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RESEARCH ARTICLE

Optimization of Microemulgel for Tizanidine Hydrochloride

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Abstract: ***Background*:** Tizanidine hydrochloride acts centrally as a muscle relaxant. It is used for the treatment of painful muscle spasm, spasticity associated with multiple sclerosis or spinal cord injury and treatment of muscle spasticity in spinal cord disease. Tizanidine hydrochloride belongs to BCS class II. It has low oral bioavailability and short half-life. Incorporating this drug in microemulgel is an excellent way to overcome problems associated with the drug.

***Objectives*:** Present research work was aimed to develop and optimize a microemulsion based gel system for tizanidine hydrochloride.

***Methods*:** Screening of oil, surfactant and co-surfactant was carried out. Ternary phase diagram was constructed to obtain concentration range of components. The prepared microemulsion was evaluated for pH, globule size, zeta potential, conductivity, density and viscosity. 3^2 level factorial design was applied to study the effect of concentration of carbopol 934 and HPMC K15M on % cumulative drug release and viscosity of microemulgel using software Design Expert. Microemulgel was evaluated for pH, spreadability, viscosity, syneresis, drug content, bioadhesive strength, *in-vitro* as well as e*x-vivo* diffusion study.

***Results*:** Microemulsion was prepared by using isopropyl myristate as oil, tween 80 as a surfactant and transcutol P as cosurfactant. Largest transparent microemulsion region was found with Smix ratio of 1:1. FE-SEM showed globule size 28µm for batch B1 and zeta potential was -1.27mV indicating good stability of the microemulsion. Optimised batch was F6 which showed 92% drug release within 8 hours. It followed the Korsmeyer-Peppas model.

***Conclusion*:** A stable, effective and elegant microemulgel formulation, exhibiting good *in-vitro* and *ex-vivo* drug release was formulated.

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1. INTRODUCTION

Mixing of microemulsion and gel leads to formulation called as microemulgel. This formulation system had advantages of both emulgel as well as microemulsion. Most important advantage of this system is that both hydrophilic and hydrophobic drugs can be incorporated into these systems. These systems provide a large surface area for the drug absorption. Oil portion increases bioavailability by improving permeability of the drugs. Stability of microemulsion is increased when it is incorporated in a gel system. Over to microemulsions, microemulgel has a certain degree of elegance and they are easily washable [1-3].

Tizanidine hydrochloride acts centrally as a muscle relaxant. It is used for the treatment of painful muscle spasm, spasticity associated with

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multiple sclerosis, or spinal cord injury and treatment of muscle spasticity in spinal cord disease. Tizanidine hydrochloride is also useful as an antispasmodic drug. A market survey indicated that tablet, capsule and gelatin coated capsule dosage formed with the strength of 2mg, 4mg and 6mg are given by oral route. Tizanidine hydrochloride belongs to BCS class II drug with low solubility and high permeability. Oral bioavailability of tizanidine hydrochloride is only 34% to 40%. Approximately 95% of administered dose is metabolized and 65% is excreted mainly in urine. It has a short biological half-life of only 2.5 hours due to which it needs to be administered in patient frequently to maintain the therapeutic effect. The drug had poor bioavailability after oral administration because of extensive first-pass metabolic effect. Tizanidine hydrochloride is an ideal candidate for transdermal delivery because it does not decompose in the skin; it has a low molecular weight and low water solubility. Incorporating tizanidine hydrochloride in microemulgel is an excellent way to overcome first pass metabolism, improved therapeutic efficiency and patient compliance with reducing the dosing frequency [4-6].

The onset, duration, and magnitude of therapeutic response for any topical drug product depend on the relative efficiency of three sequential processes as release of the drug substance from the product; penetration/diffusion of the drug through the stratum corneum (SC); and activation of the desired pharmacological effect at the site of action. An *in vitro* release test (IVRT) for semisolid preparations using *in vitro* diffusion cell (VDC) has been recognized in the FDA's SUPAC-SS guidance as a test for product sameness after certain manufacturing-related changes. Recently, this IVRT has also been recognized as a reasonable and useful test to be considered as a product release and stability test [7].

Patent literature had shown that work has been carried on solid oral pharmaceutical composition [8], composition in liquid dosage form which contains tizanidinehydroclorideaqueous solution suitable for the parenteral, intranasal and oral route administration [9], tizanidine formulation for treating [10] and immediate release multiparticulate composition for reducing a side effect of tizanidine [11].

2. MATERIALS AND METHODS

Tizanidine hydrochloride was gifted by J.P.N. Pharma Ltd. Mumbai. Isopropyl myristate, tween 80, transcutol P and triethanolamine were obtained from Pure Chem Laboratories. Carbopol 934 and HPMC K 15 were gifted from Colorcon Asia Pvt Ltd. Mumbai. All chemicals and solvents were of analytical grade.

2.1. Drug Characterization

Tizanidine hydrochloride was analysed using UV spectrophotometer to identify the λmax in range of 200 to 400 nm and the spectrum was recorded [12]. An IR spectrum of drug was recorded on FTIR (Jasco, FTIR model 6100, Japan) using a potassium bromide pressed disc method. The drug was mixed with previously dried IR grade KBr in 2:200 ratio and the pellet was formed in KBr press. Scanning range was 400-4000 cm^{-1}. DSC thermogram was recorded using differential scanning calorimeter (Hitachi 9020). Approximately 2-5 mg of sample was heated in a pierced aluminium pan up to 300°C at a heating rate 10°C/min under a stream of nitrogen at a flow rate of 50 ml/min. The experiments were repeated in triplicate.

2.2. Screening of Oil, Surfactant and Co-surfactant

Saturation solubility of drug in various oils (isopropyl myristate, oleic acid, vegetable oil, olive oil and liquid paraffin), surfactant (tween 80, tween 20, cremophor RH40 and lauroglycol FCC) and co-surfactant (propylene glycol and transcutol P) was determined. Excess of tizanidine hydrochloride was added to 5 ml of each oil, surfactant and co-surfactant. It was stirred for 48 hours on a magnetic stirrer at 500 rpm at room temperature. The solutions were then filtered through Whatman filter paper and scanned by UV spectrophotometer at λmax 228 nm. The experiments were repeated in triplicate. The solubility of drug in solvents was determined in mg/ml [13, 14].

2.3. Construction of Phase Diagram

Ternary phase diagram was constructed to obtain concentration range of components for existing microemulsion zones by using Chemix School ternary diagram software.

Table 1. Preliminary trial batches of microemulsion.

Formulation Batch	Volume of Isopropyl Myristate (ml)	Volume of Tween 80 (ml)	Volume of Water (ml)
A1	1	9	1
A2	2	8	1
A3	3	7	0.5
A4	4	6	0.4
A5	5	5	0.5
A6	6	4	0.5
A7	7	3	0.7
A8	8	2	1
A9	9	1	0.8

Table 2. Trial batches of microemulsion with Smix.

Formulation Batch	Volume of Isopropyl Myristate (ml)	Volume of Tween 80 (ml)	Volume of Transcutol P (ml)	Volume of Water (ml)	Ratio of Surfactant to Co-surfactant
B1	1	3.5	3.5	1	1:1
B2	1	4.66	2.32	3.5	2:1
B3	1	2.32	4.66	3	1:2

2.4. Formulation of Microemulsion

2.4.1. Microemulsion with Surfactant

Microemulsion was prepared using water titration method. 0.1% of tizanidine hydrochloride was added to isopropyl myristate and tween 80 mixture in conical flask as per Table **1**. Water was added drop-wise to the mixture from burette. Following each water addition, the mixture was assessed visually until clear microemulsion was obtained. The point at which mixture begins to turn turbid after further addition of water, is considered as an endpoint of titration. All the samples were evaluated for % transmittance. The study was repeated in triplicate [14-16].

From above microemulsion, batch A1 was selected for further study due to its transparency. Batch A1 showed the highest % transmittance (98.46%).

2.4.2. Microemulsion with Surfactant and Co-surfactant

Equivalent amount of drug (0.1%) was added to isopropyl myristate and a mixture of surfactant, co-surfactant (tween 80 and transcutol P). The ratio of Smix was varied as per Table **2**. A mixture in the conical flask was titrated by adding drop-wise water from the burette until clear microemulsions are obtained [14-16]. The experiment was carried in triplicate.

From above microemulsion, batch B1 with Smix ratio 1:1 was selected for further study due to its globule size and % transparency. Batch B1 showed highest % transmittance of 99.96%.

2.4.3. Preparation of Microemulsion

A predetermined amount of drug was weighed accurately and dissolved in oil by stirring on a magnetic stirrer. Water and surfactant were mixed

together. This mixture was added drop-wise to an oily solution of drug and mechanically stirred to form an emulsion. Co-surfactant was added drop-wise to the emulsion. Formation of the transparent solution indicated the formation of microemulsion.

2.5. Evaluation of Microemulsion

2.5.1. % Transmittance Measurement

Microemulsion was diluted 100 times with distilled water. % transmittance of formulation was measured using UV Visible spectrophotometer at 560 nm wavelength against continuous phase (Distilled water) as blank.

2.5.2. Globule Size Measurement

Size analysis of optimized batch of microemulsion was carried out by Digital microscope (MOTIC) in which a drop of microemulsion was placed on glass slide covered by slip which was observed under the microscope under 10X object lens. Motic digital microscope has built-in cameras that allow plug and play into a USB port on a computer. Live images were viewed and measured for globule size by the software on the computer and images were saved.

