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Formulation, Development and Optimization of Topical Emulgel by 3^2 Factorial Design

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Abstract:

Emulgel have emerged as one of the most interesting Topical delivery system as it has dual control release system i.e. gel and emulsion. Topical applications of drug offers many advantages for delivering drug directly to the site of action and deliver the drug for extended period of time at effected site. In the present study, an attempt has been made to formulate the topical drug delivery system of Pentoxifylline EP in the form of Emulgel. Pentoxifylline EP is widely used Non steroidal Anti-inflammatory drug, mostly used to relieve pain and also have significant results in the treatment of oral submucous fibrosis treatment.

Emulgel batches were formulated and developed to get the optimized batch using 3^2 Factorial Design. For the formulation of emulgel, gelling agent Acrypol 934P and Penetration enhancer Menthol were selected as independent variables whereas Viscosity and in vitro drug release were the dependent variables.

All the formulation developed were evaluated for the post formulation studies like color, phase separation, pH, rheological behaviour, spreadability, swelling index, drug content, In- vitro drug release and stability studies and all the results observed were within official limit.

Keywords: Emulgel, Topical drug delivery system, Gel based emulsion, Oral submucous fibrosis, Oral cancer.

Introduction[1,2]:

Number of medicated products are applied to the skin or mucous membrane that either enhances or restores a fundamental function of skin or pharmacologically alters an action in the underlined tissues. Such products are referred as topical or dermatological products. Many widely used topical agents like ointments, creams lotions have certain disadvantages. They are sticky in nature, causing uneasiness to the patient when applied. They have lesser spreading coefficient so they are applied by rubbing. They also exhibit the problem of stability. Due to all these factors within the major group of semisolid preparations, the use of emulgels has expanded both in cosmetics and in pharmaceutical preparations due to their advantages over the gels.

Oral submucous fibrosis (OSF) is a potentially malignant disease characterized by gradual inability to open the mouth. It is due to oral submucosal juxta-epithelial inflammatory changes, accompanied by fibro elastic changes throughout the lamina propria and epithelial atrophy, resulting in oral mucosa stiffness, trismus and feeding difficulties.

This condition is multifactorial and associated with areca nut chewing present in betel quid and gutkha. The components of the areca nut produce reactive oxygen species, that makes mucosa atrophic due to poor wound healing.

Iron and multivitamin supplements with lycopene, stabilize and deactivate the free radicals, are the treatment choice. For severe cases, intralesional steroid injection, laser ablation and surgery, including fibrotomy of jaw muscles and temporomandibular joint, have been used.

Pentoxifylline is a methyl xanthine that increases the vascularity of the mucosal layer by increasing red cell deformability, leukocyte chemotaxis, antithrombin and antiplasmin activities, and fibrinolytic activity. It inhibits neutrophil adhesion and activation, induces neutrophil degranulation, increases natural killer cell activity, and inhibits T-cell and B-cell activation.

Rajendran et al used pentoxifylline in the treatment of OSMF for 7 months and reported significant improvement in subjective symptoms, of intolerance to spices and burning sensation of the mouth in 6 - 12 months of follow-up in experimental group.

Among all these benefits of Pentoxifylline, there is a major problem associated with it which is it undergoes first pass metabolism. Due to which it's oral bioavailability is about 20 to 30% only. To overcome these problems we tried to Formulate and Develop the Pentoxifylline EP Emulgel in this research work.

Materials and Methods:

Materials:

The drug Pentoxifylline EP was received as a gift sample from Bakul Pharma Pvt Ltd, Ankleshwar, Gujarat and Acrypol ® 934P was received as a gift sample from the Corel Pharm Chem , Ahmedabad, Gujarat.

Different formulations of emulgel were prepared using varying amounts of gelling agent (Acrypol ® 934 P) and menthol.

Formulation of Emulgel :

Table: 1. Various ingredients used in the formulation of emulgel and their uses :

Ingredients	Use
Pentoxifylline EP	API
Acrypol ® 934 P	Gelling agent
Propylene glycol	Surfactant, Preservative,
Glycerol	Humectant and emollient property
Triethanolamine (TEA)	pH adjusting agent for gel formation
Distilled water	Solvent
Ethanol	Solvent
Wheat germ oil	Oil phase
Eucalyptus oil	Oil phase, also help in penetration enhancement
Menthol	Penetration enhancer
Propyl Paraben	Antimicrobial Preservative
Butylatedhydroxy Toluene (BHT)	Antioxidant
Span 80	Surfactant
Tween 80	Surfactant

Preparation of gel Phase:

For preparation of gel phase, Sufficient quantity of Acrypol ®934P was sprinkled onto distilled water with continuous stirring on a mechanical stirrer at 200 to 250 RPM. The dispersion was allowed to hydrate for 2 hours. Then Propyl Paraben was dissolved in Propylene glycol and thereafter mixed with Glycerol, the solution formed was added with continuous stirring. The pH was adjusted upto 6.5 using Triethanolamine (TEA). The gel was sonicated for 15 min and kept overnight to remove air bubbles.

Preparation of Emulsion:

Due to hydrophilic nature of the drug Pentoxifylline EP, it was dissolved in aqueous phase and further encapsulated within the oil phase by forming water in oil (w/o) type of emulsion.

For preparation of emulsion, the aqueous phase was prepared by dissolving Tween 80 in distilled water. Propyl Paraben was dissolved in Propylene glycol and mixed with the aqueous phase. The drug Pentoxifylline EP and Menthol were dissolved in ethanol and mixed with aqueous phase.

The oil phase of the emulsion was prepared using wheat germ oil. Butylatedhydroxy Toluene was mixed with Wheat germ oil, then the surfactant Span 80 was added.

Then the aqueous phase was added to the oil phase with continuous stirring to form water in oil (w/o) type emulsion.

The obtained emulsion was mixed with the gel in 1:2 ratio with gentle stirring to obtain emulgel.

Tween 80 was added for mixing of emulsion with the gel.

The ingredients used in emulgel formulation and it's uses are given in table number 1.

Development and Optimization of Topical Emulgel by using 3^2 Factorial Design :

The application of mathematical optimization in the pharmaceutical field was first reported by Fonner et al (1970), using the Lagrangian method as a constrained optimization technique. Later developments in computer science have enabled the incorporation of the optimization algorithm into the experimental design software. Experimental design, also called design of experiments (DoE), is an approach in the development and optimization of drug delivery devices. By this method, it is feasible to obtain the desired formulation as quickly as possible while avoiding unnecessary experiments.

Advantages of Factorial Design:

The major advantage of the method is the development of pharmaceutical formulations so that all the potential factors could be studied simultaneously, systematically and quickly.

By using design of experiments, the effect of each formulation factor on each response can be evaluated and critical factors can be identified based on statistical analysis.

When the formulation and manufacturing process of a pharmaceutical product are optimized by a systematic approach using DoE, scale-up and process validation can be very efficient because of the robustness of the formulation and manufacturing process.

In factorial design it is possible to control multiple independent variables and determine their effect on a single dependent continuous variable.

In factorial design each level of one factor is combined with each level of other factor or independent variable.

The primary advantage of the factorial design is that it allows evaluation of effects of more than one independent variable, both separately and in combination with each other.

The factorial design also offers economical advantages by reducing the total number of observations which would be needed if the two main effects were evaluated separately and in combination with each other.

