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FORMULATION AND EVALUATION OF LYCOPENE EMULGEL

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Abstract:

The present work was carried out with the grail of formulating a gellified emulsion of lycopene, an anti-oxidative agent. The distinctive feature of topical drug delivery system is the direct accessibility of the skin as a target organ for diagnosis and treatment. Emulgels have emerged as one of the most prevailing drug delivery systems for the delivery of hydrophobic drugs owing to their dual control release system i.e. gel and emulsion. Lycopene, as a natural source of anti-oxidants, has enamoured attention due to its biological and physicochemical properties. It is completely insoluble in water, so to overcome this limitation, an emulsion based approach was being used so that even this hydrophobic moiety can enjoy the unique property of gels. Lycopene, an anti-oxidative agent, has been used in the treatment of various oxidative diseases. This pigment protects the cells against damage from the free radicals formed when body cells burn oxygen for energy. In order to decrease the oxidative reactions with skin i.e. to treat acne vulgaris, lycopene emulgel was developed. This work was conducted to develop an emulgel of lycopene using three different gelling agents i.e. Carbopol 934P, HPMC LV-15 and NaCMC. Oleic acid was used as a penetration enhancer. The gellified emulsions were characterized for their physical appearance, rheology, spreadability, drug content and stability. In-vitro release studies were conducted to check the drug release through egg membrane. The formulations were evaluated for their antioxidant activity as well as their acute skin irritation potential. Formulation F1 was found to have fallen within the stipulated criteria of all the evaluation parameters. Hence, it was concluded that formulation F1, containing carbopol 934P (1% w/w), was the optimized formulation. It exhibited the maximum drug release and antioxidant activity, in addition to the least skin irritation potential.

Keywords: *Lycopene, Emulgel, Gelling agents, Anti-oxidant, gellified emulsion.*

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INTRODUCTION

Lycopene, the predominant carotenoid in tomatoes, exhibits the highest antioxidant activity and singlet oxygen quenching ability of all dietary carotenoids. It inhibits the free radical formation in the body. Oxidative stress, induced by reactive oxygen species (ROS), is associated with the incidence of chronic diseases. ROS are the highly reactive oxidant molecules that are generated endogenously through normal metabolic processes, life style activity and diet. Lycopene shows considerable scientific interest in prevention of degenerative diseases [1].

When gel and emulsion are used in combined form, the dosage form is referred to as emulgel. Emulgels have emerged as one of the most interesting topical delivery systems as they have dual release control system i.e. gel and emulsion. The emulsifying agent, the oil phase and a gelling agent constitute the major component of gellified emulsion formulation as they contribute to the physicochemical properties of the final formulation. In spite of having so many advantages gels are having one major disadvantage i.e. being aqueous based they cannot solubilize hydrophobic drugs and to overcome this difficulty emulgels have been developed. The major objective behind this formulation is delivery of hydrophobic drugs to systemic circulation via skin. In fact presence of a gelling agent in water phase converts a classical emulsion in to emulgel. The emulgel for dermatological use has several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life, bio-friendly, transparent and pleasing appearance. Further stability of emulsion is increased when incorporated into gel. The excipients which are to be optimized in the formulation of emulgels is the concentration of oils and gelling agents. The conventional and topical preparations such as ointments, creams, lotions, have certain disadvantages such as being sticky causing uneasiness to the patient, have lesser spreading coefficient, need to be applied with rubbing and exhibit the problem of stability. Due to all these factors within the group of semisolid preparations, the use of emulgels has expanded in pharmaceutical preparations [2].

MATERIALS AND METHODS

Lycopene was procured from Vashishtha Pharmaceuticals, Gudgaon as a gift sample. Carbopol 934P, NaCMC, HPMC-LV 15, Triethanolamine, Propylene glycol, Span 80, Tween 80, Olive oil, Oleic acid, Methyl Paraben and other chemical and solvents were of analytical grade/IP/equivalent gradeand procured from laboratory.

Identification by IR Spectroscopy

The I.R absorption spectrum of lycopene was recorded using dispersive powder technique. Drug sample was directly scanned as powder over range of 4000-400 cm^{-1}.

Determination of Solubility of Lycopene by Analytical Method Using UV-Spectrophotometer

An excess quantity of lycopene was dissolved in 5ml of chloroform and stirred magnetically at 100 rpm, then allowed to stand for 24 hr at room temperature. The solution was then passed through a Whatmann (No.42) filter paper and the amount of the drug dissolved was analyzed spectrophotometrically (UV-Visible spectrophotometer 1800).

Determination of Melting Point by Capillary Method Using Melting Point Apparatus-

Melting point of lycopene was determined by capillary method using the melting point apparatus.

Determination of Viscosity of Polymers (1% w/v)

A 1% w/v dispersion of the three polymers i.e. Carbopol 934P, NaCMC and HPMC LV-15 were prepared and the viscosity of these polymers was determined using spindle 96 with the help of Brookfield Viscometer.

Determination of Absorption Maxima (λ_{max})

UV-Visible spectral analysis of lycopene was done by using a Shimazdu double beam UV-Visible spectrophotometer 1800 model with a matched pair of Quartz cell (Optiglass U.K. Limited) with double beam was used. Accurately weighed 100 mg lycopene was transferred to 100 ml volumetric flask and dissolve in chloroform. Volume was made up to mark with chloroform to make a 1000 ppm solution. 25 ml of stock solution was diluted to 100 ml with chloroform to make 250 ppm solution and the absorbance was measured between the wave-length of 200-800 nm.

Preparation of Calibration Curve of Lycopene

100 mg of lycopene was dissolved in 100 ml of chloroform to prepare a stock solution of 1000 ppm. 10 ml of this solution was placed in 100 ml volumetric flask and diluted to mark with chloroform to make a 100 ppm solution of lycopene. Further, 1 ml of solution was pipette out from 100 ppm stock solution and diluted to 10 ml with chloroform to make 10 ppm solution. Aliquots of 2, 3, 4-9 ml were transferred into a series of 10 ml volumetric flasks and volume made up to mark with chloroform, so as to produced standard solution containing 20, 30, 40-90 µg of lycopene/ml. The absorbance was measured at 483.5 nm.

Stability of solution
The stock solution of lycopene was kept for 24 hours to assess any possible degradation and again the calibration curve was plotted. There was no difference in the slope of both the calibration curves at 0 and 24 hours.