For FE-SEM (Field Emission Scanning Electron Microscopy FEI-NOVA NANOSEM 450), sample was prepared by drop casting method with a dilution of 1 ml of microemulsion with 10 ml of distilled water. A drop of this solution is then placed on aluminium foil in a petri dish with the help of a micropipette. The foil is allowed to dry at room temperature for 24 hours. The sample was then placed in the sample holder of FE-SEM and images were captured.

In zetasizer (Malvern), disposable sizing cuvette at 25.1°C was used where 1 ml microemulsion sample was diluted with 10 ml water and the result was recorded.

2.5.3. Zeta Potential Measurement

Zeta potential of microemulsion was determined by Zetasizer (Malvern). Samples were placed in clear disposable zeta cell and results were recorded. Before putting a fresh sample, cuvettes were washed with methanol and rinsed using the sample to be measured before each experiment.

2.5.4. Dilution Test

This test was carried out to find out which type of microemulsion was formed. The prepared microemulsion was diluted with water which was external /continuous phase.

2.5.5. Conductivity Measurement

Solubilisation of water phase in the selected mixture containing oil, surfactant and co-surfactant was measured by an electrical conductivity meter.

2.5.6. pH

pH of microemulsion was measured by digital pH meter (Lab India PICO^{+}).

2.5.7. Density

Density was measured using a density bottle.

2.5.8. Viscosity

Viscosity of microemulsion was determined using Brookfield's viscometer.

2.5.9. Centrifugation

This parameter was measured to evaluate the physical stability of microemulsion. The prepared microemulsion was centrifuged at ambient temperature at 5000 rpm for 10 minutes to evaluate the system for creaming or phase separation. The microemulsion was observed visually for appearance.

2.5.10. Refractive Index

Refractive index of microemulsion was measured with the help of Abbe's refractometer.

2.5.11. Ex-vivo Drug Release Study

Ex-vivo drug release study was carried out to investigate physicochemical *ex-vivo* characteristics. *Ex-vivo* drug release study was carried out using chick ileum in USP type II dissolution test apparatus. Ileum part from intestine of the chicken was separated and washed with the freshly prepared tyrode solution. Ileum was tied at one end with a thread. 2 ml microemulsion containing 0.1% tizanidine hydrochloride was filled in ileum and other end is sealed with thread. Similarly, plain drug solution and optimised microemulgel were added in two other pieces of the ileum. These

Table 3. Preliminary trial batches of gel.

Formulation Batch	Carbopol 934 (%)	HPMC K 15M (%)	Xanthan Gum (%)	Sodium Alginate (%)
C1	2	8	-	-
C2	4	6	-	-
C3	-	-	1	3
C4	-	-	3	2
C5	2	-	2	-
C6	2	-	3	-
C7	3	-	-	1
C8	4	-	-	2
C9	-	2.5	-	1.5
C10	-	4	-	2

Table 4. Independent variables.

Variables (Level)	Independent Variables	
	Concentration of Carbopol 934 (%)	Concentration of HPMC K15M (%)
Low (-1)	2	4
Medium (0)	4	6
High (+1)	6	8

ileum parts were then dipped in dissolution media in jars of dissolution apparatus such that all of them are at equal distance from the bottom. Continuous aeration is provided. Dissolution media used was 900 ml of distilled water. Temperature of bath was maintained at 37 ± 0.5°C with aeration speed 1-2 bubbles/sec. Samples were withdrawn at predetermined times interval. Amount of drug released was estimated spectrophotometrically at 228 nm. The experiment was carried out in triplicate.

2.6. Formulation of Gel Phase

Carbopol 934, HPMC K15M, xanthan gum and sodium alginate were selected for preparation of gel phase. Polymers were dispersed in warm water separately and soaked for 24 hours before use. Combination of gel was prepared by mixing the polymers as shown in Table **3**.

2.7. Development of Microemulsion Based Gel (MBG)

Carbopol 934 and HPMC K15M polymers were used as a gelling agent in the formulation of microemulsion based gel. Both polymers were soaked separately in water for 24 hours. They were mixed to form gel phase. The prepared microemulsion was then added to the gel phase to form microemulsion based gel and mixed until the smooth, elegant gel was obtained.

2.8. Experimental Design

3^2 level factorial design was applied to study the effect of independent variable (Table **4**) *i.e*. concentration of carbopol 934 and concentration of HPMC K15M on dependent variables *i.e*. % cumulative drug release and viscosity of microemulgel using software program Design-Expert version 10 [13-17]. Independent variables were

Table 5. Formulation of factorial batches for microemulgel.

Ingredients (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Isopropyl myristate	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Tween 80	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Transcutol P	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Carbopol 934	0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6
HPMC K15M	0.4	0.4	0.4	0.6	0.6	0.6	0.8	0.8	0.8
Triethanolamine	Adjust pH 6 to 7								
Water	q.s.								

listed while all batches were prepared according to experimental design as shown in Table **5**.

2.9. Evaluation of Microemulsion Based Emulgel

2.9.1. Physical Examination

Prepared microemulsion based emulgel was inspected for their colour, homogeneity and consistency.

2.9.2. Syneresis Measurement

After visual inspection of microemulgel, it was checked for any possible phase separation. Upon standing, gel sometimes shrinks and little liquid is pressed out. This phenomenon is called syneresis. It is expressed as percent syneresis. Test involves the use of a centrifuge machine. The formulation was added in a cylindrical tube which had a perforated bottom which was covered with Whatman filter paper. The tube was placed in the centrifuge and centrifuged for 15 minutes. Tube and liquid separated from microemulgel were weighed. Percent syneresis was calculated using formulas shown in Eq. (1).

$$\%\ Syneresis = \frac{weight\ of\ liquid\ separated\ from\ microemulgel}{total\ weight\ of\ microemulgel\ before\ centrifugation} \times 100 \quad (1)$$

2.9.3. pH

1 % aqueous solutions of microemulsion based emulgel was prepared.pH was measured by using digital pH meter.

2.9.4. Spreadability Test

Spreadability is an important criterion for inspecting uniformity, ease and application of transdermal formulations. It is expressed as the spreading coefficient (S). Spreading coefficient was determined using a modified apparatus [17]. Apparatus consisted of two glass slides one of which was fixed onto a wooden board and other was movable, tied to a thread which passes over a pulley, carrying a weight. 1 gm of microemulgel was placed between two glass slides. 100g weight was allowed to rest on the upper slide for 1 to 2 minutes to expel entrapped air between slides and to provide a uniform film of formulation. Weight was removed and top slide was subjected to a pull obtained by attaching a known weight over the pulley. Time (sec) required for moving slide to travel a pre-marked distance (cm) was noted and expressed as spreadability. Spreadability was calculated by using Eq. (2).

$$\text{Spreadability} = \frac{M \times L}{T} \ldots \quad (2)$$

where, M=weight required to move the slide (gm).

L=distance travelled by the glass slide (cm).

T= time required by the slide to travel distance L (sec).

2.9.5. Rheological Study

Viscosity of microemulgel was determined by using Brookfield's viscometer.

2.9.6. Drug Content

Determination of drug content is necessary to evaluate the uniform distribution of drug in the

formulation. It ensures a minimum batch to batch variation. Tizanidine hydrochloride content in microemulgel was determined by dissolving the known quantity of formulation in methanol by sonication. The solution was stirred for 3-4 hours on magnetic stirrer to ensure complete dissolution of formulation in methanol. Later, the solution was filtered through Whatman filter paper. Their absorbance was measured at 228 nm using UV-Visible spectrophotometer. All the experiments were performed in triplicate.

2.9.7. In-vitro Measurement of Bioadhesive Strength

Bioadhesive strength of formulation was measured in a simulated environment using a modified test assembly. The method is based on measurement of tensile strength or shear stress required to break the adhesive bond between model membrane and test formulation. Freshly separated egg membrane was used as model membrane. Assembly consists of a pan balance. 100 ml beaker was placed on the left side of balance. Balance weight was tied to right hand side. A glass slide was hanged to balance, to which one egg membrane was spread upon. The formulation was applied to this membrane. Another glass slide was with egg membrane spread upon it and was attached to the previous slide with the help of adhesive force of gel sandwiched between two membranes. Water was added drop-wise in a beaker placed on left side until membrane separates and comes off. Weight water required to separate membranes was noted and bioadhesive strength was calculated using Eq. (3).

$$\text{Bioadhesive strength} = \frac{m \times g}{A} \ldots\ldots \quad (3)$$

where, m = weight required to detached the slides.

A = area of rat skin attached to slides.

g = acceleration due to gravity (980 cm/s^2).

2.9.8. In-vitro Diffusion Study

All the experiments were performed in triplicate.

2.9.8.1. Cellophane Membrane

Franz diffusion cell was used for this study. Cellophane membrane (12, pore size of 2.4 nm, mol. wt. approx. 12,000 Dalton and capacity of approx. of 1.61 ml/cm) was used. Cellophane membrane (cut to suitable size) was stored in phosphate buffer pH 6.8 for 24 hours before use and was placed in between donor and receptor compartment. An equivalent amount of gel (0.1% Tizanidine hydrochloride) was spread on cellophane membrane. The entire surface of membrane containing formulation was in contact with receptor compartment containing 25 ml of phosphate buffer pH 6.8. Cell was agitated on a magnetic stirrer at 50 rpm and maintained at 37 ± 1°C. Aliquots of 2 ml were withdrawn at predetermined intervals up to 480 minutes. The absorbance of samples was measured at 228 nm [18].