The factorial designs also offer economical advantages by reducing the total number of subjects or observations which would be needed if the two main effects were evaluated separately.

A factorial design is used to evaluate two or more factors simultaneously.

The treatments are the combinations of levels of the factors.

The advantages of factorial design over one factor at a time experiment are that they are more efficient and they allow interactions to be detected.

3^2 Factorial design :

Instead of repeating experiments for each independent variable or factor, we can design a more efficient experiment that evaluates the effect of two or more factors at the same time. These types of designs are referred to as factorial designs.

Following terms are used in factorial design:

i) Factor: It is the variable which affects the results of the experiment. The determination of factor for particular experiment depends mainly on the objective of the experiment and it needs to be determined after careful evaluation of results.

ii) Level: It is the limit of the variables below or beyond which an experiment cannot give significant change in results.

Intervention studies with 2 or more categorical explanatory variables leading to a numerical outcome variable are called as “Factorial design".

A factor is simply a categorical variable with 2 or more values referred to as levels.

A study in which there are 2 factors with 3 levels is called as 3^2 Factorial design.

For present work 3^2 Factorial design was selected with 2 dependent and 2 independent factors.

In this design, 2 factors were evaluated each at 3 levels and experimental trials were performed at all 9 possible combinations as reflected table

On the basis of the observations from the trial formulations, the combination of Acrypol® 934P and Menthol was fixed to further optimize the combination 3^2 Factorial design was applied. The results were statistically analyzed to report the optimzed combination.

The two independent variables selected were Acrypol® 934P (X_1) and Menthol (X_2). The combinations were evaluated for the dependent variable viz. Viscosity ($Y_{1)}$) and In vitro drug release (Y_2).

Table 2.: Variables in Optimization Study

Sr. No	Variables	Factors
1	Independent X_1 X_2	 Acrypol® 934 P Menthol
2	Dependent Y_1 Y_2	 Viscosity In vitro Drug release

Based on the results of preliminary batches the concentration ranges were determined (Shown in Table), these factors were evaluated at three levels (shown in Table 3 and 4.)

Table 3.: Selected Concentration ranges of Independent Variables :

Sr. No.	Independent variables	Concentration range (%w/w)
1	Acrypol ® 934 P	0.5 to 1.5
2	Menthol	2.5 to 4.5

Table 4.: Translational Coded Factor level

Coded value	Actual value (%w /w)	
	Acrypol ®934 P	Menthol
-1	0.5	2.5
0	1	3.5
+1	1.5	4.5

Following are combinations of the Acrypol ®934 P and Menthol are possible as per the 3^2 factorial design.

Table 5.: Experimental Design as per 3^2 Factorial design

Formulation code	X_1 (Coded value)	X_2 (Coded value)
F_1	-1	-1
F_2	0	-1
F_3	+1	-1
F_4	-1	0
F_5	0	0
F_6	+1	0
F_7	-1	+1
F_8	0	+1
F_9	+1	+1

In these combinations the concentration of drug and other excipients was kept constant. Different batches of emulgel formulation are shown in figure number 1.

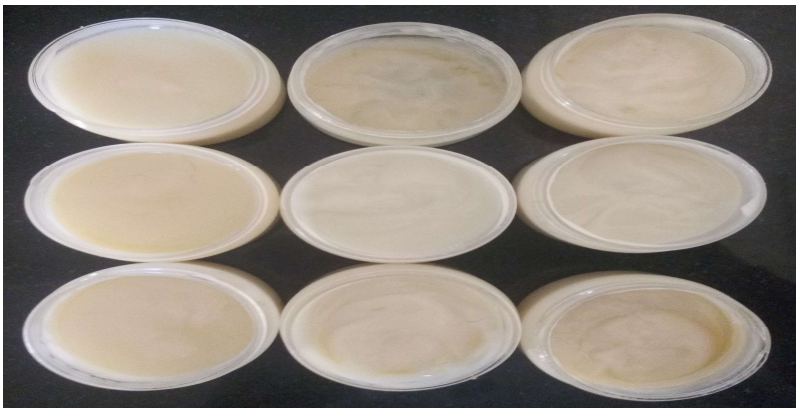


Fig 1. Different formulated batches of Pentoxifylline EP Emulgel.

The formulation batches alongwith the actual concentration of their ingredients are given in table number 6.

Table 6.: Formulation of Emulgel batches using 3^2 Factorial design.

Ingredients (%w/w)	Formulation codes								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F9
Pentoxifylline EP	1	1	1	1	1	1	1	1	1
Acrypol ® 934 P	**0.5**	**1**	**1.5**	**0.5**	**1**	**1.5**	**0.5**	**1**	**1.5**
Menthol	**2.5**	**2.5**	**2.5**	**3.5**	**3.5**	**3.5**	**4.5**	**4.5**	**4.5**
Ethanol	3	3	3	3	3	3	3	3	3
Wheat germ oil	15	15	15	15	15	15	15	15	15
Eucalyptus oil	1	1	1	1	1	1	1	1	1
Propylene glycol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glycerol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Span 80	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Tween 80	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Butylatedhydroxy Toluene	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Propyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Triethanolamine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100

Preformulation study of the Drug and Excipient:

Preformulation studies [3,4]:

Preformulation Study is the first step in rational development of dosage forms of a drug substance. Preformulation study is the process of optimizing the delivery of drug through determination of physicochemical properties of the new compound that could affect drug performance and development of an efficacious, stable and safe dosage form. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form. Hence, preformulation studies were performed on the obtained sample of drug for confirming the identity of drug.

Identification and Confirmation of Pure Drug Sample:

The Pentoxifylline EP drug sample was received from Bakul Pharma Pvt. Ltd., Ankleshwar. This sample was drug standardized by carrying out the following tests:

Description: The Pentoxifylline EP sample was evaluated visually for its appearance and color, odour, taste, nature.

Solubility determination: The solubility of Pentoxifylline EP was checked in different solvents like distilled water, phosphate buffer solution, ethanol and different oils.

Melting point determination: Melting point determination of the obtained drug sample was done as it is a first indication of purity of the drug sample. It was determined by capillary tube method.

Calibration curve of Pentoxifylline EP Drug in Phosphate Buffer pH-6.8 :

a) Preparation of Phosphate Buffer pH 6.8 :

28.7 gm of Potassium dihydrogen Phosphate was weighed accurately and dissolved in distilled water. The volume was made upto 1000 ml in volumetric flask. This Solution formed was labelled as Solution"A".

4 gm of Sodium Hydroxide was weighed accurately and dissolved in distilled water. The volume was made upto 500 ml in volumetric flask. This solution formed was labelled as solution "B".

Then 250 ml of solution "A" and 112 ml of Solution "B" was pipette out in 1000 ml volumetric flask and the volume was made upto 1000 ml with distilled water. This solution formed was labelled as phosphate buffer pH 6.8.

b) Determination of wavelength of maximum absorbance(λ max) of Pentoxifylline EP :

In order to ascertain the wavelength of maximum absobance (λ max) of the drug,

10mg of pure drug (Pentoxifylline EP) was weighed accurately and transferred into 10ml volumetric flask. The volume was made upto 10ml with phosphate buffer of pH 6.8 . This solution was labelled as stock solution- 1. (1000mcg/ml)

1 ml of sample from this stock solution-1 was pipette out and transferred to another 100ml volumetric flask and the volume was made upto 100ml mark with the phosphate buffer of pH 6.8 and formed solution was labelled as stock solution- 2. (10mcg/ml).