Preparation of Lycopene Emulgel
The steps involved in the preparation of the gellified emulsion include the preparation of the emulsion phase, followed by the addition of the emulsion into an aqueous solution of the gelling agent, to form a semisolid formulation. The oil phase of the emulsion was prepared by dissolving the lipophillic surfactant (span 80) in olive oil while the hydrophilic surfactant (Tween 80) was dissolved in de-ionized water to obtain the aqueous phase. The selection of oil for preparation of lycopene emulgel was based on solubility studies conducted initially using different oils such as light liquid paraffin, eucalyptus oil, clove oil and olive oil. A clear homogenous solution resulted using olive oil and lipophillic surfactant span 80. In formulations F_1, F_2 and F_3 the gel bases were prepared by dispersing carbopol 934P in de-ionized water with constant stirring at a moderate speed using magnetic stirrer. Formulations F_4, F_5 and F_6 were prepared by using NaCMC as a gelling agent and formulations F_7, F_8 and F_9 were prepared by dispersing HPMC-LV15 in heated distilled water (75^{o}C) and the dispersion was cooled and left overnight. The pH of all the formulations was adjusted to 5.5 to 6.5 using triethanolamine. Methyl and propyl paraben were dissolved in propylene glycol and mixed with aqueous phase. Lycopene, being hydrophobic was dissolved in oil phase. Oleic acid was also mixed in oil phase as a penetration enhancer. Both the oil and aqueous phases were separately heated to 70^{o}C to 80^{o}C, then the oily phase was added to the aqueous phase with continuous stirring and allowed to cool to room temperature. The prepared emulsion was mixed with the gel in 1:1 ratio with gentle stirring to prepare the emulgel. The formulation composition of different batches of lycopene emulgel is presented in Table 6.

Physical examination

The prepared gellified emulsions were inspected visually for their color, appearance and homogeneity.

pH Determination
The pH of the prepared gellified emulsion was determined by using a digital pH meter. 1 gm of the gellified emulsion was stirred in distilled water, until a uniform dispersion was formed. It was kept aside for 2 hours. The volume was then made up to 100 ml. Then, the pH was measured. The test was performed in triplicate using a digital pH meter and the mean ± SD was calculated.

Spreading Coefficient
Spreading coefficient was determined by the apparatus suggested by Multimer *et al* (1956). It consists of a wooden block, which is attached to a pulley at one end. Spreading coefficient was measured on the basis of 'slip' and 'drag' characteristics of emulgel. A ground slide was fixed on the wooden block. An excess of emulgel (about 2 gm.) under study was placed on this ground slide. Emulgel preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 1 kg was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of emulgel between the two slides. Measured quantity of weight was placed in the pan attached to the pulley with the help of hook. The time (in sec) required by the top slide to move a fixed distance was noted. A shorter interval indicates better Spreading coefficient. It is calculated by using the formula:

$$S = M.L / T$$

Where, **M** = weight tied to upper slide.

L = length of glass slides/distance of travel.

T = time taken to travel a fixed distance.

Rheology
The viscosity of the formulated batches was determined using a Brookfield Viscometer with spindle 96. The formulation whose viscosity was to be determined was placed in the beaker and was allowed to settle down for 30 min. at room temperature before the measurement was taken. Spindle was lowered into the centre of Emulgel taking care that spindle does not touch bottom of the beaker and rotated at a speed of 10, 20, 50 and 100 rpm. The viscosity reading was noted down and the averages of three readings were taken.

Extrudability
Extrudability test is based upon the determination of the weight required to extrude 0.5 cm ribbon of emulgel in 10 seconds from lacquered collapsible aluminum tube. The test was performed in triplicate and average values were calculated using the following formula.
Extrudability=Weight applied to extrude emulgel from tube (gm)/area (cm^2)

Determination of drug content

Accurately weighed one gm of emulgel was dissolved in q. s. 100 ml chloroform. The volumetric flask was kept for 2 hours and shaken well to mix properly. The solution was filtered through Whatmann filter paper and suitably diluted. The absorbance of the solution was measured spectrophotometrically at 483.5 nm.

***In - vitro* drug release**

Franz diffusion cell (with effective diffusion area 3.14 cm^2 and 15 ml cell volume) was used for the drug release studies. Egg membrane was used for the release study. The egg membrane was obtained from egg by keeping an egg in a 10% HCl solution until the egg shell dissolved completely. Further, the egg was punctured and egg yolk was discarded and egg membrane was obtained, washed completely with de-ionized water and used. The membrane was previously treated with chloroform and soaked overnight in the chloroform at refrigeration temperature.

The emulsified gel (1gm) was applied onto the surface of egg membrane evenly. The membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with chloroform solution to solubilize the drug and was stirred magnetically. The samples (1 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content spectrophotometrically at 483.5 nm after appropriate dilutions. Cumulative amount of drug release at each time interval was computed. The cumulative amount of drug released across the egg membrane was determined as a function of time.

RESULTS AND DISCUSSION

Transmittance peaks exhibited in the recorded IR spectrum of lycopene were compared with peaks exhibited in the reported spectrum of lycopene and were found to be similar as presented in Table 1.

Table 1: Comparison between the Reported and Recorded Ir Peaks of Lycopene

Peak No.	Wave no.(cm^{-1})	Absorption Frequency Band	Characteristic Functional group/vibration
1.	3196	3040-3010	C-H Stretching(sp^2)
2.	2916	2960-3010	C-H Stretching(sp^3)
3.	1639	1680-1620	C=C Stretching(trans)
4.	1339	1300-1350	CH_2 (Bending)
5.	990	1080-1120	CH (Trans OOP)
6.	857	1000-650	=C-H bend
7.	804	1000-650	=C-H bend
8.	612	700-610	R_2C=CR