2.9.8.2. Egg Membrane

Raw egg was taken and a small hole was made at the bottom to remove all its contents. Then the eggshell was dipped into 0.1 N HCl for 2 hours. Eggshell got dissolved and the membrane was collected. This fresh egg membrane was then washed with distilled water followed by phosphate buffer pH 6.8. This was used for the study. Freshly separated egg membrane is used each time for the study. All experimental conditions were maintained as they were for study with cellophane membrane.

2.9.9. Ex-vivo Diffusion Study

Fresh dorsal skin of goat was obtained from slaughterhouse. Skin hair was removed and skin was hydrated by keeping it in a physiological salt solution. Subcutaneous fat was carefully removed with scissor. This skin of goat was mounted on donor compartment with epidermis facing donor compartment. Receptor compartment was filled with phosphate buffer solution pH 6.8 and was maintained at 37 ± 0.5°C. Cell was agitated by using magnetic stirrer. 1gm of microemulgel was placed over it and spread evenly. Drug permeation study was carried out in a similar manner as with cellophane membrane.

2.9.10. Drug-excipient Compatibility Study

Drug, microemulsion and microemulsion based gel was stored in stability chamber (Thermolab) at 40°C ± 2°C /75% ± 5% RH for one month. They were evaluated by UV, FTIR, and DSC. Solution

of all three samples was scanned using UV spectrophotometer in the range of 200-400 nm and spectrum was recorded. IR spectra of tizanidine hydrochloride, carbopol 934, HPMC K15M and physical mixture of drug with these excipients in a ratio of 1:1 were obtained using FTIR. Scans were obtained in range of 4000-400 cm^{-1}. DSC thermogram of tizanidine hydrochloride, carbopol 934, HPMC K15M and physical mixture of drug with these excipients in a ratio of 1:1 was obtained using differential scanning calorimeter.

2.10. Release Kinetics

Drug release from all the batches of the microemulgel was evaluated for best fit model. Various kinetic models are zero order, first order, Higuchi, Hixson Crowell and Korsmeyer Peppas. PCP disso v3 software was used for this study.

2.11. ANOVA Study

Statistical validation of polynomial equations generated by Design Expert 10 was established on the basis of ANOVA provision in software.

2.12. Permeation Data Analysis (Flux)

Permeation rate of drug *i.e.* flux of drug calculated using PCP disso v3 software. Flux is the amount of drug permeated through skin per unit time at a steady state.

2.13. Stability Study

Stability study for the optimised batch was carried out as per ICH guidelines. Short term accelerated stability of gel was carried at 40°C ± 2°C /75% ± 5% RH for 1 month. Long term stability study was carried out at 1, 2 and 3 months at 30°C ± 2°C /65% ± 5 % RH. After storage, samples were tested for their physical appearance, consistency, viscosity and drug content.

3. RESULTS AND DISCUSSION

3.1. Drug Characterization

Tizanidine hydrochloride was white to yellowish powder with melting point 279°C which was matching with the reported melting point 280°C. This sample confirmed IP specifications [19]. IR spectrum is highly specific for each chemical structure since small structural differences result in significant spectral changes. FTIR spectrum is characteristic of entire molecule and provides structural information referring to peaks associated with characteristics group. Tizanidine hydrochloride, exhibited characteristic peaks as shown in Fig. (**1**). Sharp characteristic peak was observed at 3245cm^{-1} which indicated NH stretching. Peaks at 3073cm^{-1} suggested there was aromatic CH stretching. Peak at 1938cm^{-1} indicated the presence of secondary amine group shown by NH bending. Peaks at 1604cm^{-1} and 1484cm^{-1} suggested there was aromatic C=C stretching. Peak at 1067cm^{-1} indicated C-Cl stretching. Observed spectrum of tizanidine hydrochloride (Fig. **1**) matches to standard spectra of tizanidine hydrochloride reported in Indian Pharmacopeia 2014 thus indicating that drug is in pure form [19].

DSC thermogram of tizanidine hydrochloride is as shown in Fig. (**2B**). Sharp endothermic peaks were observed at 290.1°C which corresponds to a reported range of melting temperature of tizanidine hydrochloride (Fig. **2A**).

3.2. Screening of Oil, Surfactant and Co-surfactant

Amongst oil, surfactant and co-surfactant that were screened, tizanidine hydrochloride had shown good solubility in isopropyl myristate, tween 80 and transcutol P (Table **6**). They were further selected to formulate the microemulsion. The solubility of tizanidine hydrochloride in distilled water was 0.92 mg/ml and phosphate buffer pH 6.8 was 2.04 mg/ml.

3.3. Construction of Phase Diagram

Pseudo-ternary phase diagram was constructed to determine the region of transparent microemulsion. To obtain appropriate components and their concentration ranges for microemulsion, pseudo-ternary phase diagram was constructed for microemulsion with different Smix ratio. Microemulsion region could be identified and microemulsion formulations could be optimized. The shaded area shows transparent or clear microemulsion region and remaining area shows turbid microemulsion (Fig. **3**). Largest transparent microemulsion region was found with Smix ratio 1:1 and therefore it was selected for preparation of microemulsion [20].

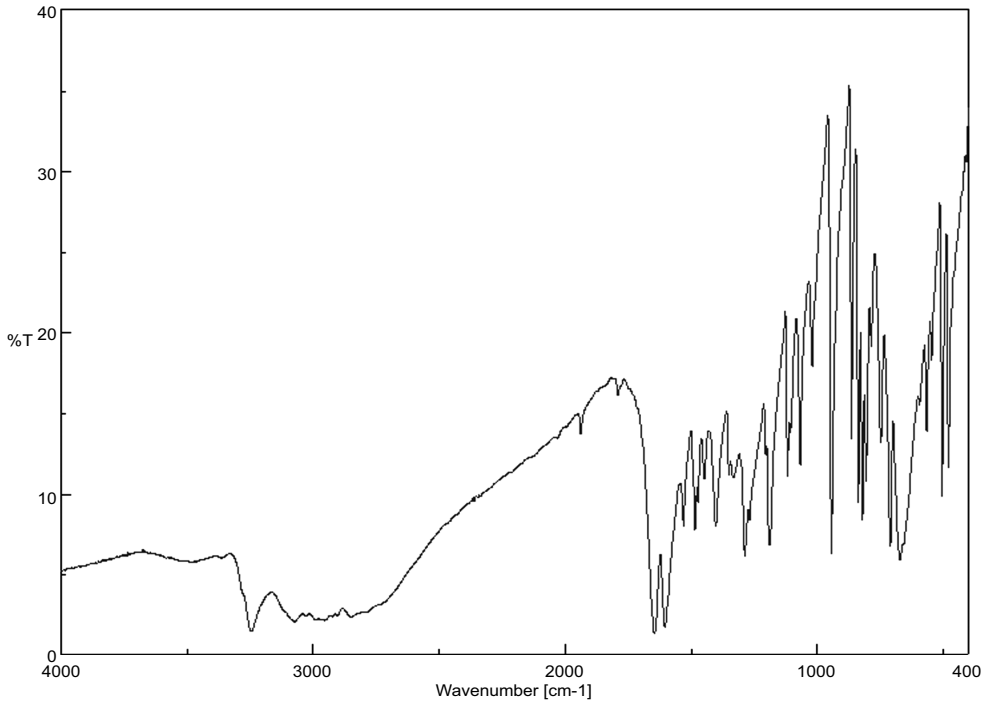


Fig. (1). Observed IR spectra for drug.