This solution-2 was scanned using spectrophotometer within the wavelength region of 400-200nm against phosphate buffer pH 6.8 as blank for determination of wavelength of maximum absorbance (λ max).

c) Standard Calibration Curve of Pentoxifylline EP in Phosphate Buffer pH 6.8 :

10mg of pure drug (Pentoxifylline EP) was weighed accurately and transferred into 10ml volumetric flask. The volume was made upto 10ml with phosphate buffer of pH 6.8 . This solution was labelled as stock solution- 1. (1000mcg/ml)

1 ml of sample from this stock solution-1 was pipette out and transferred to another 100ml volumetric flask and the volume was made upto 100ml mark with the phosphate buffer of pH 6.8 . This Solution was labelled as stock solution- 2. (10mcg/ml)

From this stock solution-2, samples of 0.2 ml, 0.4 ml, 0.6 ml, 0.8ml and 1ml were pipette out and the volume was made upto upto 10ml each for making the resultant concentration in the range of 2, 4, 6, 8 & 10mcg/ml respectively. All dilutions were made with Phosphate buffer of pH 6.8. Then the absorbance of all these solutions was measured against the blank solvent (i.e. phosphate buffer of pH 6.8) at the same wavelength at which maximum absorbance (λ max) was determined using UV- spectrophotometer. A standard curve was plotted with concentration on X-axis and absorbance on Y-axis.

d) Drug-Excipient Compatibility [5]:

Fourier Transform Infrared (FTIR) Spectroscopy Study:

The study carried out by FTIR Spectroscopy is to verify whether the drug and excipients are compatible with each other or not. IR spectroscopy can be used to investigate and predict any physiochemical interaction between different components in a formulation and therefore it can be applied to the selection of suitable chemical compatible excipients while selecting the ingredients, which are stable, compatible, cosmetically and therapeutically acceptable. The possible interaction between the selected gelling agent Acrypol ® 934 with drug Pentoxifylline EP was identified.

10mg of the sample and 400mg of KBr were taken in a mortar and triturated. A small amount of triturated sample was taken into a pellet maker and was compressed at 10 kg/cm^2 using a hydraulic press. The pellets was kept onto the sample holder and scanned from 4000 cm^{-1} to 400 cm^{-1} in FT-IR Spectrophotometer. Samples were prepared for pure gelling agent (Acrypol ® 934 P), pure drug (Pentoxifylline EP) and a physical mixture of drug and gelling agents (Pentoxifylline EP+ Acrypol ® 943P). The spectra obtained through those samples were compared and interpreted for the shifting of functional peaks and disappearance or appearance of new functional peaks.

Evaluation of the Emulgel:

The prepared formulation of emulgel was evaluated for it's different parameters like Physicochemical Studies, In vitro drug release study and Accelerated stability study. The detailed description is given below.

1) Physicochemical [6,7] :

a) Physical Examination :

The Emulgel was visually inspected for its different physical parameters like Colour, Homogeneity, Consistency and Texture, Phase separation, Odour, Taste, Grittiness.

Colour: Colour of the emulgel formulation was checked against white and black background.

Homogeneity : Homogeneity of the emulgel was checked by direct observation with naked eye under the microscope

Consistency and Texture : The consistency and texture of the emulgel was checked by applying it to the skin.

Phase Separation Study: The emulgel was centrifuged at 3000 RPM for 10 minutes and observed by visual inspection to evaluate if there is any creaming or phase separation.

b) pH Determination:

The pH of the emulgel was determined using Digital pH meter. 1% aqueous solution of Emulgel (1 gm of emulgel in 100 ml distilled water) was prepared and subjected to measure the pH by the Calibrated Digital pH meter. The test was performed in triplicate and the average pH value was noted.

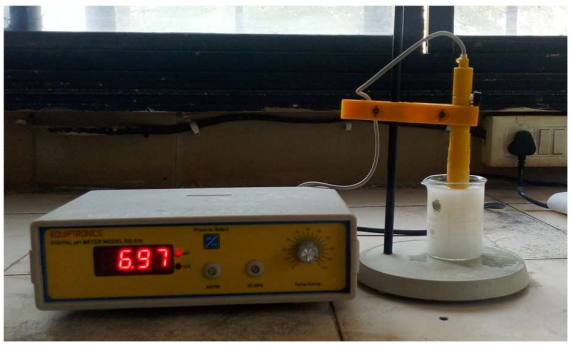


Figure 2.: Digital pH Meter

c) Rheological Characterization :

The emulgel contains aqueous phase, oil, surfactants and gelling agent as formulation components.The rheological properties of a dosage form like Viscosity and Flowability can be greatly affected by a small change in the physiological properties of the formulation components.The change in viscosity affect the stability factors , drug release and other biological functions.Taking these factors consideration, it is very essential to understand the rheological properties of emulgel.

Rheological characterization of samples were performed using Brookfield viscometer. The measurements were performed using spindle number 3 . Viscosity parameters were collected at different RPM with 1 minute equilibration time at every RPM. Different torque values at respective spindle speeds were obtained for an ascending and descending curve. Rate of shear and shearing stress were calculated by using the formula given below. Rheogram was constructed by plotting the shear stress verses shear rate. Results obtain are noted.

$$\text{Shear rate } (\dot{\gamma}) = \frac{2\omega.Rc^2.Rb^2}{X\,(Rc^2-Rb^2)}$$

Where,ω = Angular velocity of spindle (rad^{-1})

$$\omega = \frac{2\pi}{60} \times \text{Spindle speed (RPM)}$$

Rb = Radius of spindle

Rc = Radius of container

X = Radius at which shear rate is being calculated

$$\text{Shear Stress } (\sigma) = \frac{M}{2\pi.Rb^2.L}$$

Where,

M = Torque input by instrument

Rb = Radius of Spindle

L = Effective length of spindle

d) Spreadability/ Spreading Coefficient [8]:

For topical preparations spreadability is one of the important parameters. The delivery of the correct dose of drug is highly dependent on the spreadability of emulgel formulation. The spreadability is important for the ease of application of topical preparation and better patient compliance. It indicates whether the emulgel is easily spreadable by small amount of shear or not.

For the determination of Spreadability, A weighed quantity of emulgel (1gm) is placed on within a circle of 1 cm diameter pre-marked on a glass plate. Another glass plate was placed upon it and thus the emulgel was sandwiched between two glass plates. The spread in emulgel diameter was noted down.

The experiment was repeated in triplicate (n=3) and the average of such determinations was calculated for each formulation.

Figure 3. Spreadability Test Apparatus

e) Swelling Index [9]:

To determine the swelling Index of the prepared topical emulgel, 1 gm of Topical Emulgel was taken on the porous Aluminum foil which was then placed in a Petri dish containing 5 ml of phosphate buffer of pH 6.8. The sample was removed from Petri dish at different time intervals i.e. 10, 20, 30, 40, 50, 60 minutes respectively and put on a dry place for some time, then it is re-weighed. The average weight was used for further calculations. The Swelling Index was calculated by using the following formula:

Swelling Index (SW%)= [(Wt-W_0)/W_0 ×100]

Where,

SW% = Percentage of Swelling Index

Wt= Weight of Swollen Emulgel after time t,

W_0= Initial weight of Emulgel at zero time,

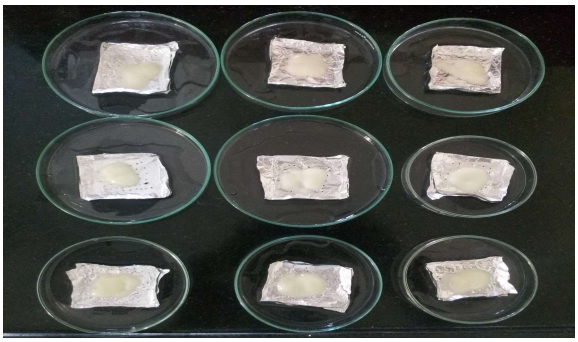


Fig.4.Determination of Swelling index.

