GNK Ganesh et al /J. Pharm. Sci. & Res. Vol. 11(4), 2019, 1638-1645

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Design and Development of Curcumin Nanogel for Squamous Cell Carcinoma

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Abstract

The aim of the present study was to formulate and evaluate the nanoemulgel for squamous cell carcinoma. This nanoemulsion acts as a transdermal delivery system for poorly soluble and permeable drug. Curcumin is used in order to overcome the troubles associated with its oral delivery. The novelty of the work was to enhance the solubility, permeability, biocompatibility and overcome the problem associated with curcumin anticancer drug. Nanoemulsions were prepared using the spontaneous emulsification mechanism. Different nanoemulsion components (oil, surfactant, and co-surfactant) were selected on the basis of solubility and emulsification ability. Carbopol 940 was added as gel matrix to convert nanoemulsion into nanoemulgel. Drug-loaded nanoemulsions and nanoemulgels were characterized for particle size by scanning electron microscope *(*SEM), viscosity, conductivity and spreadability. Transdermal permeation of curcumin from nanoemulgels was determined by using Franz diffusion cell method. Nanoemulgel containing capmul MCM as oil, Tween 20 and polyethylene glycol (PEG) 400 as surfactant. Co-surfactant mixture contains water, drug and carbopol and concluded as optimized formulation. Nanoemulgel showed significantly higher drug release and less toxicity.

Keywords: Nanoemulsion, Squamous cell carcinoma, Transdermal delivery system, Curcumin, Characterization, *In-vitro* cytotoxicity.

1. INTRODUCTION

Nanoemulsion is also used as transdermal drug delivery and defined as a dispersion consisting to oil, surfactant, co-surfactant and aqueous phase, which is optically isotropic and thermodynamically stable liquid solution with a droplet diameter usually in the range of 10–200nm (1). The ascendancies associated with the transdermal use of nanoemulsion which enhanced drug solubility, good thermodynamic stability, and enhanced the effect on transdermal ability. The aptness of nanoemulsion was to increase the concentration gradient and thermodynamic activity towards skin along with permeation enhancement activity, and to make it's the system expedient for transdermal delivery (2). Curcumin is an orange-yellow crystalline powder obtained from the rhizome of *Curcuma longa*, family *Zingiberaceae*. Angiogenesis (blood vessel formation) is essential for tumor growth and metastasis (3). The precise mechanism that leads to angiogenesis is not fully understood, but growth factors that cause proliferation of endothelial cells have been shown to play a critical role in this process. Curcumin has been shown to suppress the proliferation of human vascular endothelial cells and abrogate the fibroblast growth factor-2–induced angiogenic response suggesting that curcumin have an antiangiogenic factor (4, 5). As curcumin has low solubility and low permeability, therefore, transdermal drug delivery system has been chosen to overcome these issues and also lower the toxicity and for patient compliance (6).

Squamous cell carcinoma (SCC) is an epithelial malignancy that occurs in organs that are normally covered with squamous epithelium. It includes several different anatomic sites, like skin, lips, mouth, esophagus, urinary tract, prostate, lungs, vagina, and cervix. Of these anatomic sites, there are four sites which make up the majority of SCC cases: non-melanoma skin cancer, head and neck cancer, esophageal cancer, and non-small cell lung cancer (7). Given the range of tissues in which it arises, SCC represents the most common cancer capable of metastatic spread in the US and worldwide. Despite advances in diagnostic methods and combined treatment modalities, the survival rate has not improved significantly over the last 30 years. Due to lack of reliable early diagnostic cancer biomarkers and limited number of molecularly targeted therapeutic strategies (8).

2. MATERIALS AND METHODS

Materials

The Curcumin, dialysis bags were procured from Hi-Media Ltd, (Mumbai, India). Polysorbate/ Tween 20, Capmul, carboxylic methyl cellulose (CMC) and Polyethylene glycol (PEG) 400 were procured from Gattetosse India Pvt Ltd, (Mumbai, India). Potassium dihydrogen ortho-phosphate, Sodium hydroxide pellets, Triethylamine were procured from Qualigen Fine Chemicals, (Mumbai, India). Ortho-phosphoric acid was procured from Rankem, (New Delhi, India). Carbopol was procured from Sigma Aldrich.