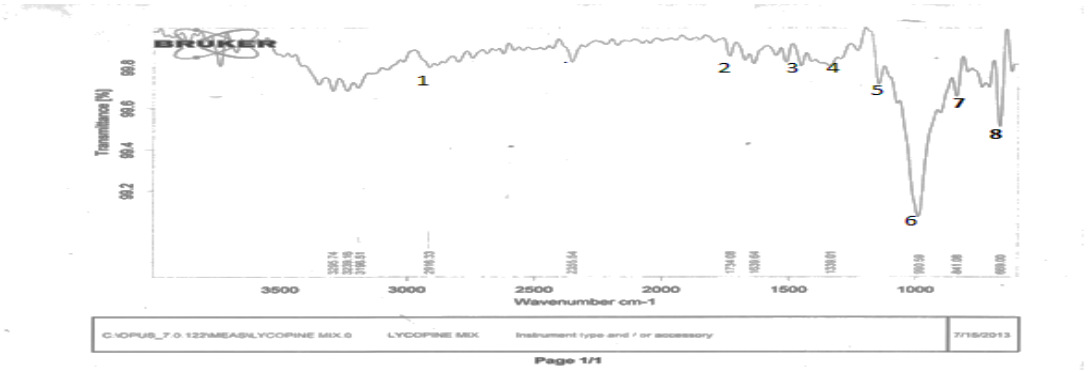

Fig.1: Recorded IR Spectrum of Lycopene

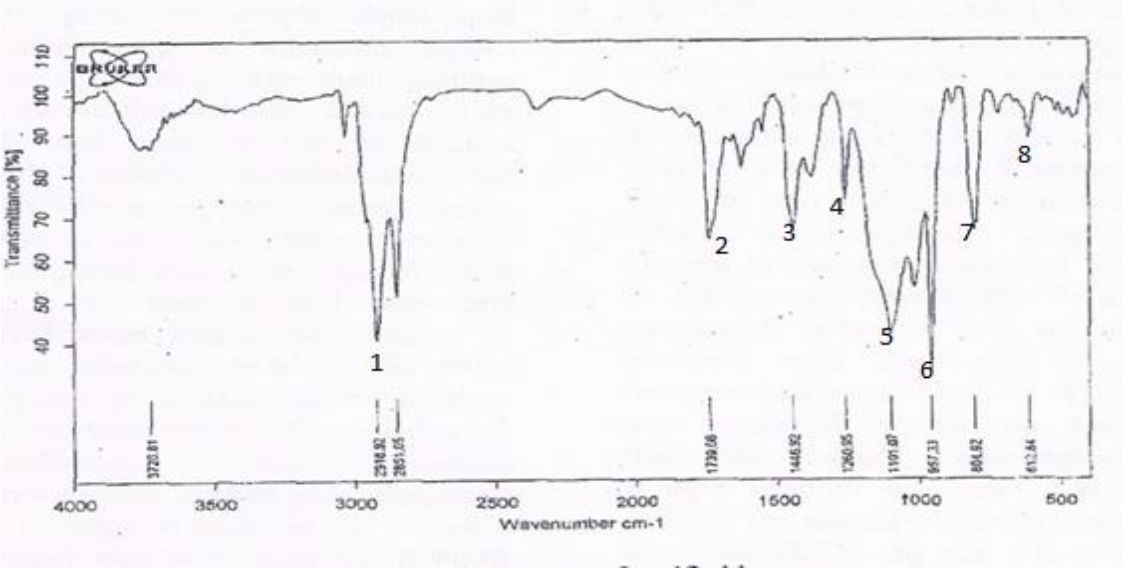

Fig. 2: Reported IR Spectrum of Lycopene

0.15 ml of chloroform is required to dissolve 50 mg of lycopene; hence, it is justified that 15 ml of chloroform shall be used as medium in receptor compartment of Franz diffusion Cell for release studies. 2.95 parts of chloroform are required to solubilize 1 part of lycopene; hence, lycopene is freely soluble in chloroform and can be suitably used for determination of drug content in suitably prepared emulgel formulations. The solubility of lycopene is presented in Table 2.

The melting point of lycopene was found to be 171ºC which is within the reported range hence the sample of lycopene is free of impurities (Table 3).

Table 2: Solubility of Lycopene

Solvent	Solubility (mg/ml)	Volume of the medium required to dissolve one dose of the drug (ml)	Parts of the solvent required to dissolve one part of the drug
Chloroform	338.57	0.15	2.95

Table 3: Melting Point Of Lycopene

Name of Drug	Reported melting point	Observed melting point
Lycopene	172-173ºC	171ºC

The λ_{max} of the Lycopene was found to be 483.5 nm. The spectrum is presented in Fig. 3.

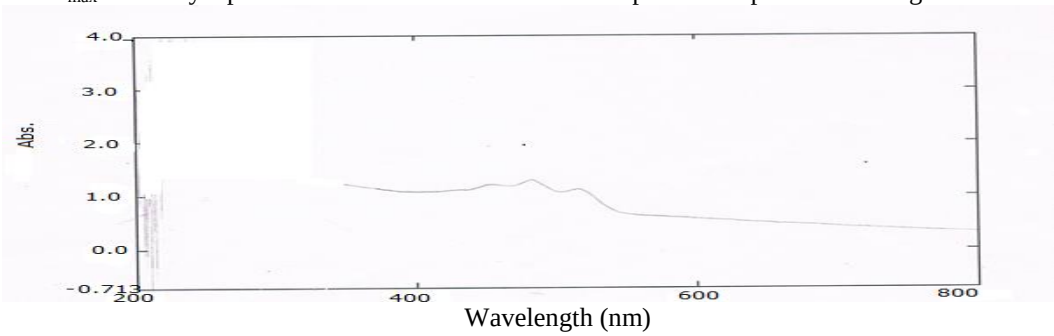

Fig. 3: UV-Spectrum of Lycopene in Chloroform

Table 4: Viscosity of Polymers (1% W/V)

rpm	Carbopol 934P	NaCMC	HPMC LV-15
	Viscosity (cPs)*	Viscosity (cPs)*	Viscosity (cPs)*
10	40068.7±166.2	10257.9±185.1	12393.7±93
20	35087.5±4.2	7563±54.2	9020±71
50	24290.6±2.4	5432±64	6417±37.5
100	19206.8±2.6 1.6	2437.5±59.3 0.4	4118.7±54.9 0

* Data indicates mean ± std. deviation of triplicate determinations.

Table 5: Data for Calibration Curve of Lycopene In Chloroform

S.No. No	Conc. (µg/ml)	Absorbance			
		A_1	A_2	A_3	$A_{average}$
1.	20	0.1654	0.1657	0.1660	0.1657
2.	30	0.2344	0.2345	0.2347	0.2345
3.	40	0.3029	0.3032	0.3035	0.3032
4.	50	0.3854	0.3858	0.3862	0.3858
5.	60	0.4590	0.4595	0.4600	0.4595
6.	70	0.5425	0.5429	0.5433	0.5429
7.	80	0.6105	0.6109	0.6113	0.6109
8.	90	0.6926	0.6928	0.6930	0.6928

Beer's law was obeyed over the concentration range of 20 to 90 µg/ml at 483.5 nm wavelength for lycopene. The slope and intercept of calibration curve (Fig. 4) were found to be 0.0007 and 0.007 respectively with correlation coefficient of 0.999.