2) Drug Content Determination [10]:

Drug concentration in emulgel was measured by using spectrophotometer. 1 gm of emulgel formulation was dissolved in 10 ml of solvent i.e. saline phosphate buffer pH 6.8. The formed solution was then diluted upto 1000 times. Filtration was done to obtain a clear solution. The absorbance of the resulting solution was analysed using UV visible spectroscopy. The Drug Content was determined by comparing it with the standard calibration curve of the Pentoxifylline.

3) *in vitro* drug release [11] :

For the In vitro drug release study, the diffusion cell apparatus was used. It is shown in figure number. A dialysis membrane is used as the separation membrane for receptor and donor compartments. The dialysis membrane separating the two compartments was obtained from egg shell membrane. Like human stratum corneum, egg shell is mainly made up of keratin. The content of the egg was detached by making a small hole to egg and the outer shell of the egg was dissolved by putting it in the concentrated hydrochloric acid for about 15 minutes. Then the membrane was put into the fresh water and washed gently prior use.

Fig.6.Franz Diffusion Cell apparatus

1 gm of Emulgel formulation was applied onto the surface of the egg shell membrane separating the donor and receptor compartments of the diffusion cell. A freshly prepared phosphate buffer (pH 6.8) was filled in the receptor chamber to solubilize the drug. The fluid in the receptor chamber was continuously stirred at 300 RPM by using a magnetic stirrer. The temperature of the receptor chamber was maintained at 37˚ C. The samples from the receptor chamber were collected for 6 hours at suitable time interval i.e. 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 minutes respectively from the sampling port and it was replaced with equal amount of fresh buffer. The collected samples were analyzed for drug content with the help of UV-visible spectrophotometer after appropriate dilutions. To obtain the total amount of drug released at each time interval, Cumulative corrections were made. The cumulative amount of drug release across the membrane was determined as a function of time. Then the cumulative percentage drug release was calculated using standard calibration curve.

4) Accelerated Stability Study [12]:

Accelerated stability study was carried out to assess the stability of the emulgel after storage. For this study, Stability Chamber was used.

Emulgel formulation was put in triplicate in stability chamber for observation under accelerated condition of 40°C±2°C/75%±2%RH for a period of 3 months. The Samples then withdrawn at an interval of 15 days, 30 days, 60 days and 90 days for Accelerated Stability Conditions. The collected samples were evaluated for it's physical appearance (visually inspected for any change in colour, odour and appearance) , pH, Rheological Properties (Viscosity, Flow behaviour) and Drug content.

Results and Discussion:

Preformulation studies of drug and excipient:

Table 7: Physical Characterisation of Pentoxifylline EP :

Experimental	Property Studied	Results
Organoleptic Properties	Colour	White
	Odour	Odourless
	Taste	Bitter
	Nature/ Texture	Smooth Amorphous Powder
	Solubility	Soluble in Water and Ethanol
Identification and Confirmation of Pentoxifylline EP	Melting Point	105°C

Standard Calibration curve of Pentoxifylline EP in Phosphate Buffer pH 6.8 :

The drug Pentoxifylline EP showed maximum absorbance (λ max) at the wavelength of 265 nm in Phosphate Buffer pH 6.8. The standard calibration curve of Pentoxifylline EP was taken from the absorbance obtained at different dilution concentrations.

Table 8.: Calibration curve of Pentoxifylline EP in Phosphate Buffer pH 6.8

Sr. No	Concentration (mcg/ml)	Absorbance
1	2	0.025
2	4	0.039
3	6	0.055
4	8	0.067
5	10	0.083

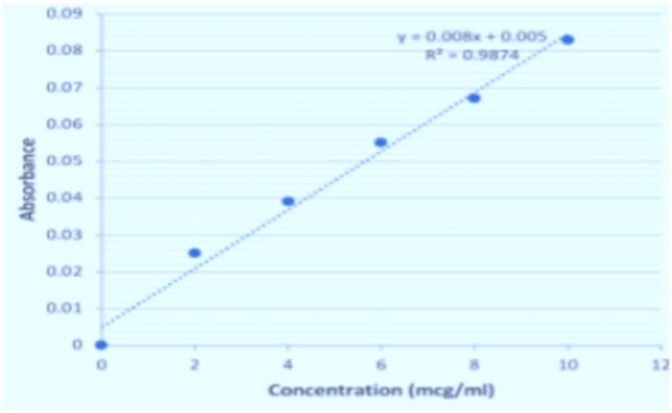


Fig.7.Calibration curve of Pentoxifylline EP in Phosphate Buffer pH 6.8 :

I.R Spectroscopy to predict the Compatibility of gelling agent with drug :

An I.R study was carried to check the compatibility between the selected gelling agent (Acrypol ® 934P) and Pentoxifylline EP. The spectra obtained for I.R studies at wavelength from 4000 cm^{-1} to 400 cm^{-1}. After interpretation through the spectra it was confirmed that there were no major shifting as well as no loss of functional peaks between the spectra of drug, gelling agent, physical mixture of drug and gelling agent. From the I.R studies it was concluded that, the selected gelling agent (Acrypol ® 934P) is compatible with the selected drug Pentoxifylline EP. The FTIR Spectrum are given in following figures.

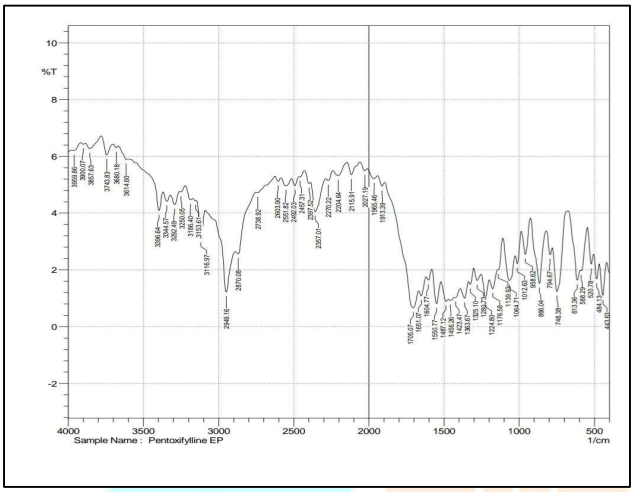


Fig. 8. FTIR Spectrum of Pentoxifylline EP.