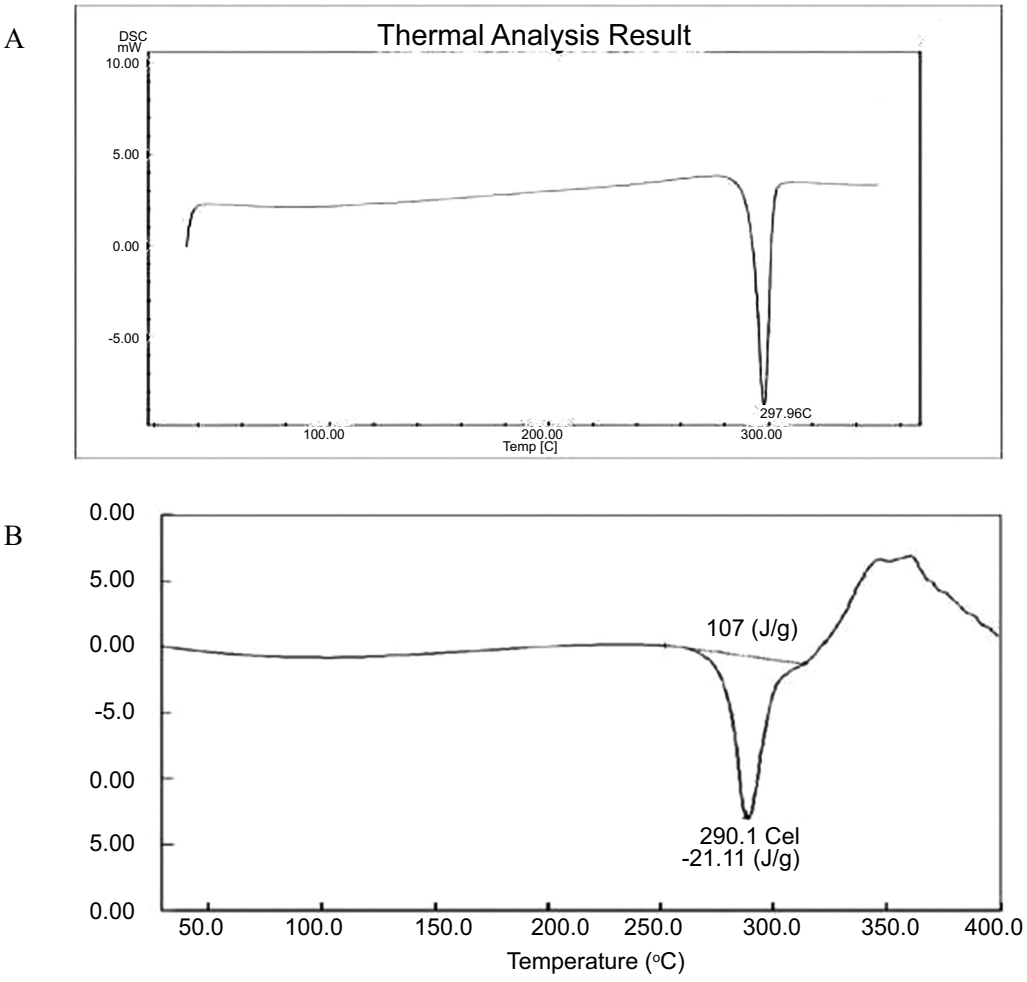


Fig. (2). **A**: Standard DSC and **B**: Observed DSC.

Table 6. Solubility study.

Oil/ Surfactant / Co-surfactant	Solubility (mg/ml)
Vegetable oil	1.82
IPM	4.21
Olive oil	3.1
Labrafill M 1944C	3.0
Paraffin oil	2.8
Oleic acid	2.6
Tween 20	1.43
Tween 80	3.7
Cremophor 40	2.6
Lauroglycol 90	1.68
Propylene glycol	2.7
PEG 400	1.5
Transcutol P	2.8

3.4. Evaluation of Microemulsion

3.4.1. % Transmittance

Batch A1 showed highest % transmittance which was 98.73%. Amongst batches consisting of Smix, batch B1 showed highest % transmittance *i.e.* 99.85% indicating transparent microemulsion. This showed that Smix ratio 1:1 forms most transparent microemulsion and therefore was chosen to prepare microemulsion.

3.4.2. Globule Size Measurement

Microemulsion images showed spherical shape globules of a size range between 247nm by digital microscope (MOTIC) with 10X magnification objective lens (Fig. **4A**). FE-SEM image of microemulsion for batch B1 showed globule size 28µm (Fig. **4B**).

3.4.3. Zetasizer

Globule size of microemulsion batch B1 was found to be 223nm (Fig. **5A**). PDI is a measure of particle homogeneity and it varies from 0.0 to 1.0. If PDI value is closer to 0.0, it indicates a narrow size distribution of microemulsion. PDI of optimized microemulsion was found to be 0.448; hence it indicated that prepared microemulsion is monodisperse which remains stable and not converted to macro-emulsion.

3.4.4. Zeta Potential Measurement

Zeta potential measurement gives an idea about the potential stability of the colloidal system. If zeta potential of particles in the suspension had large negative or positive value, then particles will repel each other. This indicates there will be no tendency of particles to come together. However if zeta potential of particles has low value, then particles will come together and flocculate. The general dividing line between stable and unstable suspensions is taken at either +30 or -30 mV. Particles with zeta potential value more positive than +30 mV or more negative than -30 mV are normally considered as stable [17, 21, 22]. Zeta potential of optimized batch B1 was found to be -1.27 mV which showed a good stability of the microemulsion (Fig. **5B**).

3.4.5. Dilution Test

Upon dilution with water no phase separation was observed indicating B1 is o/w type.

3.4.6. Conductivity Test

5% of water addition showed no significant change in conductivity due to oil concentration which was more than water concentration so resistance was more. Upon 5% to 40% water addition conductivity increased significantly showing the bi-continuous region of microemulsion. Above 40% of water addition, no significant change in conductivity was observed. Conductivity remains constant upon further addition of water which indicated that o/w type microemulsion was formed from optimized batch (B1).

3.4.7. pH

pH of microemulsion (in the range of 6.7-7) indicated that it is suitable for use on human skin.

3.4.8. Density

All microemulsion batches showed density close to 1g/ml.

3.4.9. Viscosity

Microemulsion possess low viscosity. Microemulsion batches B1-B2 showed viscosity in range of 4500-6000 cps.

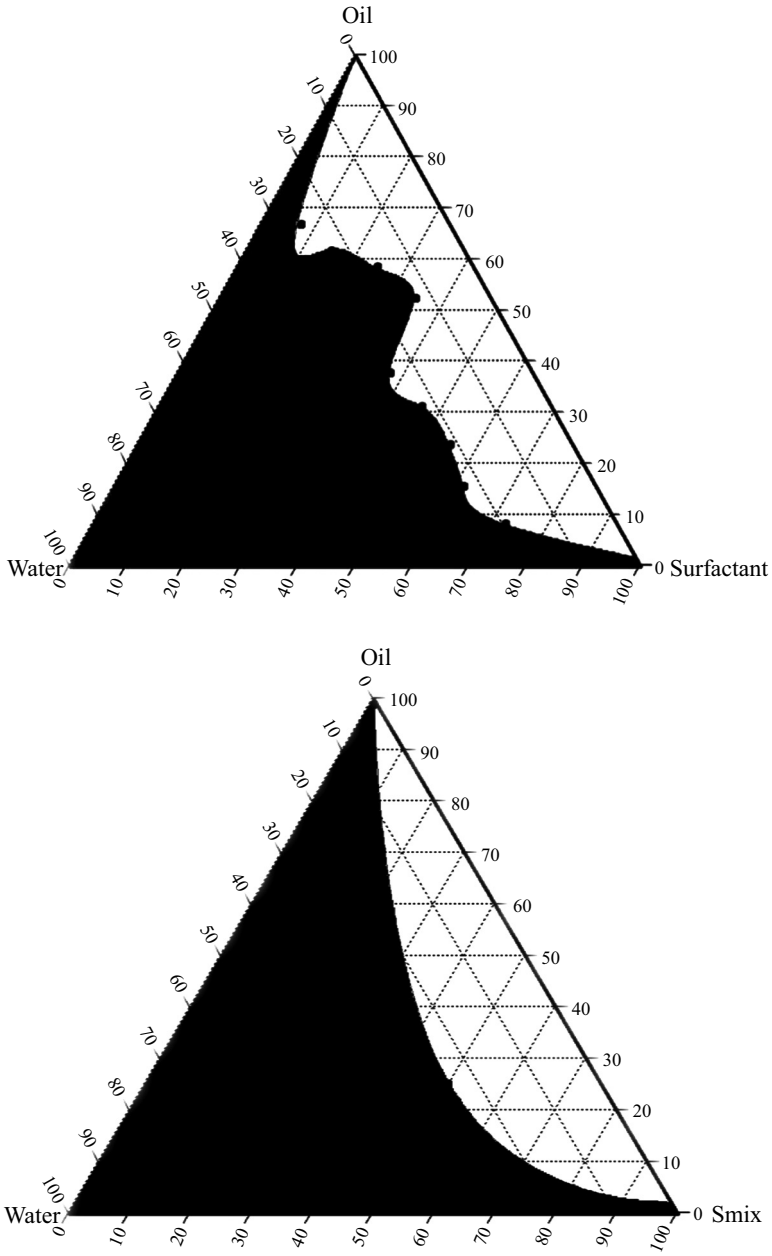


Fig. (3). Pseudo-ternary phase diagrams of micro-emulsions.

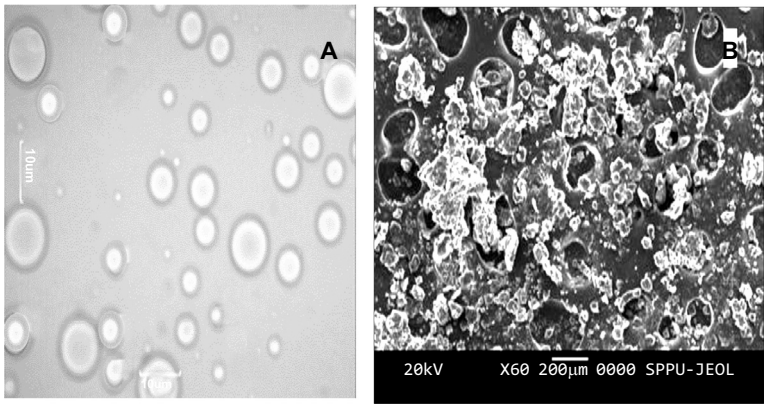
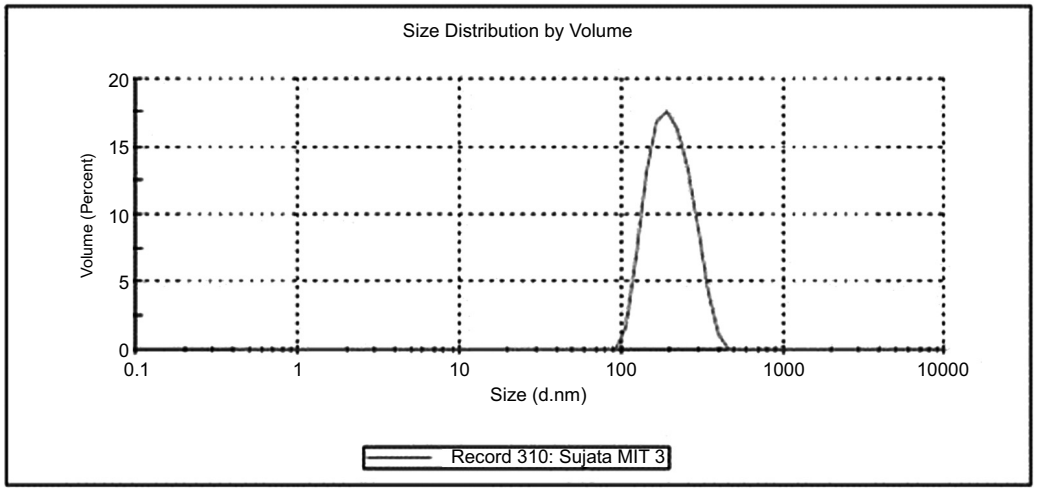


Fig. (4). **A**: Globule size by digital microscope and **B**: FE-SEM.