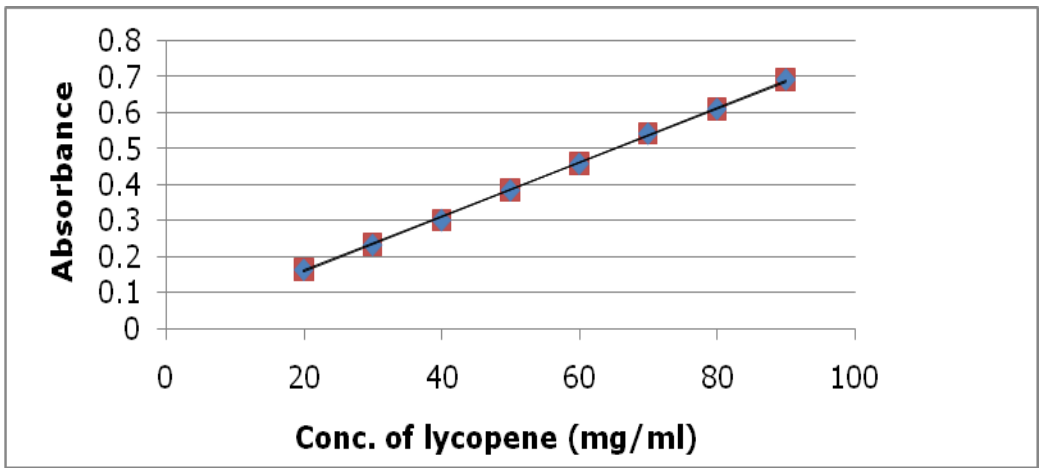

Fig. 4: Calibration curve of Lycopene in Chloroform at 483.5 nm.

Table 6: Formulation Composition of Lycopene Emulgel (%W/W)

	Batch code	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Oil phase	Lycopene	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Oleic acid	2	2	2	2	2	2	2	2	2
	Olive oil	4	4	4	4	4	4	4	4	4
	Spans 80	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aqueous phase	Propylene glycol	5	5	5	5	5	5	5	5	5
	Tween 80	1	1	1	1	1	1	1	1	1
	Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Propyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gel phase	Carbopol 934P	**1**	**1.25**	**1.5**	-	-	-	-	-	-
	Sodium CMC	-	-	-	**5**	**5.5**	**6**	-	-	-
	HPMC-LV15	-	-	-	-	-	-	**6**	**7**	**8**
	Water	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
	Triethanolamine was added to adjust pH of all the formulations from 5.5 to 6.5									

The prepared gellified emulsions were pink, viscous, and creamy preparations, with a smooth and homogenous appearance. The pink color of formulation diminishes as the concentration of polymer increases, as given in Table 7.

Table 7: Physical Appearance of Lycopene Emulgel

Formulation	Color	Homogeneity	Texture
F1	Pink	Homogeneous	Smooth
F2	Pink	Homogeneous	Smooth
F3	Light pink	Homogeneous	Smooth
F4	Pink	Homogeneous	Smooth
F5	Pink	Homogeneous	Smooth
F6	Light pink	Less homogeneous	Smooth
F7	Pink	Less homogeneous	Smooth
F8	White	Homogeneous	Smooth
F9	White	Not homogeneous	Less smooth

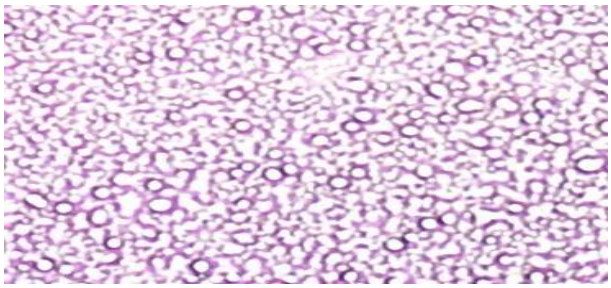

Fig. 5: Optical Micrograph of Formulation F1 Containing 1% Carbopol 934P (Gelling Agent)

The pH of all the gellified emulsions was found to be in the range of 5.9 to 6.6, which lies in the normal pH range of the skin. The results are shown in Table 8.

The spreadability indicates the ease with which emulgel is spreadable by the amount of shear. From Figure 5 it is clear that the spreading coefficient for the prepared lycopene emulgel formulation in the descending order was F1>F7>F8>F9>F2>F4>F5>F3>F6. Formulation F1 gave the highest value for spreadability. The effect of various polymers on spreading coefficient is shown in Figure 9. From Figure 8, it is seen that with increase in concentration of polymers: Carbopol 934P, NaCMC and HPMC-LV15 a significant decrease ($p \leq 0.05$, *df* 2 and 6, F_{crit}= 5.143, F= 722896.2 for Carbopol 934P, F=66.102 for NaCMC and F=174.699 for HPMC LV-15, single factor ANOVA) was noted in the spreadability of the prepared emulgel formulations. The spreadability is very important since it shows the behaviour of the emulgel when it comes out from the tube. Spreadability coefficient of prepared lycopene emulgel is shown in Table 9 and Figure 7.

Table 8: pH of Prepared Lycopene Emulgel

Formulation	Mean pH ± S. D.	Formulation	Mean pH ± S. D.
F1	6.3±0.3	**F6**	6.2±0.1
F2	6.6±0.2	**F7**	6.3±0.2
F3	6.3±0.3	**F8**	5.9±0.6
F4	6.1±0.1	**F9**	6.4±0.4
F5	6.5±0.5		

* Data indicates mean ± std. deviation of triplicate determinations

Table 9: Spreadability Coefficient of Lycopene Emulgel

Formulation	Spreading coefficient* (gm.cm/sec)
F_1	27.5±2.02
F_2	3.47±0.63
F_3	2.17±0.73
F_4	2.94±1.05
F_5	2.68±0.96
F_6	1.51±0.37
F_7	13.8±3.70
F_8	8.92±2.64
F_9	4.63±1.43

* Data indicates mean ± std. deviation of triplicate determinations.