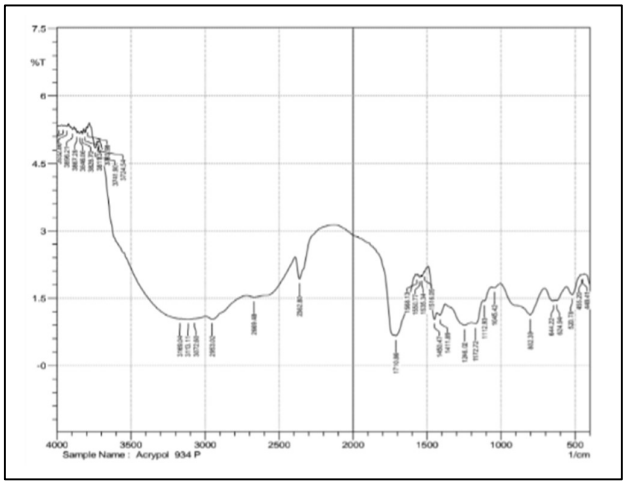


Fig. 9. FTIR Spectrum of Acrypol ® 934P

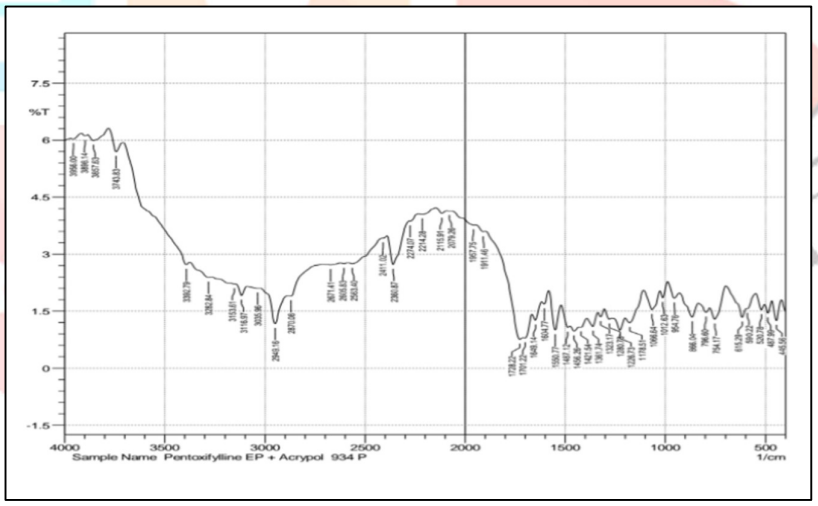


Fig. 10. FTIR Spectrum of Pentoxifylline + Acrypol ® 934P

Evaluation of Emulgel:

Physicochemical Studies:

Physical examination: All formulated batches of Pentoxifylline EP Emulgel were examined visually for their colour, homogeneity, Consistency, phase separation & texture. The results are shown in Table no. 9

Table 9: Physical Appearance of Pentoxifylline EP Emulgel:

Formulation Codes	Colour	Consistency	Homogeneity	Phase Separation	Texture
F_1	Creamy White	Uniform	Good	None	Smooth
F_2	Creamy White	Uniform	Good	None	Smooth
F_3	Creamy White	Uniform	Good	None	Smooth
F_4	Creamy White	Uniform	Good	None	Smooth
F_5	Creamy White	Uniform	Good	None	Smooth
F_6	Creamy White	Uniform	Good	None	Smooth
F_7	Creamy White	Uniform	Good	None	Smooth
F_8	Creamy White	Uniform	Good	None	Smooth
F_9	Creamy White	Uniform	Good	None	Smooth

pH determination :

pH of all the formulations of emulgel was measured by Digital pH meter, the readings were noted in triplicate and average values were calculated. The pH of the formulation was in the range of 6.86 to 7.23, which lies in the normal pH range that of salivary pH and would not produce any irritation to buccal mucosa. The results are shown in Table number 10.

Table 10.: pH values of F_1 to F_9 Formulation batches

Formulation Codes	pH
F_1	6.86
F_2	7.04
F_3	6.97
F_4	7.12
F_5	7.23
F_6	6.94
F_7	7.10
F_8	7.21
F_9	7.15

Rheological Characterization:

Rheological characterization of all the emulgel formulations was studied using Brookfield viscometer. The rheogram of all the formulated batches was constructed by plotting the Shear stress verses Shear rate. The data obtained is given in table number 11 and 12.

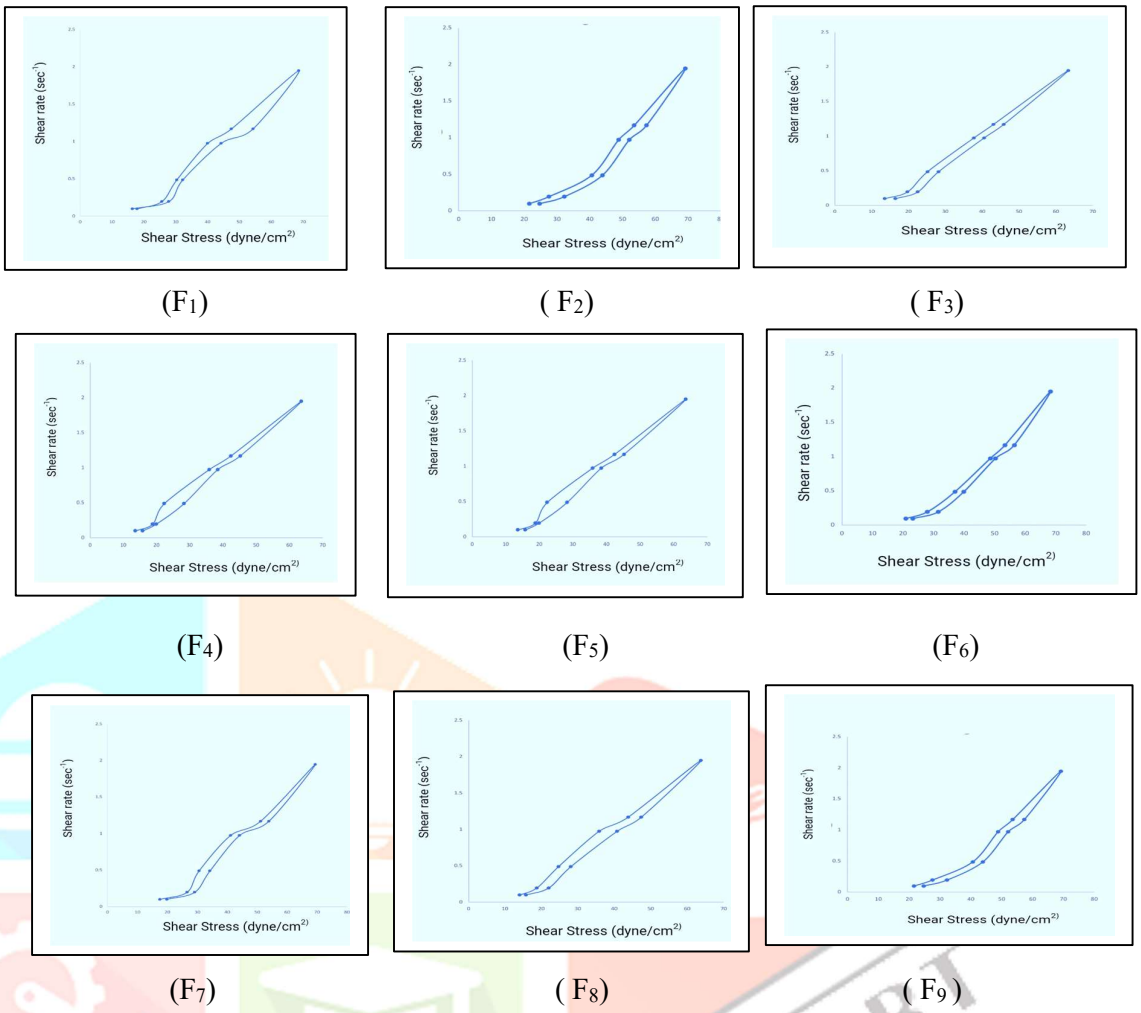
Table 11. Viscosity of different emulgel formulations (F_1 to F_9)