A

			Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm):	223.1	Peak 1:	205.5	100.0	61.90
PdI:	0.448	Peak 2:	0.000	0.0	0.000
Intercept:	0.937	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				



B

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-1.27	Peak 1:	-1.27	100.0	4.74
Zeta Deviation (mV):	4.74	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.203	Peak 3:	0.00	0.0	0.00
Result quality	See result quality report				

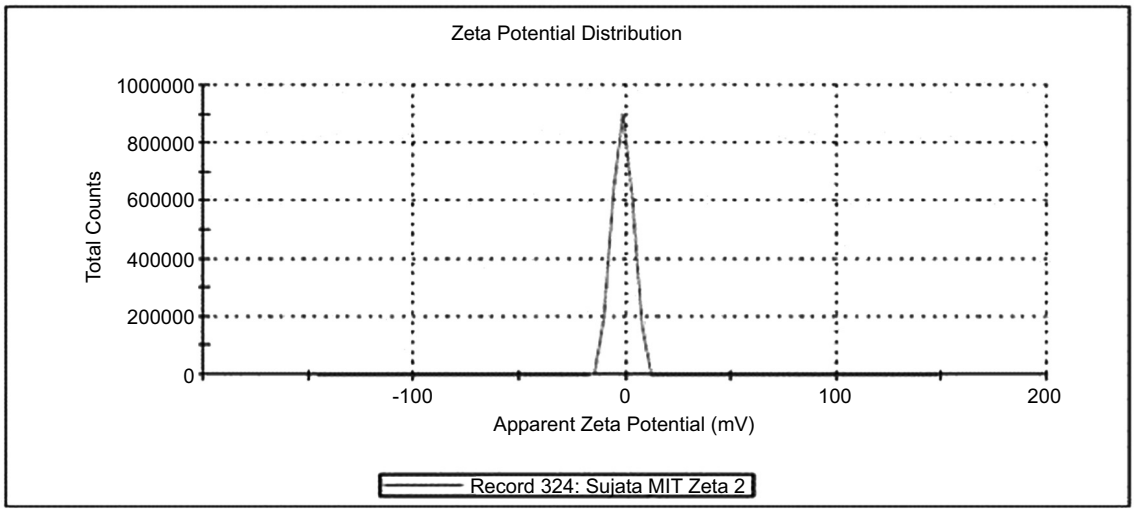


Fig. (5). **A**: Globule size by Zetasizer and **B**: Zeta potential.

3.4.10. Centrifugation

After centrifugation, no phase separation was observed which indicated that microemulsion B1, B2 and B3 were stable.

3.4.11. Refractive Index

Refractive index of microemulsion was found to be 1.456. These values of R.I. correspond to the R.I. of water. This indicates that prepared microemulsion was isotropic in nature.

3.5. Microemulsion Based Gel

Carbopol 934P and HPMC K15M were chosen as gelling agents based on their ability to form a smooth, spreadable gel and controlled release of drug. Preliminary trial batch C2 was chosen. Batch

Table 7. Evaluation Parameters for microemulsion based gel.

Formulation Batch	% Syneresis	pH	Spreadability (gm.cm/s)	Viscosity (cps)	Drug Content %	Bioadhesive Force (dynes/cm^2)
F1	1.78 ± 0.76	5.8 ± 0.08	19.74 ± 1.27	8000 ± 1.45	91.09 ± 0.31	2055.27 ± 0.42
F2	4.93 ± 0.43	5.9 ± 0.05	18.17 ± 1.20	15000 ± 1.80	88.05 ± 0.22	2296.87 ± 0.17
F3	2.75 ± 0.91	5.8 ± 0.43	19.36 ± 1.57	9000 ± 1.56	89.02 ± 0.71	2569.31 ± 0.13
F4	3.38 ± 0.58	5.8 ± 0.09	20.87 ± 1.03	12000 ± 1.47	92.06 ± 0.12	2451.42 ± 0.19
F5	1.79 ± 0.38	5.8 ± 0.023	19.74 ± 1.65	11000 ± 1.44	95.01 ± 0.33	2631.93 ± 0.09
F6	1.54 ± 0.35	6.0 ± 0.00	20.71 ± 1.34	16000 ± 1.45	92.08 ± 0.41	2536.35 ± 0.10
F7	1.90 ± 0.39	5.9 ± 0.09	19.55 ± 1.82	17000 ± 1.53	85.02 ± 0.38	2485.18 ± 0.16
F8	1.72 ± 0.87	5.8 ± 0.07	18.14 ± 1.12	14000 ± 1.77	87.04 ± 0.52	2375.43 ± 0.13
F9	3.90 ± 0.67	5.8 ± 0.08	19.48 ± 1.19	13000 ± 1.97	81.05 ± 0.15	2078.65 ± 0.18

C1 also formed a smooth gel but drug release from this batch was retarded due to the high concentration of HPMC K15M. Other batches C3-C10 produced gel with either very low or very high viscosity making them less consistent.

3.6. Evaluation of Microemulsion Based Gel

3.6.1. Physical Examination

Microemulsion based gel was found to be white, viscous, creamy and smooth in consistency. F6, F7, F8 batches were found to have very good consistency and smooth appearance. Other batches were either too thick or had very less viscosity due to varying polymer concentration [23, 24].

3.6.2. Syneresis Measurement

% syneresis of microemulgel were found to be very less (Table **7**). This indicated that microemulgel showed stability at room temperature.

3.6.3. pH

pH of microemulgel were found from 5.8 to 6.0. Batch F2, F6, F7 were found to have more stable pH which was 5.9, 6.0 and 5.9 (Table **7**).

3.6.4. Spreadability

Spreadability of microemulgel were found to have in the range of 18 to 21gm.cm/s indicating good spreadability (Table **7**).

3.6.5. Viscosity

Viscosity of microemulgel batches were found from 8000 to 17000 cps. Batch F2, F6 and F7 showed highest viscosity 15000, 16000 and 17000 respectively (Table **3**).

3.6.6. Drug Content

Drug content of all batches were in the range of 85-96%. Batch F1, F4, F5, F6 showed highest drug content (Table **5**).

3.6.7. Bioadhesive Force

Batch F3, F4, F5 and F6 showed maximum bioadhesive force which was 2569.31, 2451.42, 2631.93 and 2536.35 dynes/cm^2 (Table **7**).

3.6.8. Ex-vivo Drug Release Study

After 2 hours, amount of drug released from plain drug solution, microemulsion and microemulgel was found to be 79.33%, 87.74% and 86.38% respectively (Fig. **6A**). From this study, it can be concluded that the extent of release of tizanidine hydrochloride released from microemulgel and microemulsion was greater than plain drug solution. Increase in drug release was due to enhancement in penetration which may be combined effect of surfactant and cosurfactant.