2.1 Pre-Formulation Studies

Preformulation of the studies is like phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of a new drug substance, by which may develop stable, safe and effective dosage forms. Preferably, the Preformulation phase starts early in the discovery process such that appropriate physical, chemical data is obtainable to add in the selection of novel chemical entities that enter the development process. This evaluation shows potential interaction with different inert ingredients planned for use in the final dosage form was considered (9).

2.1.1 Solubility studies

The solubility of the curcumin was determined in various oils by adding an excess amount of drug to 1mL of selected oils in stoppered vials. The vials were kept at 25± 0.5°C in an isothermal shaker for 72h to reach equilibrium (10). The equilibrated samples were removed from the shaker and centrifuged at 3000 rotation per minutes (rpm) for 15min. The supernatant was taken and filtered through a 0.45µ membrane filter and concentration of curcumin was determined in the oils after dilution using UV-Visible spectrophotometer at 421nm (11).

2.1.2 Melting point determination

Capillary tube has been used to determine melting point, small amount of the drug was taken in a capillary tube and closed at one end and placed in Vigo melting point apparatus and the temperature.

2.1.3 Development of calibration curve of curcumin with ethanol

a) Preparation of stock solution:

For the preparation of the stock solution, 100mg of curcumin was exactly weighed into 100 mL volumetric flask and dissolved in small amount of ethanol. The volume of solution was made up with the ethanol to 100mL to produce a stock solution having a concentration of 1 mg/mL (12).

b) Preparation of standard solution:

An aliquot of 10mL from stock solution was diluted to 100mL to get a standard solution having a concentration of 100mcg/mL using ethanol.

c) Preparation of working standard solution:

Working standard solutions were prepared by appropriately diluting the standard solution with ethanol ranging in concentration from 4 to 14mcg/mL. The absorbance of every working standard solution was measured at 421nm using a Shimadzu UV-1800 spectrophotometer, ethanol used as a blank. The absorbance of every batch was composed in triplicates and plotted against concentration (13).

2.1.4 Development of calibration curve of curcumin using phosphate buffer

a) Preparation of stock solution:

100mg of curcumin was accurately weighed into a 100mL volumetric flask and dissolved in a small quantity of phosphate buffer 5.5pH to prepare a stock solution. The volume of solution was prepared using the phosphate buffer 5.5pH to 100mL to make a stock solution having a concentration of 1mg/mL.

b) Preparation of standard solution:

An aliquot of 10mL from stock solution has been taken and diluted to 100mL to get a standard solution having a concentration of 100mcg/mL. using phosphate buffer 5.5pH (14).

c) Preparation of working standard solution:

Working standard solutions were prepared by appropriately diluting the standard solution using phosphate buffer ranging in concentration from 4 to 14mcg/mL. The absorbance of every working standard solution was measured at 421nm by using a Shimadzu UV-1800 spectrophotometer, ethanol used as a blank. The absorbance of every batch was performed in triplicates and plotted against concentration (15).

2.2 Preparation of nanoemulsion

Nano-emulsions were prepared using the spontaneous emulsification mechanism which is taking place when an organic phase and an aqueous phase were mixed together. The organic phase contains homogeneous solution of oil, lipophilic surfactant and co-surfactant, the aqueous phase consists of distilled water (16). The selected oil phase was heated gently at 45-50^{o}C for 5min. Surfactant and co-surfactant (Smix) were mixed together in different volume ratios (1:1, 1:2, 1:3) and heated at the same temperature. These Smix ratios were chosen to reflect the increasing concentration of co-surfactant with respect to surfactant increasing concentration of surfactant with respect to co-surfactant for the detailed study of the phase diagrams for the formulation of NE. Mixture of oil and Smix were prepared in different volume ratios (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9) in screw-cap glass tubes and were vortexed to form homogenous isotropic mixtures (17). Each of the mixtures was then slowly titrated with aqueous phase (double distilled water) and under vortex mixing at room temperature to attain equilibrium (18).