RPM	Viscosity of Emulgel formulations								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
1	36089	51574	63030	36218	50451	61452	35913.2	50812	63126
2	16834	26072	38213	17120	27218	35920	17326.3	26271	36742
5	5129	5607.9	9714	5236	5735	9216	6216.6	5718.6	9423
10	3016.3	3114.4	7253	3142.8	3314	6840	3158.2	3236.2	6875
12	2600.3	2762.4	4766	2826.3	2979.6	5118	2746	2967.1	5339
20	2589.1	2563.1	3887	2606.2	2693.2	4251	2614.3	2736.4	3976
12	2610	2686.7	4513	2913.2	2731.5	4962	2781.2	2941.6	5146
10	2916	3069.9	6839	3180.6	3436.2	6645	3276.5	3250.1	6684
5	5010	5314.6	8532	5325.2	5813.1	8421	6280.5	5662	9127
2	17025	25312	36704	18103	27629	32976	17433	27164	33986
1	35480	50406	57320	36862	51338	57614	36120.3	51631	59374

Table 12.: Shear rate and Shear stress of different emulgel formulations (F_1 to F_9)

Shear Rate (sec^{-1})	Shear Stress (dyne / cm^2)								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
0.097385283	23.21	15.84	19.71	27.75	16.44	16.38	24.66	15.72	19.83
0.194770566	31.55	19.95	27.08	34.58	22.49	27.81	32.28	22	29.14
0.486926416	39.90	28.29	34.58	41.23	28.17	32.25	43.89	28.05	34.22
0.973852832	50.29	38.45	45.22	51.99	40.50	44.25	52.11	40.74	44.13
1.168623398	56.46	45.22	50.90	58.52	45.82	54.28	57.31	47.39	53.92
1.947705663	68.31	63.59	69.64	68.55	63.35	68.67	69.16	63.71	69.40
1.168623398	53.44	42.44	48	56.58	43.04	47.51	53.56	43.77	51.14
0.973852832	48.60	35.91	41.35	49.81	37.72	40.01	48.84	35.79	41.11
0.486926416	36.99	22.37	27.81	39.53	25.15	30.35	40.62	24.66	30.59
0.194770566	27.93	18.86	22.49	33.01	19.71	25.63	27.57	18.74	26.60
0.097385283	20.92	13.66	15.72	23.58	13.54	17.89	21.52	14.02	17.53

The rheological behaviour of all emulgel formulations is shown in following figures.



(F1) (F2) (F3)

(F4) (F5) (F6)

(F7) (F8) (F9)

Figure 11. Rheological behaviour of all emulgel formulations.

Viscosity is an expression of the resistance of a fluid to flow; the higher the viscosity, the greater resistance. The rheological behaviour of the emulgel formulations was studied using a Brookfield viscometer. In all the rheograms a non-linear relationship was observed between shear stress and shear strain indicating a Non Newtonian system .

In case of Newtonian systems, if the rate of shear was reduced once the desired maximum rate had been reached, the downcurve would be identical to the superimposed up curve, whereas the down curve for non-Newtonian systems could be displaced with regard to the up curve. This indicates a breakdown of structure (and hence shear thinning) that does not reform immediately when the stress is removed or reduced. The recovery process is not instantaneous; rather, there is progressive restoration of consistency.

Non Newtonian fluids are further classified as Rheopectic fluids and Thixotropic fluids. Rheopecty or rheopexy is the property of non-Newtonian fluids to show a time-dependent increase in viscosity (time-dependent viscosity); the longer the fluid undergoes shearing force, the higher its viscosity. Whereas Thixotropy is a reversible, isothermal, time-dependent decrease in the apparent viscosity when a material is subjected to increased shear rate.

The reversibility of the process results that the viscosity recovers, i.e. structure builds up, again when the shear rate is eliminated or reduced.

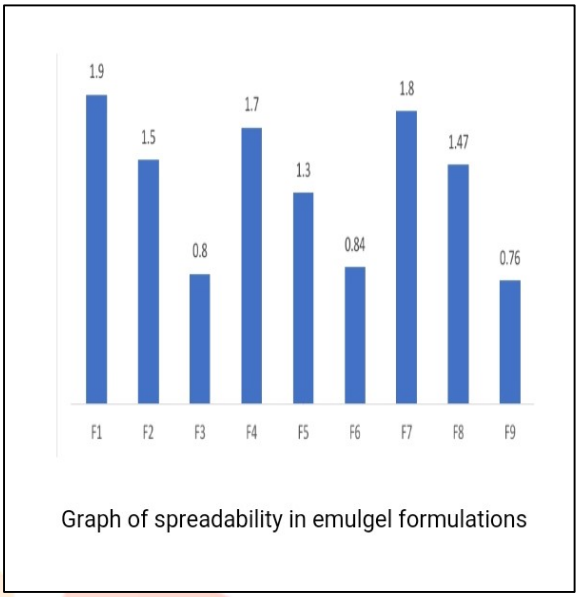
From the above rheogram curves we could conclude that all the emulgel formulation exhibited thixotropic flow behavior.

Spreadability :

Spreadability is important for absorption of drug through the skin. Spreadability was measured as discussed in experimental work. The readings were noted in triplicate and average values were calculated. The results are shown in Table no. 13. and in graph (Fig 12.)

Table 13: Spreadability values of F_1 to F_9 Formulation batches

Formulation Codes	Spreadability (cm)
F_1	1.9
F_2	1.5
F_3	0.8
F_4	1.7
F_5	1.3
F_6	0.84
F_7	1.8
F_8	1.47
F_9	0.76



Graph of spreadability in emulgel formulations

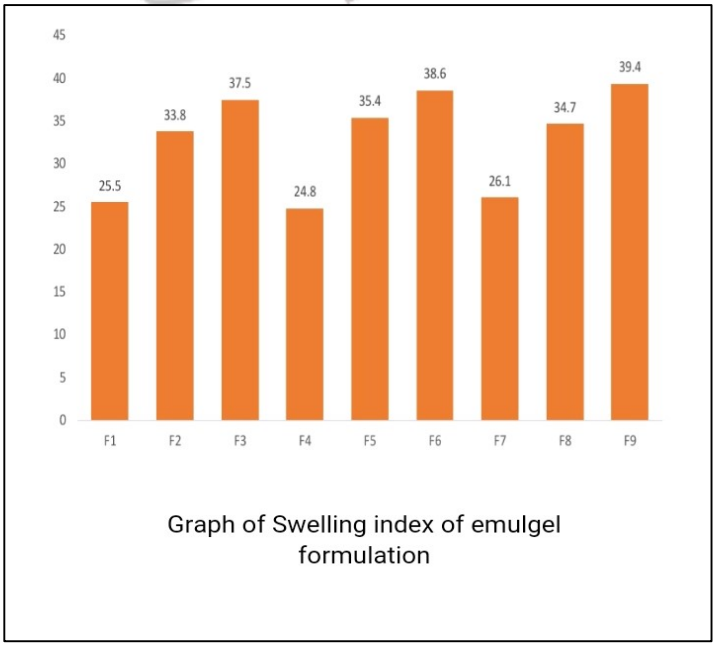
From the above table and graph it could be concluded that, the higher the concentration of gelling agents in the emulgel, lower will be Spreadability of the formulation.

