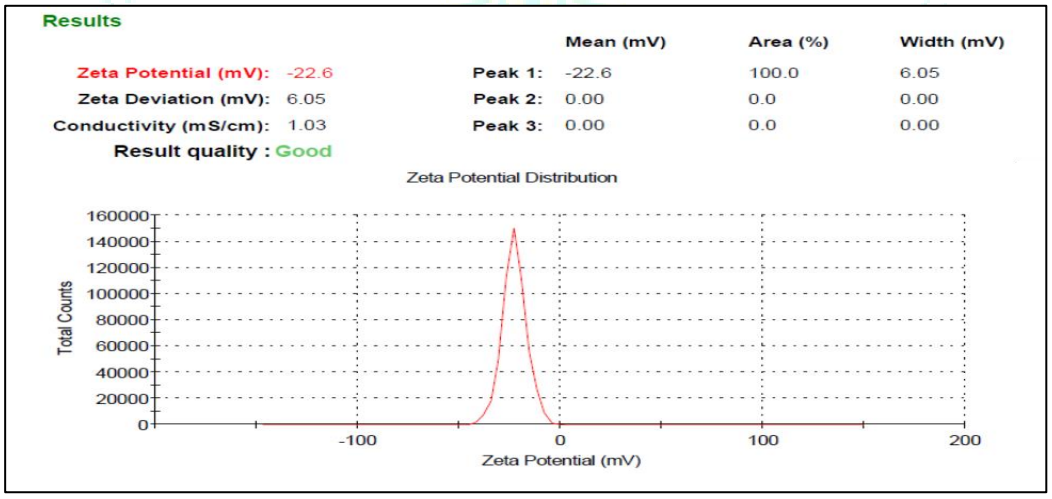


Figure 9: Zeta potential measurement of optimized formulatio

Drug Content

Microemulsion of Ebastine with blend of surfactant and cosurfactant were prepared by Phase Titration Method (Water titration) method. The percentage of drug content of optimized formulations was found to be 97.09 ± 0.12%

Transmission Electron Microscopy

The morphology of the droplets of optimized formulation measured using TEM showed spherical shape and uniform droplet size of optimized microemulsion. Because the loaded ebastine microemulsion globules are nanometric and morphologically spherical, they are not expected to cause ocular irritation. (Figure 10)

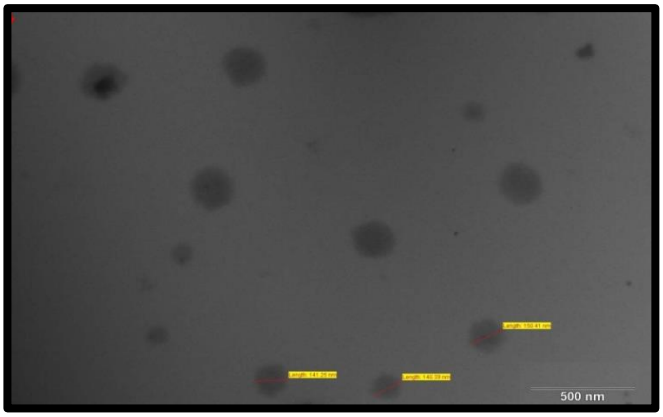


Figure 10: Transmission electron microscopy (TEM) of optimized formulation

In vitro Drug Release Study

It is difficult to mimic diffusion cell in vitro method with the real situation in vivo because cellulose membrane cannot exhibit the barriers of ocular multilayered epithelium as well as the constant volume of diffusion cell will not be able to eliminate the drug released by tear fluid turnover and nasolacrimal leakage. This phenomenon affects the concentration gradient and the diffusion of the drug through the epithelium. Therefore, possibility exists that formulations would have a different release *in vivo*.

In drug release profile (Figure 11), Maximum % Ebastine released from Microemulsion was found 89.19 ± 2.45% compared to Microemulsion gel 71.34 ± 2.34% within 8 hr. However, microemulsion gel was able to sustain the release of the remaining Ebastine for up to 24 h. It is found that drug release from Microemulsion is comparatively more than Microemulsion gel. This might be possible matrix effect on release of Ebastine due to incorporation of microemulsion in CMC and HA gel, a micro gel layer forms around the droplets that can hinder drug diffusion from the oil phase, so the rate and the amount of the released drug may decrease, while the release rate of the drug from microemulsion depends on the rate of diffusion of the drug from oil droplets. The possibility of the drug partition between the oil and the water phases in the presence of the surfactant positioned at the oil–water interface prior to release. Formulation provided the highest in vitro drug release with the ability of providing a sustained release over 24 hr, thus reducing frequency of application and improving patient compliance. But, microemulsion gel has better consistency for topical drug delivery.

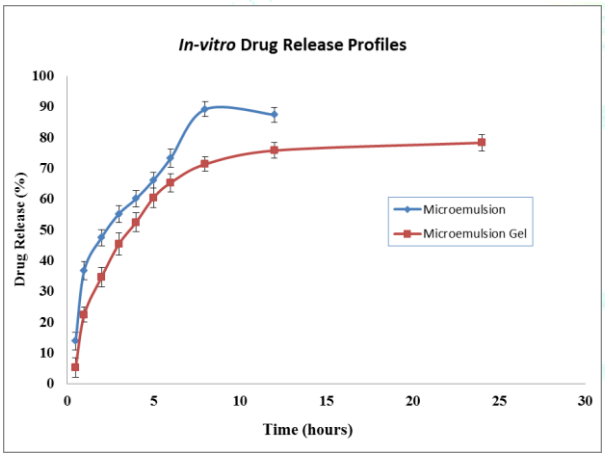


Figure 11: *In vitro* Release profile of optimized microemulsion formulation and microemulsion gel, Data expressed were of mean ± SEM

Sterility Testing

After specified incubation period, both fluid thioglycolate and soyabean casein digest media showed absence of turbidity which is a sign of growth of microorganisms in the test sample of the optimized formulation and negative control while the turbidity or growth was found in the sample for positive control of *Staphylococcus aureus* and *Candida albicans*.

Accelerated Stability Tests by Centrifugation Stress Test

Stress stability study of the microemulsion sample was carried out by subjecting to centrifugation. A formulation shows no sign of phase separation when subjected to centrifugation at 9,000 rpm for 20 minutes. Thus, it was concluded that the Microemulsion formulation was stable under stressful conditions.

CONCLUSION

Studies of equilibrium solubility were conducted in different oils, surfactants and co- surfactants to rationally optimize the formulation using D- optimum mixture design. The developed microemulsion was found in the limit of acceptable droplet size range for ocular use and presented physical stability. Physicochemical parameters like pH, osmolarity, isotonocity were found in the range which favors its ophthalmic suitability. The addition of the gelling agent increased the viscosity in comparison to parent microemulsion. The results of the release study indicated that formulation could prolong the precorneal retention owing to mucoadhesion by polymer. Hence, bioavailability at the site of action of said drug was found to be significantly increased. In conclusion, Ebastine microemulsion could be offered as a promising strategy for ocular drug delivery for allergic manifestation. These findings further warrant *in vivo* investigation.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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