Table 1: the samples were visually observed for the following categories

Translucent and easily flowable	o/w nanoemulsion (NE)
Milky or cloudy/phase separation	Emulsion (E)
Milky gel	Emulsion gel (EG)
Translucent gel	Nanoemulsion gel (NG)

2.3 Preparation of nanoemulsion gel

1% of carbopol was mixed with distilled water and triethnolamine together and kept for continuous mixing using magnetic stirrer. In the carbopol gel, the prepared nanoemulsion was added and kept for continuous moving using magnetic stirrer (19).

2.4 Physicochemical characteristics of the nanoemulsion

2.4.1 Percentage transmittance

At 421nm the percent transmittance of the system was measured using a UV spectrophotometer and double distilled water used as a blank (20).

2.4.2 Drug content

The dose of the drug was well below the saturation point. The release of the amount of drug incorporation should be presumed. Because surfactant and cosurfactant (Smix) were added, this can lead to precipitation. Hence, the drug content was calculated by UV visible spectrophotometer. The formulation was diluted to get required concentration using methanol as solvent and the absorbance was measured at 421nm (21). The drug content was calculated as:

$$Drug\ content = Analysed\ content / Theoretical\ content \times 100$$

2.4.3 Globule size and zeta potential

Zetasizer ZS 90 (Malvern Instruments, UK) have been used to determine the mean Globule size (GS) and Zeta potential (ZP) of NEs. Based on photon correlation spectroscopy technique the mean globule size was measured. This analyzes the fluctuations in dynamic light scattering due to the brownian motion of the particles. The mean diameter was obtained at an angle of 90° in 10mm diameter cells at 25°C. The zeta potential, reflecting the electric charge on the particle surface and it was very useful way of evaluating the physical stability of any colloidal system. It was determined based on an electrophoretic light scattering technique**.** All GS and ZP measurements were conceded at 25^{o}C using disposable polystyrene cells, disposable 5plain folded capillary zeta cells and after suitable dilution of all samples with the original dispersion medium (22, 23).

2.4.4 Determination of viscosity

The viscosity of the formulations was determined using Brookfield DVE viscometer. 0.5g of the sample was taken for analysis without diluting the sample by using spindle no. 63 at different rpm at 25±0.5°C (24).

2.4.5 Determination of pH

The prepared neutral, positive, negative liposomal gels were measured using (Systronics, 361-micro pH meter) (25).

2.4.6 Physical examination and homogeneity:

The final prepared Emulgel formulation was inspected visually for their color intensity variation. All developed gels were tested for homogeneity by visual inspection after the gels have been placed in the container. They were also examined for their appearance and presence of any aggregates (24).

2.4.7 Spreadability

Spreadability was determined by apparatus recommended by Mutimer which was properly customized in the laboratory and used for the study. It consists of a wooden block provided by a pulley at one end. By this method, spredability was calculated on the basis of 'Slip' and 'Drag' characteristics of Emulgel (25). A ground glass slide was fixed on the block. An excess of Emulgel (about 2g) below study was placed on the ground slide. The Emulgel was then sandwiched between ground slide and another glass slide having the measurement of the fixed ground slide, provided with the hook. 1kg weight was placed on the peak of the two slides for 5 minutes to expel air and to supply a uniform film of the Emulgel between the slides. Excess of the Emulgel was scrapped off from the edges. The peak plate was then subjected to the weight of 100g with the help of string attached to the hook and the time (in seconds) necessary by the peak slide to cover a distance of 7.5cm was noted. A shorter interval indicates better spreadability (24).

Spreading coefficient was calculated by using the formula:

$$S = M.L/T$$

Where, S = spreadability,

M = Weight tied to upper slide,

L = Length of glass slides T = Time taken to separate the slides completely from each other.