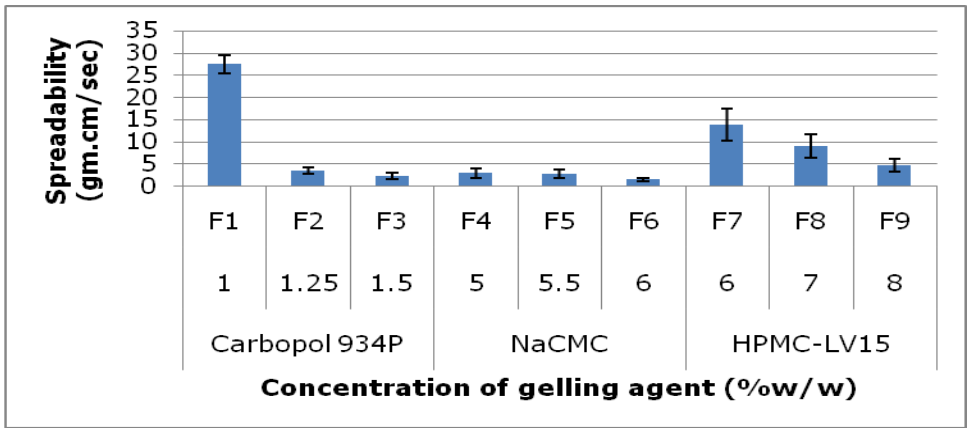

Fig. 6: Spreadability of Prepared Lycopene Emulgel

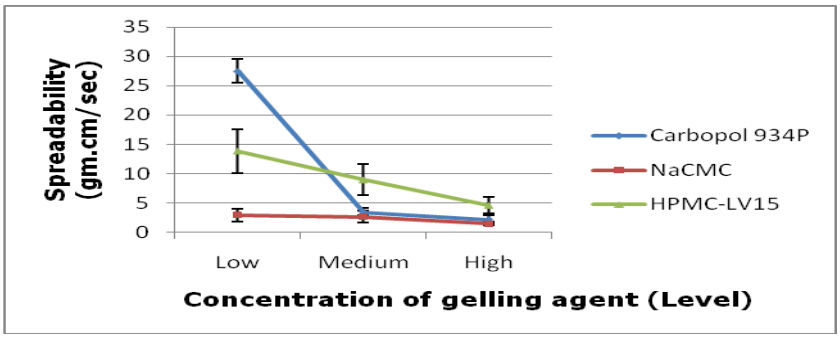

Fig 7: Effect of Concentration of Gelling Agent on Spreadability of Lycopene Emulgel

The viscosity of the prepared lycopene emulgel was found to increase in general with increase in concentration of gelling agent. The effect of rpm on viscosity is depicted in Figure 9. It is seen that there is a shear thinning effect, that is, the viscosity falls on increasing shear rate from 10 to 50 rpm. All the prepared formulations possessed optimum viscosity. Inclusion of different gelling agents in different concentrations seems to have brought about a noticeable difference in the viscosity of the gellified emulsions. While F3 was the most viscous formulation, F7 had the least viscosity. It is seen from Fig. 10 that increase in concentration of gelling agents results in a significant ($p \leq 0.05$, *df* 2 and 6, $F_{crit}=5.143$, $F=101942.9$ for Carbopol 934P, $p \leq 0.05$, *df* 2 and 6, $F_{crit}=5.143$, $F=1385288$ and $p \leq 0.05$, *df* 2 and 6, $F_{crit}=5.143$, $F=158027.1$, single factor ANOVA) increase in viscosity of formulations. The viscosity of the prepared emulgel at 20 rpm is depicted in Table 10 and Figure 8

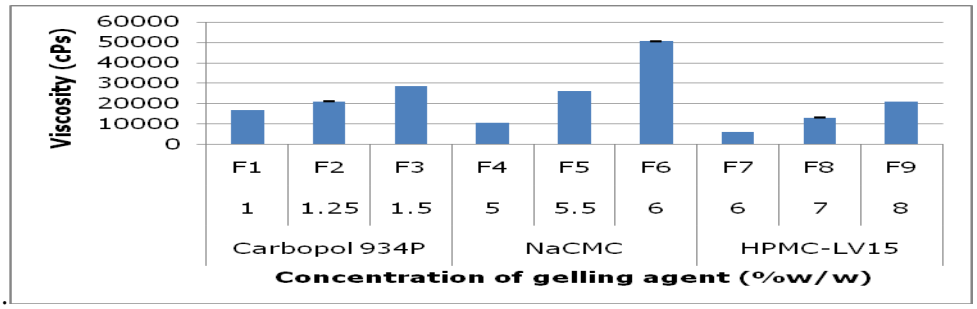

Fig.8: Effect of Concentration of Gelling Agent on Viscosity at 20 rpm

Table 10: Viscosity of Prepared Lycopene Emulgel

RPM	Viscosity(cPs)*								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
10	34024±35	37042±35	46048±38	20591±32	40398±24	61456±23	7452±27	16540±21	29481±48
20	17012±31	21141±32	28651±33	10592±23	26198±16	50545±43	6370±35	13171±39	21041±18
30	11341±25	15449±24	21412±27	9976±25	18402±23	43789±34	4850±26	9776±21	15415±57
40	9708±23	11641±21	15881±24	7158±29	15259±20	38656±29	3556±13	4413±15	9042±16
50	6805±21	8956±19	11413±18	6577±39	12903±51	34127±37	2152±28	2882±22	3576±38

***** Data indicates mean± S.D. of triplicate determinations

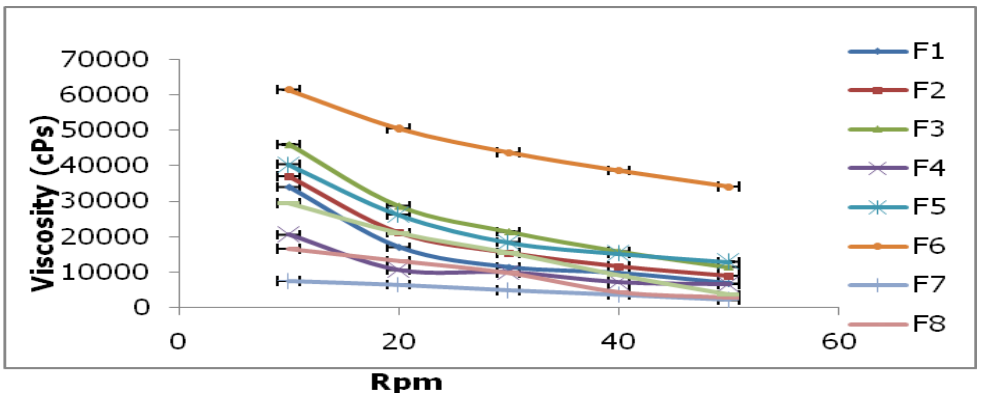

Fig 9: Effect of RPM on Viscosity of Lycopene Emulgel

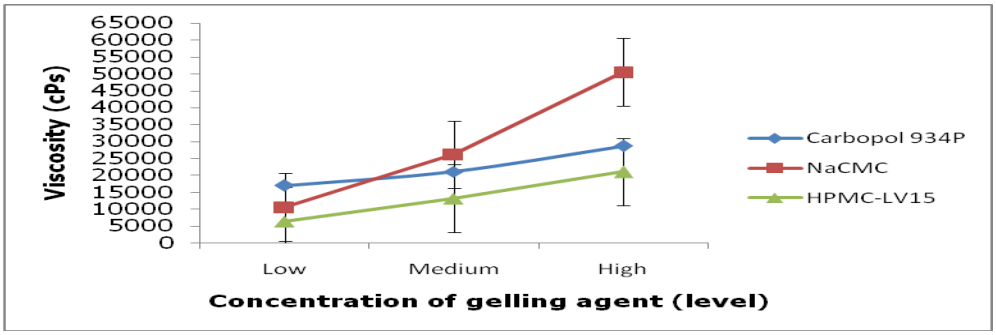

Fig 10: Effect of Concentration of Gelling Agents on Viscosity of Lycopene Emulgel