3.6.9. In-vitro Drug Diffusion Study

3.6.9.1. Cellophane Membrane

Diffusion study showed that an increase in the concentration of HPMCK15 and Carbopol 934

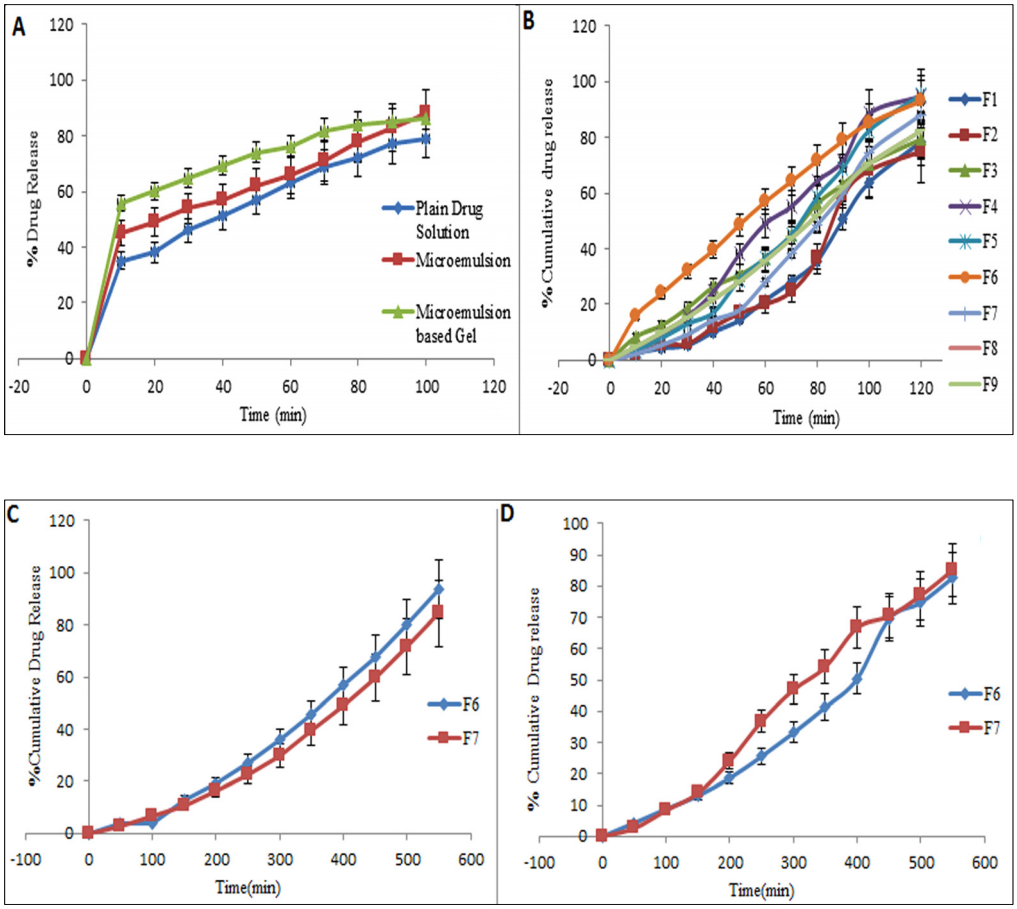


Fig. (6). A: *Ex-vivo* drug release and **B**: *In-vitro* drug release through Cellophane membrane. **C**: *In-vitro* drug release through Egg membrane and **D**: *Ex-vivo* drug release through goat skin.

retard the drug diffusion from the formulations due to high viscosity of the formulation. This may be due to the cross-linking effect of polymers at higher concentration (Fig. **6B**). Drug release from batches F1-F9 was compared to an ideal drug release range for extended-release products [25, 26]. Drug release from batches F4, F5, F6and F7 showed more drug release. Batches F4, F5, F6 and F7 pass and F1, F2, F3, F8 and F9 failed.

3.6.9.2. Egg Membrane

Formulations F6 and F7 showed a slight increase in drug release through egg membrane than cellophane membrane after 8 hours as shown in Fig. (**6C**). This may be due to a change in the thickness of the membrane.

3.6.9.3. Ex-vivo Diffusion Study

Ex-vivo study for F6 and F7 batches were performed using goatskin. It was found that *ex-vivo* drug release of these formulations was more than *in-vitro* drug release through cellophane membrane and egg membrane. F7 showed better diffusion than F6 (Fig. **6D**). This may be due to the difference in a polymer concentration of HPMC K 15 and Carbopol 934 in F6 and F7 microemulgel.

3.7. Drug Excipient Compatibility Study

3.7.1. UV Spectroscopic Study

Tizanidine hydrochloride showed λ max at 228nm. Microemulsion as well microemulsion based gel showed a peak at 228nm indicating that excipients do not affect the stability of drug (Fig. **7A**).

3.7.2. FTIR Study

Scan 'a' (Fig. **7B**) of tizanidine hydrochloride showed a characteristic peak of secondary amine at 3100 cm^{1}, aromatic C=C at 1604cm^{1}, aromatic C-Cl stretching at 1067 cm^{-1} and aromatic bending

Table 8. Analysis of variance.

Response Model	Sum of Squares	Df	Mean Square	F Value	P Value	R^2	Adequate Precision
Drug Release at 8 Hours	491.10	1	98.22	22.51	0.0139	0.9740	11.483
Viscosity	1248.95	3	416.32	6.69	0.0335	0.8006	6.319

at 710 cm^{-1}. Scan 'b' (Fig. **7B**) of carbopol 934 showed a characteristic peak of hydroxyl group at 2988 cm^{-1}, C=O group at 1717 cm^{-1}, acrylates at 1247 cm^{-1}, etheral C-O-C group at 1190 cm^{-1}. Scan 'c' (Fig. **7B**) of HPMC K15 showed characteristic peak of hydroxyl group a 3435 cm^{-1}, methyl and hydroxypropyl group at 2922 cm^{-1}, cyclic anhydrides at 1376 cm^{-1}, epoxides at 1278 cm^{-1}, CH_2 group at 829 cm^{-1}. Scan 'd' (Fig. **7B**) of tizanidine hydrochloride and carbopol 934 showed a characteristic peak of secondary amine at 3245 cm^{-1}, aromatic C-H stretching at 3074 cm^{-1}, aromatic bending at 710 cm^{-1}, hydroxyl group at 2989 cm^{-1},carbonyl group at 1716 cm^{-1}, ether at 1190 cm^{-1}. Scan 'e' (Fig. **7B**) of tizanidine hydrochloride and HPMC K15 showed characteristic peak of secondary amine at 3161 cm^{-1}, C=C bond at 1604 cm^{-1}, hydroxyl group at 3460 cm^{-1}, secondary amine bending at 1939 cm^{-1}, hydroxypropyl group at 2951 cm^{-1}, cyclic anhydride at 1358 cm^{-1}, epoxides at 1189 cm^{-1}. Scan 'f' (Fig. **7B**) of tizanidine hydrochloride, carbopol 934 and HPMC K 15 showed characteristic peak of secondary amine at 3245 cm^{-1}, aromatic C-H stretching at 3074 cm^{-1}, secondary amine bending at 1939 cm^{-1}, aromatic C-Cl stretching at 1067 cm^{-1}, aromatic bending at 710 cm^{-1}, hydroxyl group at 2951 cm^{-1}, carbonyl group at 1819 cm^{-1}, acrylates at 1267 cm^{-1}, ethereal C-O-C group at 1190 cm^{-1}, hydroxyl group at 3043 cm^{-1}, methyl and hydroxypropyl group 3074 cm^{-1}, cyclic anhydrides at 1358 cm^{-1}, epoxides at 1274 cm^{-1}, CH_2 group at 830 cm^{-1}.

3.7.3. DSC Analysis - DSC

Thermogram is shown in Fig. (**7C**). Sharp endothermic peak (Fig. **7C-a**) was observed at 290.15°C which corresponds to the melting temperature of tizanidine hydrochloride. Carbopol 934 showed a melting point at 241.0°C (Fig. **7C-b**). HPMC K15M showed a melting point at 112.2°C (Fig. **7C-c**). Fig. (**7C-d**) of tizanidine hydrochloride + HPMC K15M + Carbopol 934P showed no change in melting point of drug which indicated that stability of drug was not affected by the presence of polymers.

3.8. Release Kinetics for Microemulgel

Release kinetics data based on R^2 values indicated that batch F1 followed Matrix as the best fit model. All other batches from F2 to F9 followed Korsmeyer-Peppas model as best model. Value of 'n' is used to characterize drug release mechanism, as the release exponent (n) showed a value less than 0.5, the drug followed Fickian diffusion transport mechanism which was due to swelling of polymer in a controlled manner.

3.9. ANOVA Study

Data obtained was treated using Design Expert 10 software and analysed statistically using analysis of variance (Table **8**).

Data was also subjected to the 3-D response surface methodology to study the interaction of X_1 and X_2 on dependent variables.

A. Effect of variables on drug release at 8 hours

Final equation in terms of actual factors for drug release at 8 hours is given in Eq. (4):

$$\text{Drug release (at 8 hours)} = +93.5+2.60A-1.86B-2.79AB-14.08A^2+1.05B^2..... \quad (4)$$

where A: Conc. of Carbopol 934; B: Conc. of HPMC K15M

A positive sign of polymer A indicated that it had a positive effect on drug release at 8 hours. The negative sign of polymer B indicated its negative effect on drug release at 8 hours. From polynomial equations, response surface and contour