2.2.6 Percentage entrapment efficiency

Nanoemulsion gel was centrifuged and the supernatant was diluted with an aliquot quantity of ethanol and the concentration

was calculated by UV-Visible spectrophotometer. The total amount of drug entrapped was calculated by the formula (23):

Drug entrapped

= Total amount of drug in solution - Amount of drug present in Supernatant

% of Entrapment efficiency = (Amount of drug entrapped / label claim) X 100

2.4.8 *In-vitro* drug release studies

Franz diffusion cells have been used to perform Release studies of nanoemul gels with a diffusion area of 2.545cm^2. 15mL of Phosphate buffer 5.5P^h solutions was used to fill the receptor chamber and continuously stirred with a magnetic stirrer at 600rpm. The water temperature should be maintained approximately 32±0.5◦C. The known amount (100mg) of formulations was applied on the dialysis membrane (26). Two Franz diffusion cells were switched simultaneously i.e. two formulations of nanoemul gel (F6 and F8). 1 ml of sample was withdrawn from every cell during the programmed time intervals of 0.5, 1, 2, 4, 8, 12, 24 hours correspondingly. The amount of curcumin in the composed samples was calculated by UV-spectrophotometer (27, 28).

2.4.9 *In vitro* cytotoxicity studies

Determination of mitochondrial synthesis by MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x10^5 cells/ml using *Dulbecco's Modified Eagle's Medium* (DMEM) medium containing 10% fetal bovine serum (FBS). To every well of 96 well microtitre plates, 100µl of the diluted cell suspension (approximately 10,000cell/well) was added. After 24h, a partial monolayer was formed and the supernatant was flicked off. The monolayer was washed once using medium and 100µl of different sample concentrations were prepared in preservative media. Later added per well to the partial monolayer in microtitre plates (29). The plates were then incubated at 37^{o}C for 24h in 5% CO_2 atmosphere. Microscopic examination was conceded and observations were recorded. After 24h, the sample solutions on the wells were discarded and 20 µl of MTT (2mg/ml) in MEM-PR (MEM without phenol red)/PB was added to every well (30).

1. The plates were smoothly shaken and incubated for 3 hours at 37^{o}C in 5% CO_2 atmosphere.
2. The supernatant was separated and 100µl of isopropanol was added and the plates were softly shaken to solubilize the formed formazan.

The absorbance was calculated using a microplate reader at a wavelength of 540nm. The percentage growth inhibition was calculated using the following formula. Concentration of drug or test samples required to inhibit cell growth by 50% values were generated from the dose-response curves for every cell line (11).

% growth inhibition

= 100 – (mean OD of individual test) / (mean OD of control group)

× 100

2.4.10 Thermodynamic stability tests: all selected formulations were subjected to keep on different thermodynamic stability tests.

a) Heating cooling cycle: the studies were performed up to 48 hrs on the refrigerator temperature at 4^{o}C and 45^{o}C for six cycles. The stable formulations, at these temperatures, were subjected to centrifugation (31).

b) Centrifugation: all the chosen formulations were centrifuged at 3500rpm for 30min. The formulations which have not shown any phase separation was taken for freeze-thaw cycle test (32).

c) Freeze-thaw cycle: all three formulations were kept for 48 h at the storage temperature of freeze-thaw cycles between – 21^{o}C and +25^{o}C. Formulations which passed these thermodynamic stress tests were further taken for the percent transmittance tests for assessing the efficiency of emulsification (33).

3. Results And Discussion

3.1 Solubility studies of the drug in different oils:

Solubility is a significant standard in the formulation of NE, as the drug leftovers in liquid form solubilized in the oil phase. Hence, the oil phase in which the drug will be showed maximum solubility was chosen for the reason. Capmul CMC showed maximum solubility of Curcumin 90 ± 0.18mg/mL [Table 2]. Hence Capmul CMC was chosen for the formulation of NE. The improved solubility of the drug could be due to the more affinity towards the particular oil.

Table 2: solubility study of the drug in different oils

OIL	CURCUMIN (mg/mL.)
Labrafac PG	3.557 ± 0.410
Capmul CMC	90 ± 0.18
Labrafac Lipophile WL 1349	3.567 ± 0.351
peanut oil	0.163 ± 0.030
Lauoglycol 90	5.25±1.52
Oleic acid	22±1.06

3.2 Melting point

Table 3: Melting point of curcumin

Drug	Melting point
Curcumin	181 ^{o}C

3.3 Screening of surfactants and co-surfactants

The SCoS mixture of Tween20 and PEG400, Capmul CMC (oil) was found to make clear and uniform o/w emulsion. Hence, Tween20 and Capmul were preferred for the formulation.