The extrudability of the prepared lycopene emulgel was found to vary from 2.34±0.27 gm/cm^2 to 20.7±2.6 gm/cm^2. Figure 12 depicts the effect of concentration of gelling agent on extrudability of prepared lycopene emulgel. It is seen that with increase in concentration of Carbopol 934P and HPMV LV-15 there is a significant ($p \leq 0.05$; *df* 2 and 6; F_{crit}=5.143; F=6.655, $p \leq 0.05$, *df* 2 and 6; F_{crit}=5.143; F=113.317, single factor ANOVA)) decrease in extrudability whereas in case of NaCMC there is no any significant ($p \leq 0.05$; *df* 2 and 6; F_{crit}=5.143; F=13.57, single factor ANOVA) decrease or increase in extrudability. As we increase the concentration of gelling agents consequently the viscosity of the gellified emulsion increases which leads to decrease in the extrudability of the lycopene emulgel. Decrease in extrudability implies application of higher weight/area to extrude the gel which correlates to the higher viscosity of the formulation. The results are shown in Table 11 and Figure 11.

Table 11: Extrudability of Lycopene Emulgel

Batch code	Extrudability (gm/cm^2)*	Batch code	Extrudability (gm/cm^2)*
F1	14.2±1.9	F6	3.80±0.38
F2	18.5±2.1	F7	12.02±0.51
F3	20.7±2.6	F8	7.31±1.5
F4	2.34±0.27	F9	2.73±0.61
F5	2.45±0.47		

* Data indicates mean± S.D. of triplicate determinations

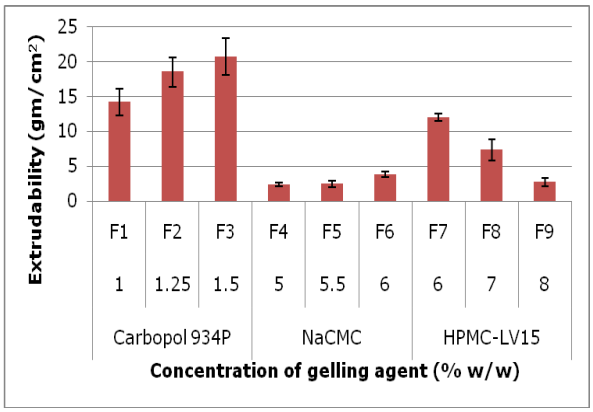

Fig 11: Effect of Concentration of Gelling Agents on Extrudability of Prepared Lycopene Emulgel

The drug content of the formulations ranged between 80.42±1.41 to 112.8±2.00. The drug content of all the nine formulations was determined which is shown in Table 12.

Table 12: Drug Content of Prepared Lycopene Emulgel Formulations

Batch code	Drug content (%)*	Batch code	Drug content (%)*
F1	112.8±2.00	F6	98.59±3.64
F2	87.57±2.22	F7	99.04±4.68
F3	80.42±1.41	F8	99.98±2.45
F4	100.63±5.46	F9	98.59±2.05
F5	99.02±2.74		

***** Data indicates mean ± S.D. of triplicate determinations

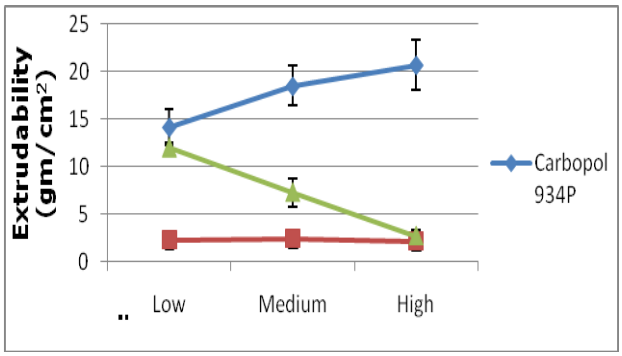

Fig 12: Effect of Concentration of Gelling Agent on Extrudability

The release of the drug through egg membrane from its emulsified gel formulation can be ranked in the following order: In case of Carbopol 934P the cumulative amount of drug release was in a descending order i.e. F1>F2>F3, the cumulative amount of drug released after 4 hour were 76.88±3.49%, 72.45±3.18% and 67.61±4.12% respectively. In case of NaCMC, the cumulative amount of drug release at fourth hour was 73.98±3.22, 66.13±2.75 and 64.94±3.77 respectively. Hence, it was in descending order i.e. F4>F5>F6. In case of HPMC-LV15 the drug release was in descending order i.e. F7>F8>F9 and the cumulative drug release at the fourth hour was 66±2.65, 63.43±3.81 and 58.46±2.59 respectively. The influence of concentration of gelling agent on release and permeation of lycopene across egg membrane as at fourth hour from the prepared formulation is depicted in Figure 15. It was seen that with increase in the concentration of Carbopol 934P and HPMC LV-15 there was no significant ($p \leq 0.05$; *df* 2 and 6; $F_{crit}=5.143$; $F=4.927$, $p \leq 0.05$; *df* 2 and 6; $F_{crit}=5.143$; $F=4.681$, single factor ANOVA) effect in the release and permeation of lycopene across the egg membrane. In case of NaCMC there was a significant ($p \leq 0.05$; *df* 2 and 6; $F_{crit}=5.143$; $F=6.755$, single factor ANOVA) decrease in the release of lycopene across egg membrane. The progressive increase in the amount of drug diffusion through egg membrane can be attributed to gradual decrease in the concentration of gelling agents. It is noticed that, if we increase the concentration of gelling agents, the diffusion of drug through the membrane also decreases. In addition viscosity increased as the concentration of gelling agents increased. Thus, high concentration of gelling agents as well as increased viscosity is accountable for decreasing the release of active substance from the lycopene emulgel. The drug release profile data is presented in Table 13, Fig. 13.