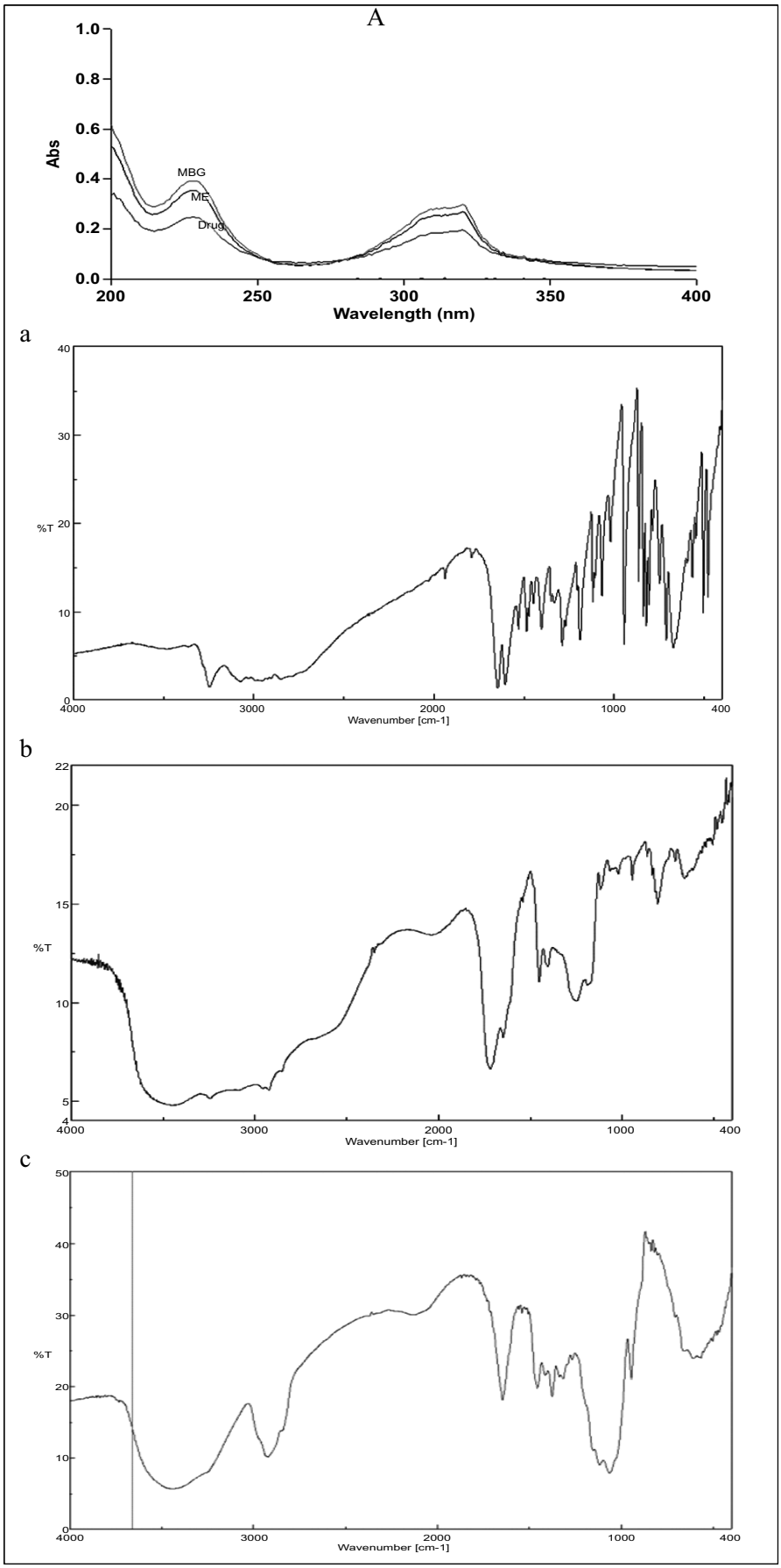

A
Abs
Wavelength (nm)
MBG
ME
Drug
a
%T
Wavenumber [cm-1]
b
%T
Wavenumber [cm-1]
c
%T
Wavenumber [cm-1]