3.4 PREFORMULATION STUDIES

3.4.1 Standard calibration curve of curcumin using ethanol

The UV absorbance of curcumin standard solutions was in the range of 10-50µg/mL of the drug in ethanol showed linearity at 425nm. The linearity was plotted for absorbance (A) against concentration(C) with the R2 value of 0.996 and with the slope equation,

y =0.0074x+0.0147. The absorbance values and standard curve.

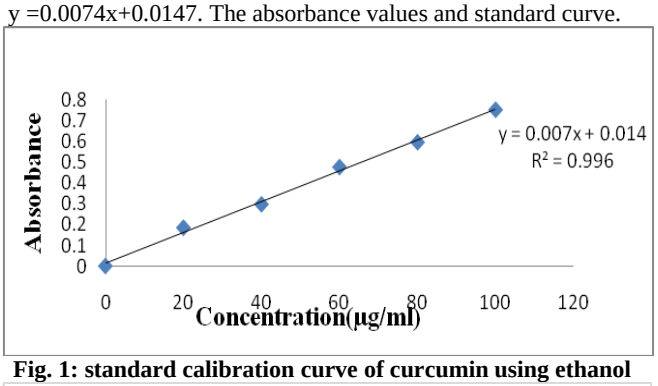


Fig. 1: standard calibration curve of curcumin using ethanol

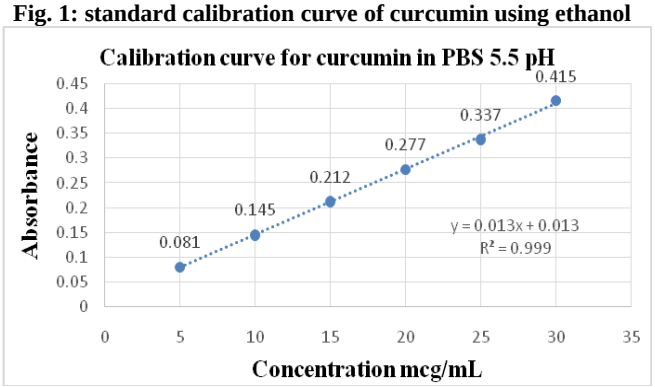


Fig. 2: standard calibration curve for curcumin with phosphate buffer 5.5 pH

3.5 Formulation of nanoemulsion (NE) using spontaneous emulsification method

The concentration of surfactant and co-surfactant is accountable for the blockade formation at the interface mandatory to prevent the coalescence of the formed NE. SCoS gets adsorbed at the interface by reducing the energy required for NE formation thus recovering the thermodynamic stability of the NE formulation. In this method following examination was recorded.

In SCoS ratio 1:1 when surfactant and co-surfactants will be in equal ratio, the ratio of Oil and SCoS i.e. none of the formulation were observed translucent. In the SCoS ratio 1:2 the ratio of Oil and SCoS i.e. from formulation (F8 to F9) translucent formation of NE was observed and final ratio of SCoS 1:3 the ratio of Oil and SCoS from formulation (F6) only was observed translucent. Therefore 1:2 and 1:3 ratio of SCoS having formulation (F8 and F6) was selected for incorporation in the final formulation of nanoemulsion gel.

3.5.1 Oil phase: Capmul CMC; SCoS: Tween 20: PEG400 Visual observations during aqueous phase titration using SCoS 1:1 to 1:3

Table 4: formulation observation

SCoS	Oil : SCoS								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1:1	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
	E	E	E	E	E	M	E	M	M
1:2	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
	M	E	E	EG	E	M	E	**NE**	**NE**
1:3	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
	E	E	M	EG	EG	**NE**	EG	E	M

NE = Nanoemulsion, E = Emulsion EG = Emulsion gel, M = Milky

3.5.2 Selection of NE formulation

From visual observations during aqueous phase titration using SCoS 1:1 to 1:3. The correct ratio of SCoS was found to be SCoS (1:2 and 1:3) ratio having the Oil: SCoS from formulation (F6 and F8), were chosen for formulation and subjected for particle size distribution, zeta potential and polydispersity index (PDI).