Swelling index :

Swelling index was performed by using the specified method which was based upon the amount of Phosphate buffer pH 6.8 that can be absorbed by the emulgel. The readings were noted in triplicate and average values were calculated. The results are shown in Table no. 14 and in graph (fig. 13).

Table 14: Swelling index values of F_1 to F_9 Formulation batches

Formulation Codes	**Swelling Index (%)**
F_1	25.5
F_2	33.8
F_3	37.5
F_4	24.8
F_5	35.4
F_6	38.6
F_7	26.1
F_8	34.7
F_9	39.4



Graph of Swelling index of emulgel formulation

From the above table and graph, it could be concluded that higher the concentration of gelling agents in the emulgel formulations, higher will be swelling index of the emulgel.

Drug Content Determination:

The drug content of the Pentoxifylline EP Emulgel formulations was estimated spectrophotometrically at 265 nm. Drug content of the emulgel formulations was within the range of 95.6 to 97.2 % which is considered as acceptable range. The results are shown in table no. 15.

Table 15: Drug Content values of F_1 to F_9 Formulation batches

Formulation Codes	Drug Content (%)
F_1	96.6
F_2	97.2
F_3	95.7
F_4	96.7
F_5	97
F_6	95.6
F_7	96.1
F_8	95.8
F_9	96.2

In vitro drug release study :

In vitro drug release profile of Pentoxifylline EP from its various formulated emulgel was determined by Franz diffusion cell and results are being depicted in Table no. 16. and figure number 14.

Table 16.: Drug release of emulgel formulations.

Time (minutes)	% Drug Release								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
0	0	0	0	0	0	0	0	0	0
30	3.63	3.06	2.37	5.51	3.34	3.27	6.13	3.58	3.19
60	7.81	6.36	5.3	11.26	7.04	6.87	12.81	7.63	6.57
90	12.28	10.1	8.96	17.67	11	10.7	19.59	12.08	10.29
120	17.41	14.04	12.84	24.68	15.35	15.04	26.6	16.89	14.23
150	23.4	18.46	17.58	31.79	19.96	19.56	33.84	21.99	18.57
180	29.82	23.39	22.56	39.11	25.08	24.53	40.88	27.63	23.13
210	36.7	28.78	27.62	46.56	30.41	29.9	48.2	33.54	28.14
240	43.97	34.88	33.39	54.14	36.31	35.67	55.84	39.86	33.66
270	51.39	41.9	39.56	61.88	42.53	41.84	63.7	46.87	39.64
300	58.96	49.32	46.33	69.7	49.42	48.26	71.78	54.19	46.49
330	66.79	57.15	53.42	77.64	56.83	55.15	80.12	61.79	53.53
360	74.82	65.35	60.82	85.17	64.91	62.88	89.29	69.86	61.26

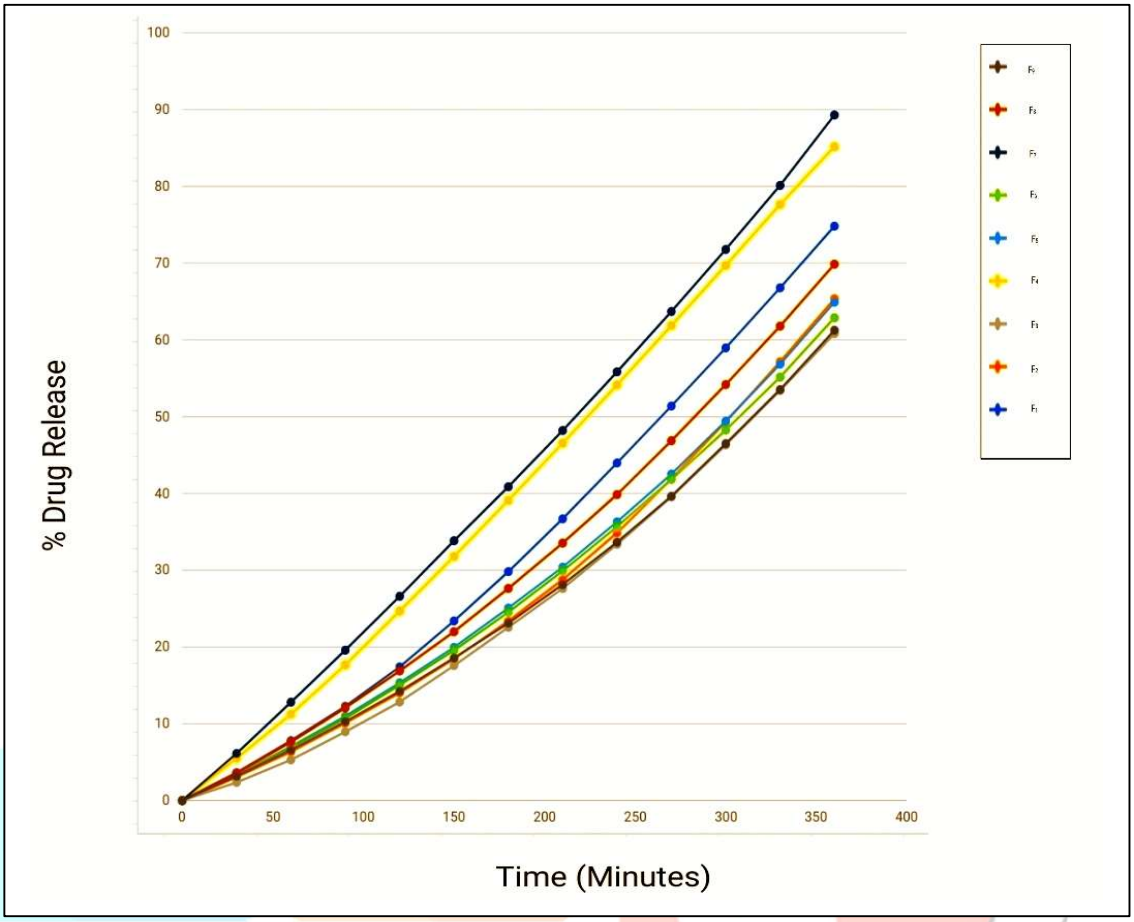


Fig .14. In vitro percentage of Drug release.

From the above table and figure it could be concluded that F_4 and F_7 Formulation has maximum percentage of in vitro drug release. This may be due to low concentration of gelling agent (Acrypol ® 934P) polymer and high concentration of penetration enhancer (Menthol). So, F_4 and F_4 Formulation batches are the Optimized emulgel formulation batches.

Accelerated stability study:

Accelerated stability study of all emulgel formulations was carried out. All the formulations of emulgel wre found to be of White Creamy colour, Uniform consistency, Good homogeneity with no phase separation, smooth texture. The pH and drug content were within the acceptable range. The results obtained are shown in table number 17.