Fig. (7) contd…

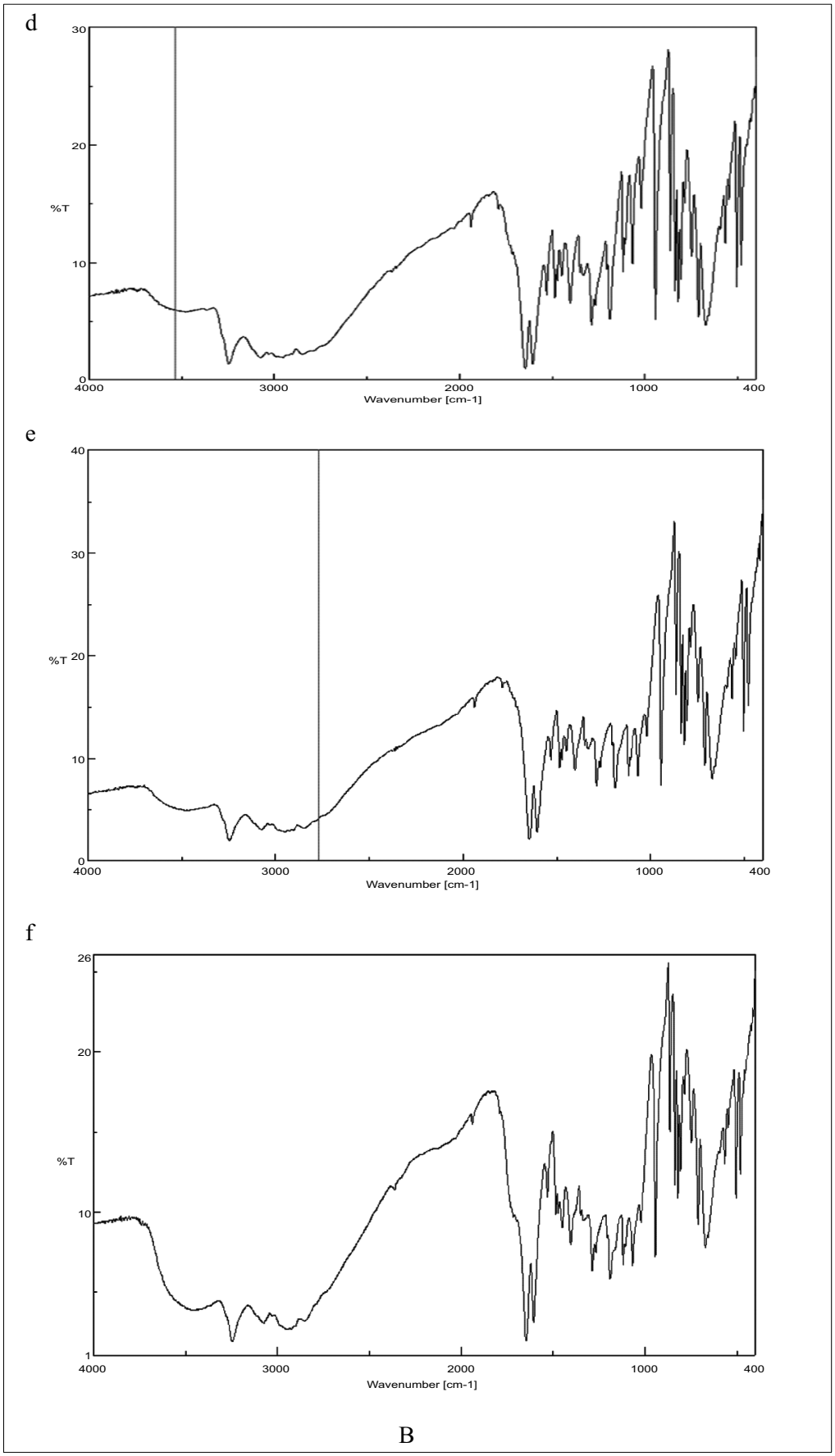


Fig. (7) contd…

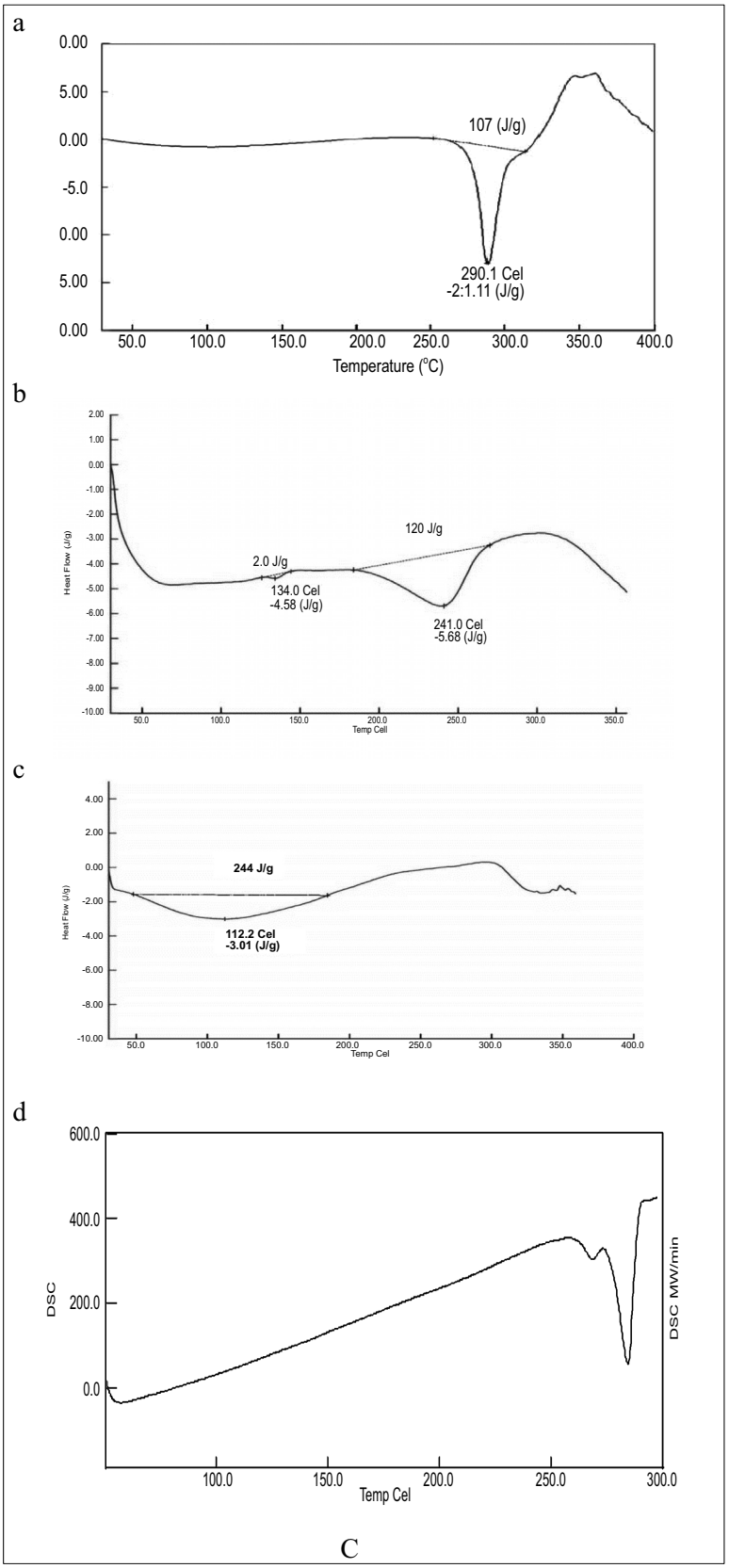


Fig. (7). A: Drug-excipient compatibility UV spectrum; **B:** Drug-excipient compatibility FTIR spectrum (**a**-Tizanidine Hydrochloride, **b**-Carbopol 934, **c**-HPMCK15, **d**-Tizanidine hydrochloride +Carbopol 934, **e**-Tizanidine hydrochloride+HPMCK15, f-Tizanidine hydrochloride+Carbopol934+HPMCK15); **C:** DSC spectrum (**a**-Drug, **b**-Carbopol 934, **c**-HPMC K15, **d**-Drug+ HPMC K15M+ Carbopol 934P).

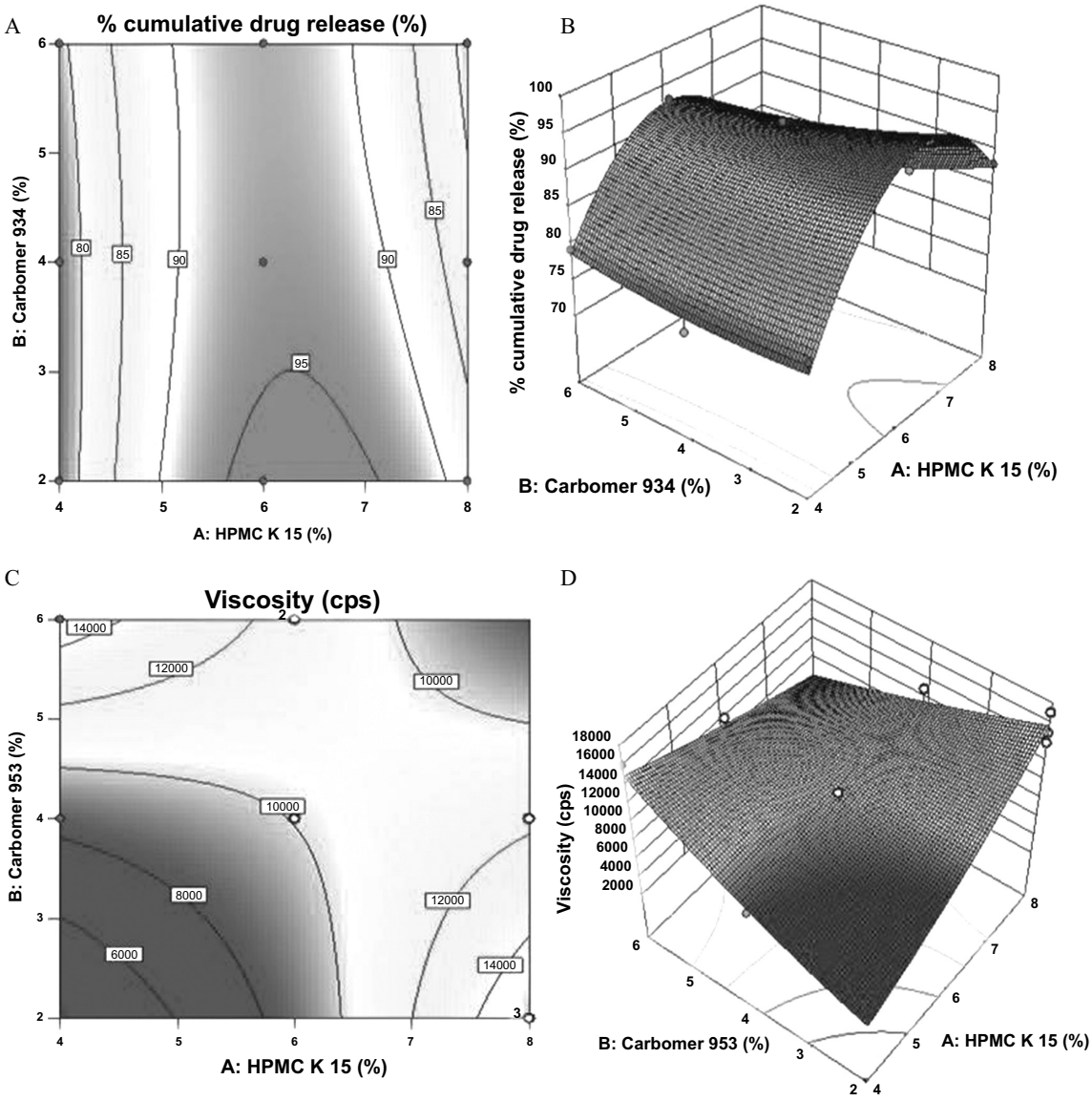


Fig. (8). Contour Plot and Response surface plot (**A**, **B**) % drug release and polymers concentration, (**C**, **D**) viscosity and polymers concentration.

plots of respective responses were generated which are as shown in the Fig. (**8A** and **8B**). Statistical optimization was carried out by software which suggested that cumulative drug release followed a linear model. Model terms for drug release at 8 hours were found to be significant with a high value of R^2 0.9740 which indicated adequate fitting to a linear model. Values of "Prob F" less than 0.0139 confirmed that model terms were significant. Also, the "Pred R-Squared" value of 0.7461 was in reasonable agreement with "Adj R-Squared" value 0.7278.The "Adequate precision" measures signal to noise ratio. A ratio greater than 4 is desirable. In this case, the ratio was 11.47 which indicated an adequate signal. This inferred that the model can be used to navigate design space [27].

B. Effect of formulation variables on gel viscosity

Final equation in Terms of Actual Factors for microemulgel viscosity is given in Eq. (5):

Microemulgel Viscosity = +99.93A+8.01A+ 6.55B-23.75AB ..... (5)

where, A: Carbopol 934 concentration; B:HPMC K15M concentration.

A positive sign indicated that both polymer carbopol 934 and HPMC K15 has a positive effect

Table 9. Validation data.

Polymers	Coded Levels	Actual Levels	Response	% Drug Release at 8 Hours	Viscosity (cps)
			Predicted value	93.5522	12000
			Observed value	89.2	8000
Carbopol 934P	4	4	Standard deviation	8.00	3073.18
HPMC K15M	6	6	Standard error mean	1.56	4.67

on viscosity of microemulgel. From polynomial equations, response surface and contour plots were generated which was indicated in the Fig. (**8C** and **8D**). Statistical optimization was carried out by software which suggested that quadratic model was followed by gel viscosity. Model terms for gel viscosity were found to be significant with a high value of $R^2$0.8006 which indicated adequate fitting to a linear model. Values of "Prob F" less than 0.05 indicated that model terms were significant. "Pred R-Squared" of 0.0335 is in reasonable agreement with "Adj R-Squared" of 0.6809. "Adequate precision" measures signal to noise ratio. A ratio greater than 4 is desirable. In this case, ratio was 6.319 which indicated an adequate signal. This inferred that the model can be used to navigate design space. In design, as the concentration of carbopol 934 and HPMC K15 was increased, gel viscosity also increased. It was observed that higher the concentration of carbopol 934 and HPMC K15 higher the viscosity of the microemulgel [27].

3.10. Validation of the Statistical Model

After statistical analysis, optimized batch was F6 by Design Expert 10 software. Experimental values for % cumulative drug release at 8 hours, viscosity and bio-adhesive strength were found very close to applied predicted values as indicated in Table **9**. Hence, the model was successfully validated.

3.11. Permeation Data Analysis (Flux)

The flux of drug through cellophane membrane with area 7.00 cm^2. Flux of batch F6 was 0.08 and batch F7 was 0.07 which was found to be more as compared to other batches. This indicated that the rate of drug diffusion of batch F6 and F7 through cellophane membrane is higher than other batches.

3.12. Stability Study

Batch F6 was white and viscous with a smooth consistency. There was no major change in % drug content and viscosity which indicated the stability of microemulgel.

CONCLUSION

Factorial design study proved that tizanidine hydrochloride release and gel viscosity were strongly dependent on the concentration of carbopol 934P and HPMC K15M. *In-vitro* drug diffusion study through egg membrane showed 93.87% of drug release for F6 Batch. *Ex-vivo* diffusion study through goat skin indicated 82.63 ± 0.78% drug release. Microemulgel formulation of tizanidine hydrochloride is a promising approach for transdermal delivery of the drug which will overcome the problems associated with drug as first pass metabolism. This will improve the therapeutic efficiency and reduce the frequency of dosing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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