3.6 Preparation of curcumin nanoemulsion gel

The nanoemulsion was formulated by a spontaneous method using various ratios of Oil: Scos. Nanoemulsion containing curcumin of optimized ratio were mixed together into the Carbopol gel using an electric mixer and lastly, the pH was adjusted to 5.5 to 6.8 with triethnolamine.

Table 5: Preparation of nanoemulsion gel

S.No	DRUG(mg)	OIL: SCOS	CARBOPOL
1	15	1:6	1%
2	15	1:8	1%

Fig. 3: Nanoemulsion gel

Physicochemical characteristics of the nanoemulsion

3.7 Drug content and transmittance

Table 6: Drug content and transmittance for nanoemulsion

Code	Transmittance (%)	Drug content (%)
F6	88.2	96.6
F8	91.4	98.8

3.7.1 Globule size, zeta potential & polydispersity index

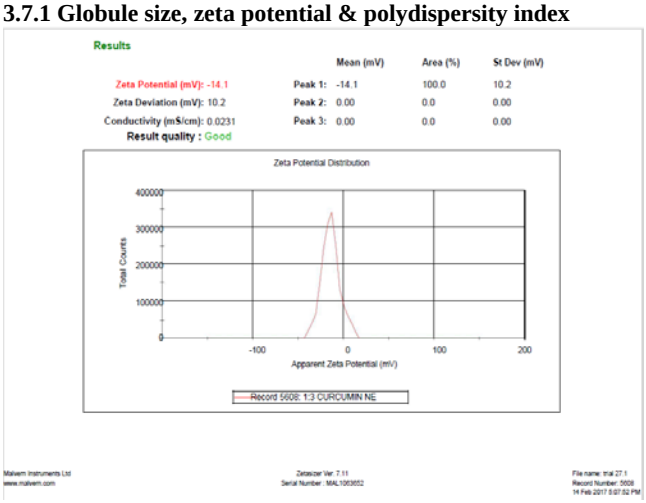


Fig. 4: zeta potential of the formulation f6

Fig. 5: size distribution of the formulation f6

Fig. 6: Zeta potential of formulation f8

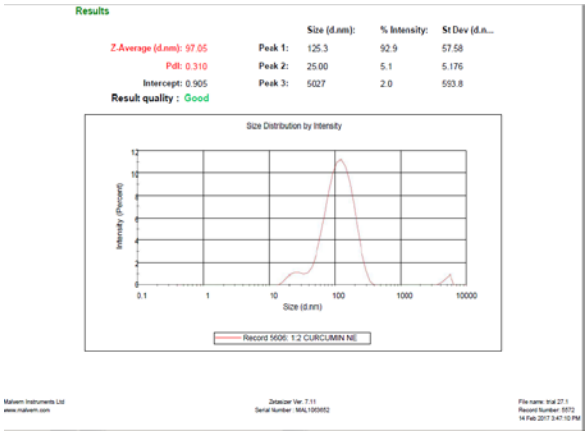


Fig. 7: size distribution of the formulation f8

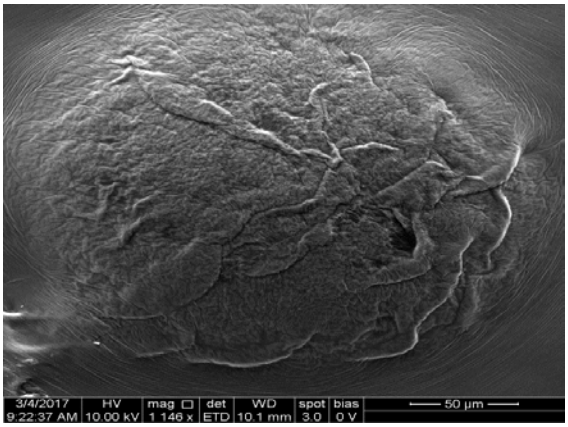
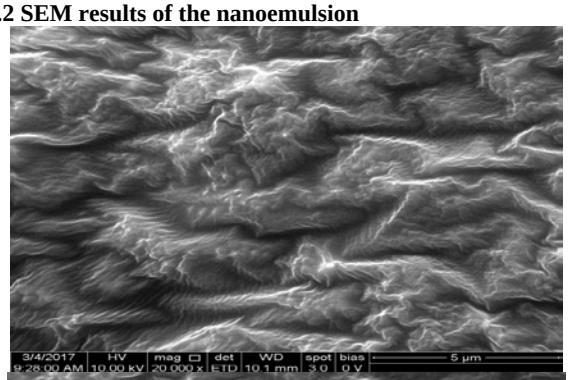
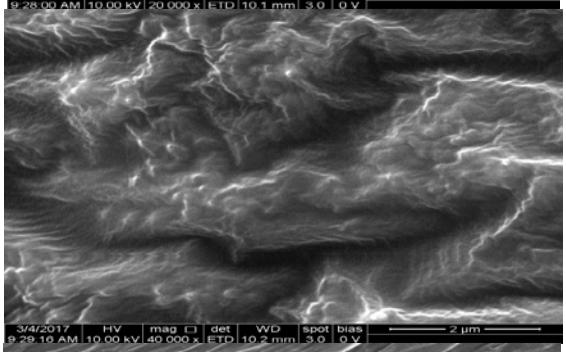
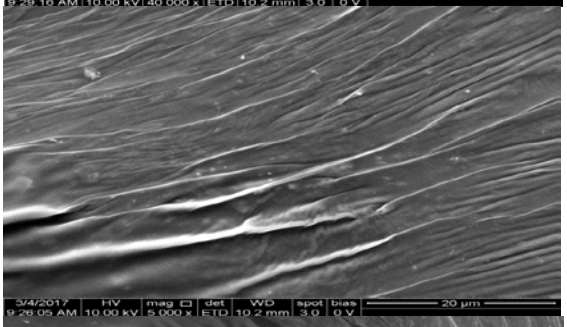


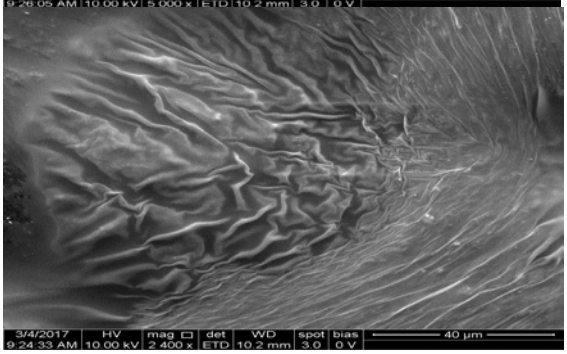
Fig. 8: SEM result of the nanoemulsion formulation f8