Table 17. Accelerated stability study of different emulgel formulations

Formulation Codes	Test Parameters						
	Colour	Consistency	Homogeneity	Phase separation	Texture	pH	Drug Content (%)
F_1	Creamy White	Uniform	Good	None	Smooth	6.81	96.1
F_2	Creamy White	Uniform	Good	None	Smooth	7.1	96.5
F_3	Creamy White	Uniform	Good	None	Smooth	7.11	95.2
F_4	Creamy White	Uniform	Good	None	Smooth	7.10	96.3
F_5	Creamy White	Uniform	Good	None	Smooth	7.21	96.7
F_6	Creamy White	Uniform	Good	None	Smooth	6.92	95.4
F_7	Creamy White	Uniform	Good	None	Smooth	7.12	95.8
F_8	Creamy White	Uniform	Good	None	Smooth	7.18	95.6
F_9	Creamy White	Uniform	Good	None	Smooth	7.17	96

Factorial design analysis[13]:

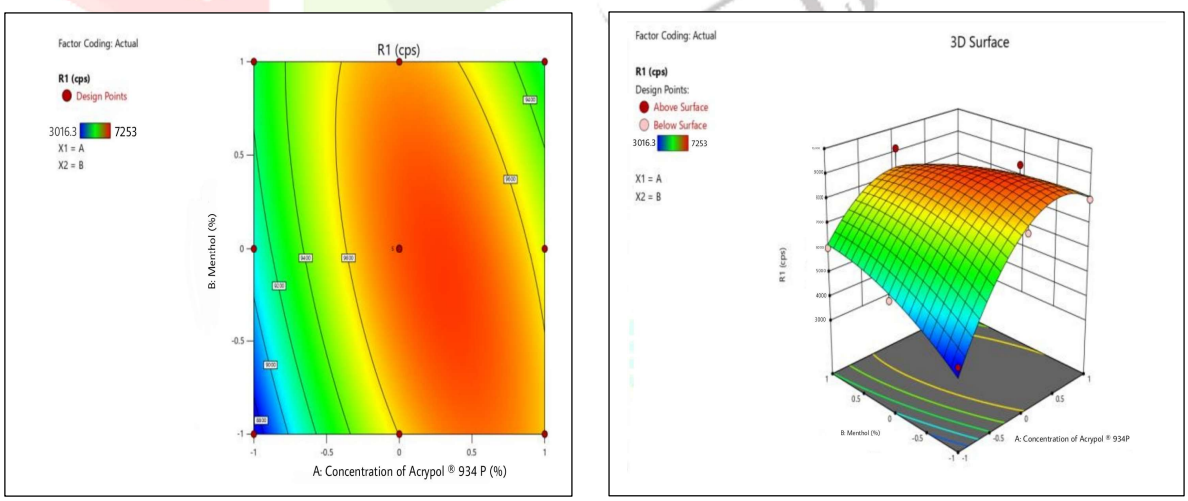


Fig. 15. Contour plot and Response 3D surface plot of Acrypol ® 934P and Menthol for Viscosity (%)

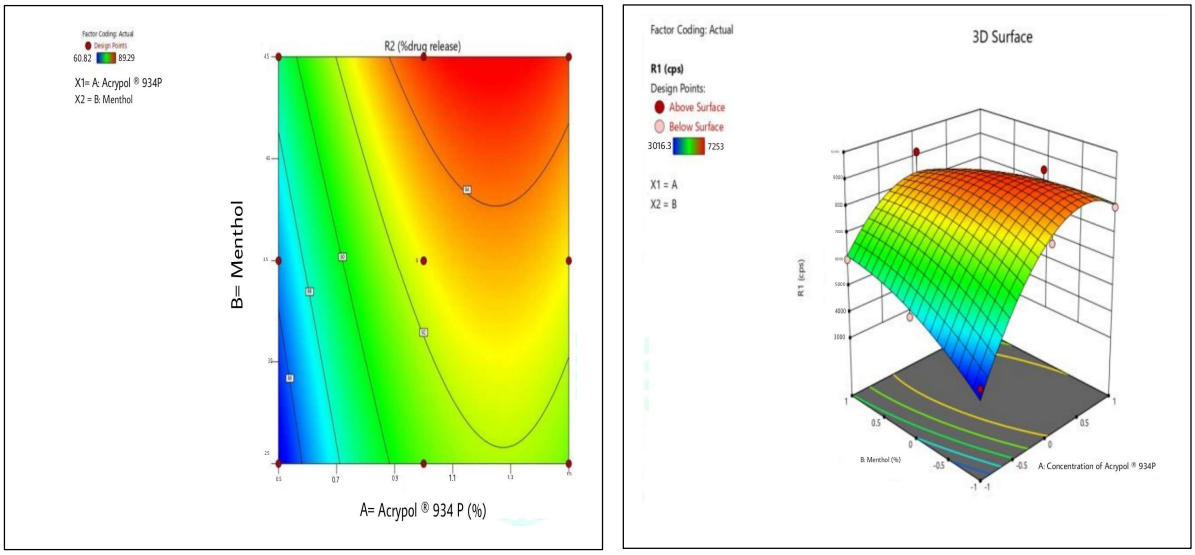


Fig.16. Contour plot and Response 3D surface plot of Acrypol ® 934P and Menthol for % drug release.

A 3^2 factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of Acrypol ® 934P (X_1) and Menthol (X_2) were selected as independent variables and the dependent variables were viscosity and *in vitro* drug release. The data obtained were treated using Minitab® statistical software 21.1.1.0 trial version. The data were also subjected to 3D response surface methodology to study the interaction of Acrypol ® 934P (X_1) and Menthol (X_2) on dependent variables.

The amount of viscosity from the F1-F9 batches of the emulgel varried from 3016.3 to 7253 cps. From the p value 0.0500 it can be concluded that Acrypol ® 934P and Menthol have prominent effect ($p < 0.05$) on viscosity.

The amount of *in vitro* percentage drug release from F1-F9 batches of emulgel varied from 60.82% to 89.29. From the p-value 0.0500 it can be concluded that Acrypol ® 934P and Menthol have prominent effect ($p < 0.05$) on %drug release.

The data clearly indicates that viscosity and percentage of in Vitro drug release were strongly dependent on the selected independent variables.

CONCLUSION :

From the Contour plot and Response surface plot of Acrypol ® 934P and menthol for the Viscosity and in Vitro drug release, it can be concluded that the viscosity and in vitro drug release were strongly dependent on these independent variables.

In vitro drug release study of the emulgel formulation had shown that F_4 and F_7 Formulation batches had maximum percentage of drug release. It may be due to the lower concentrations of gelling agent Acrypol ® 934 P i.e. 0.5% and higher concentration of penetration enhancer (menthol) i.e. 3.5 and 4.5 % in F_4 and F_7 Formulation batches respectively. So they are considered as the optimized batches.

FUTURE SCOPE :

Oral submucous fibrosis (OSMF) is a chronic and latent OSMF-relevant diagnosis and clinical malignant disease, which poses a global and regional problem reported in recent years, to public health, especially in East and Southeast Asia where areca nut chewing is popular. The malignant transformation rate of OSMF to oral squamous cell carcinoma (OSCC) accounts for 7% -13%

The Pentoxifylline EP emulgel may prevent the conversion of Oral Submucous Fibrosis form precancerous stage to Oral Cancer.

The formulated emulgel showed better results for physical parameters like pH,rheological behaviour, spreadability, swelling index, drug content, accelerated stability study and *in vitro* drug release.

Further *in vivo* study of the same may be carried out for the confirmation of the Pentoxifylline EP emulgel potency to cure the problem of Oral Submucous Fibrosis.

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