Table 13: *In-Vitro* Release of Lycopene from Emulgel Formulation

Time (Min)	% Cumulative drug release*								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	17.93±4.13	13.69±2.32	10.87±3.12	14.01±0.92	11.98±1.77	8.34±1.67	7.01±1.09	6.00±1.19	5.91±1.17
30	20.49±3.17	18.72±2.41	14.98±3.83	16.61±2.61	12.66±1.86	11.44±2.8	15.57±2.63	15.05±2.56	14.47±2.7
45	25.64±3.22	23.77±2.82	19.67±4.27	21.56±1.56	15.5±2.63	15.12±2.23	21.58±3.39	19.66±3.34	18.96±2.3
60	38.56±2.27	37.00±3.73	32.98±3.31	35.23±2.47	31.53±3.94	24.05±2.96	31.70±1.47	29.34±4.48	27.16±3.17
90	52.50±2.33	49.74±4.52	43.53±2.17	46.24±1.36	42.24±1.98	39.16±2.08	47.76±2.8	43.83±1.92	43.56±1.14
120	67.00±3.38	65.98±3.34	57.12±4.11	58.98±5.81	54.32±4.63	47.37±2.34	61.54±2.3	54.96±2.57	53.7±3.77
180	74.29±4.43	71.22±4.26	65.55±3.23	65.60±2.67	62.04±1.83	56.50±2.46	64.22±3.62	59.50±2.49	56.84±2.74
240	76.88±3.49	72.45±3.18	67.61±4.12	73.98±3.22	66.13±2.75	64.94±3.77	66±2.65	63.43±3.81	58.46±2.59

***Data represent mean ± S.D. (n=3)

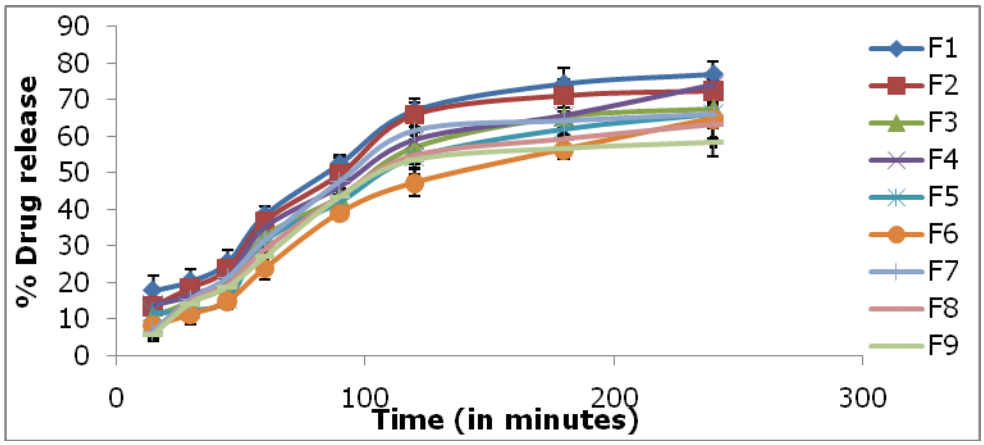

Fig13: *In-vitro* Release Of Lycopene from the Prepared Emulgel Formulations

From the above results it was found that the drug release was best fit to Korsmeyer Peppas model for F2, F3, F7, F8 and F9 since highest value of r for the formulations was seen and Higuchi model for formulation F1, F4, F5 and F6. The 'n' value for all batches is greater than 0.5, hence, it is clear that all the formulations are showing non-Fickinian and anamolous diffusion.

Table 14: Model Fitting For Release Kinetics Of Prepared Lycopene Emulgel

Batch	Release models							Best fit model
	Zero order	First order	Higuchi model	Hixon-crowell	Korsmeyer Peppas			
	R	r	r	r	r	K	n	
F1	0.936	0.702	**0.977**	0.962	0.971	0.029	0.616	Higuchi
F2	0.929	0.720	0.971	0.951	**0.977**	0.020	0.681	Korsmeyer
F3	0.945	0.749	0.974	0.964	**0.979**	0.013	0.741	Korsmeyer
F4	0.955	0.896	**0.981**	0.955	0.976	0.019	0.678	Higuchi
F5	0.942	0.882	**0.970**	0.961	0.954	0.013	0.740	Higuchi
F6	0.967	0.901	**0.986**	0.983	0.982	0.008	0.820	Higuchi
F7	0.910	0.826	0.960	0.927	**0.972**	0.008	0.844	Korsmeyer
F8	0.928	0.828	0.972	0.948	**0.973**	0.007	0.863	Korsmeyer
F9	0.910	0.957	0.906	0.925	**0.970**	0.007	0.851	Korsmeyer

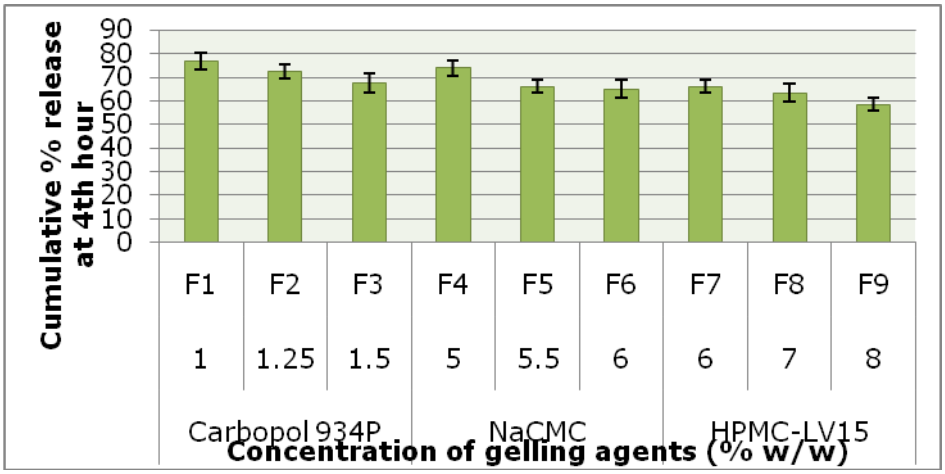

Fig 14: *In-vitro* Release of Lycopene from the Prepared Emulgel in 240 min

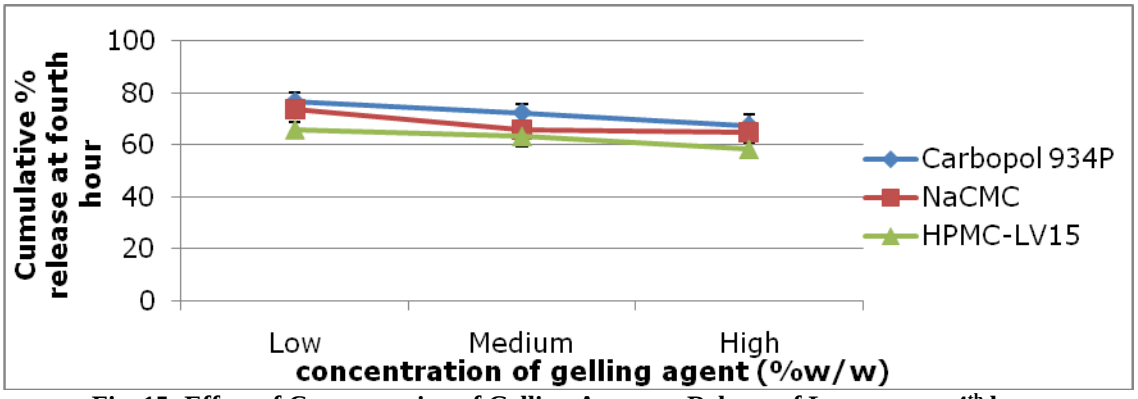

Fig. 15: Effect of Concentration of Gelling Agent on Release of Lycopene at 4th hour

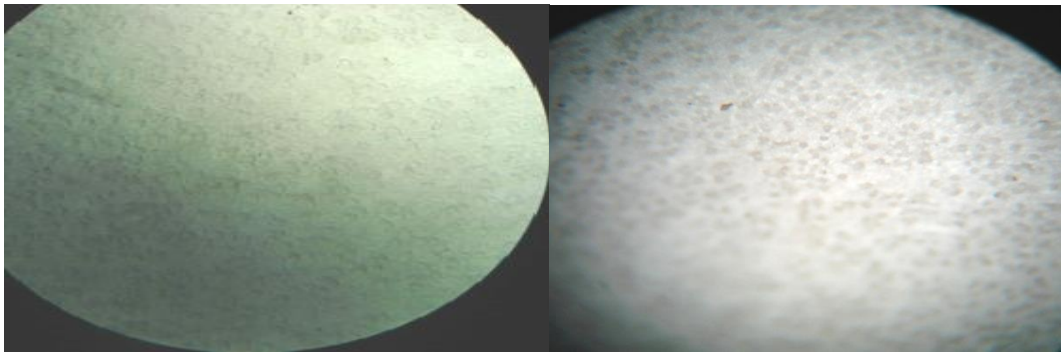

Fig.16: Micrograph of Egg Membrane After Soaking In Dissolution Media Overnight Chloroform

Fig.17: Micrograph of Egg Membrane after *In-Vitro* Release Study

CONCLUSION

This work was conducted to develop an emulgel of lycopene using three different gelling agents i.e. Carbopol 934P, HPMC LV-15 and NaCMC. Oleic acid was used as a penetration enhancer. The gellified emulsions were characterized for their physical appearance, rheology, spreadability, drug content and stability. *In-vitro* release studies were conducted to check the drug release through egg membrane. The formulations were evaluated for their antioxidant activity as well as their acute skin irritation potential. Formulation F1 was found to have fallen within the stipulated criteria of all the evaluation parameters. Hence, it was concluded that formulation F1, containing carbopol 934P (1% w/w), was the optimized formulation. It exhibited the maximum drug release and antioxidant activity, in addition to the least skin irritation potential.

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REFERENCES

1. Dimascio P, Kaiser S, Sies H. Lycopene As The Most Efficient Biological Carotenoid Singlet Oxygen Quencher. Arch Biochem Biophys 1989; 274, 532- 38.
2. Lachman L, Lieberman HA. The Theory and Practice of Industrial Pharmacy.3rd ed. Varghese Publishing House; 1990, 534.
3. Vyas SP, Khar RK. Controlled Drug Delivery, 1st ed. Vallabh Prakashan; 2002, 416-17.
4. Rieger MM, et al. The Theory and Practice of Industrial Pharmacy, 3rd ed. Lea and Febiger, Philadelphia; 1986, 502-33.
5. Baibhav J, et al. Emulgel: A Comprehensive Review on the Recent Advances in Topical Drug Delivery. International Research Journal of Pharmacy 2011; 2(11): 66-70.
6. Hyndavi N. Formulation and Evaluation of Aceclofenac-Lycopene Trans emulgel. World Journal of Pharmaceutical Research 2013; 2(4): 1036-45.
7. Dimascio P, Kaiser S, Sies H. Lycopene as the Most Efficient Biological Carotenoid Singlet Oxygen Quencher. Arch BiochemBiophys 1989; 274: 532-38.
8. Jweller AWP. Hand Book of Pharmaceutical Excipients. Second ed. The Pharmaceutical press, London: 106-9, 229-31, 204-6.
9. Espinoza HA, *et a.*, Rheological and Controlled Release Characteristic of Lycopene Gel. International Journal of Pharma Tech Research 2012; 2(3): 1632-44.
10. Darvin M, et al. Cutaneous concentration of lycopene correlates significantly with the Roughness of the Skin. European Journal of Pharmaceutics and Biopharmaceutics 2008; 69: 943-47.
11. Krishnaveni M, Kumar M, Gopinath C. A Review On Beneficial Role of Lycopene In Human Body. International Journal of Advances in Pharmaceutical Research 2010; 3(8): 1033-37.

12. Peytavi B, et al. Cutaneous Lycopene and B-Carotene Levels Measured by Resonance Raman Spectroscopy: High Reliability and Sensitivity to Oral Lacto-lycopene Deprivation and Supplementation. European Journal of Pharmaceutics and Bio-pharmaceutics 2010; 73(1): 187-94.
13. Shah D, et al. Formulation of Water Soluble Mucoadhesive Film of Lycopene for Treatment of Leukoplakia. International Journal of Pharmaceutical Sciences Review and Research 2010; 2(1): 6-10.
14. Prevec T, et al. In-vitro Anti-oxidant and Free Radical Scavenging Activity of Lycopene. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012; 3(3): 1220-28.
15. Schwarz, et al. Vehicle for Topical Delivery of Anti-inflammatory compound. Patent N0.2 US 7, 781, 429B2, 2010.
16. Gautier CJ. Photo-protective potential of lycopene, β-carotene, vitamin E, vitamin C and carnosic acid in UVA-irradiated human skin fibroblasts. Free Radical Biology and Medicine 2002; 32(12): 1293-303.
17. Akram M, et al. Design and Development of Insulin emulgel formulation for Transdermal Drug Delivery and its evaluation. Pak. Journal of Pharma Science 2012; 26(1): 323-32.
18. Dignesh M, et al. Formulation Design and Development of Piroxicam Emulgel. International Journal of Pharma Tech Research 2012; 4(3): 1332-34.
19. Ranga PM, et al. Formulation and *In-vitro* Evaluation of Ciprofloxacin Loaded Topical Emulgel. International Journal of Pharmaceutical and Chemical sciences 2012; 1(1): 324-56.
20. Singhla V, et al. Emulgel: A New Platform for Topical Drug Delivery. International Journal of Pharma and Bio Sciences 2010; 3(1): 485-s98.
21. ICH Harmonized Tripartite Guidelines, Stability testing of new drug substances and products, ICH Committee. Federal register 2003; 68:6571718.