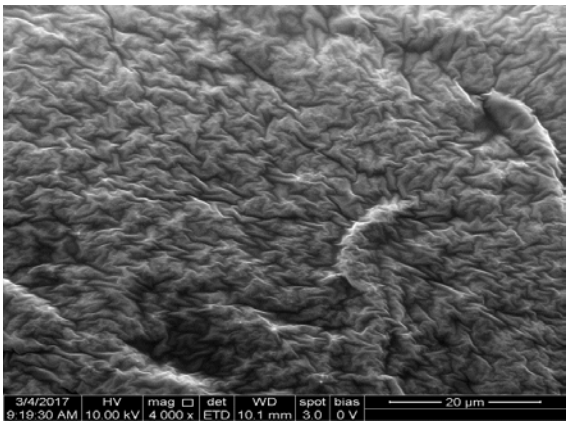
3.7.2 SEM results of the nanoemulsion

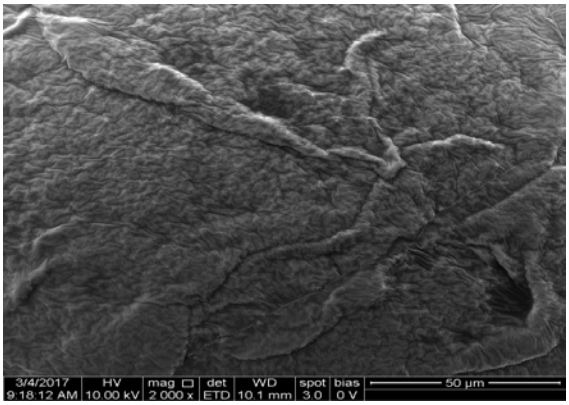












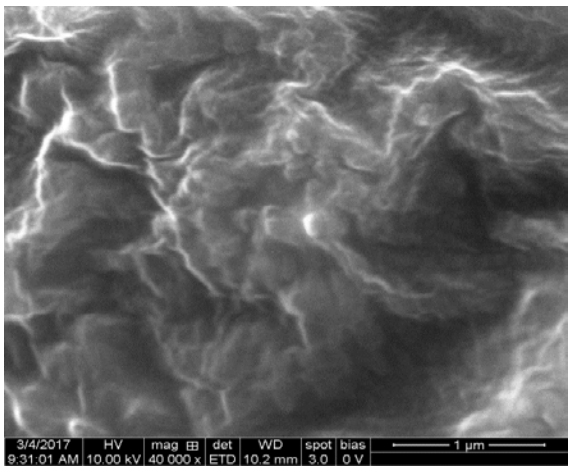


Fig. 9: SEM result of the nanoemulsion formulation f6

The SEM results exposed the nanoparticles are found to be in the range of the 120-135nm. By taking into consideration of the images, it was found that there were no particle ruminants in globules. It explains that the drug was entirely solubilized in the oil. The surface of the globule was slightly uneven.

3.7.3 Determination of the viscosity

3.7.4 Viscosity

The viscosity of the formulated nanoemulsion gel was 3152 poise.

3.7.5 pH

The pH of the formulated nanoemulsion gel was 5.7.

3.7.6 Spredability

Based on the values of prepared emulgel, F6 emulgel was less viscous as compared to F8 and also F8 formulation had high spredability coefficient. Suitability and clinical efficacy of topical preparations need to possess optimal mechanical properties (ease of removal from the container, spredability on the substrate), rheological properties (viscosity, elasticity, thixotropy, flowability).

The efficacy of topical therapy depends on the patient spreading the formulation is an even layer to distribute a standard dose. The optimum steadiness of such a formulation helps to ensure that an appropriate dose is applied or delivered to the target site. The delivery of the right dose of the drug depends highly on the spredability of the formulation.

So spredability is directly proportional to efficacy.

Table 7: Spredability of nanoemulsion gel

S.No	Formulation	Spredability
1	F8	42
2	F6	40

3.7.7 Percentage of entrapment efficiency

The percentages of entrapment efficiency in F8 and F6 were found to be 99% and 96.2% correspondingly.

Table 8: Entrapment efficiency of nanoemulsion gel

S. No	Formulation	% Entrapment Efficiency
1.	F8	99%
2.	F6	96.2%

3.7.8 *In vitro* drug release (Franz diffusion)

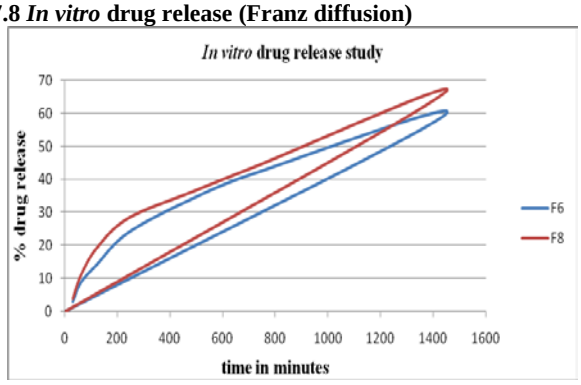


Fig. 10: *In vitro* drug release (Franz diffusion)

3.7.9 *In vitro* cytotoxicity assay:

The percentage growth inhibition was estimated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were created from the dose-response curve for every cell line.

$$\%\ Cell\ Viability = \frac{Mean\ OD\ of\ individual\ test\ group}{Mean\ OD\ of\ control\ group} \times 100$$

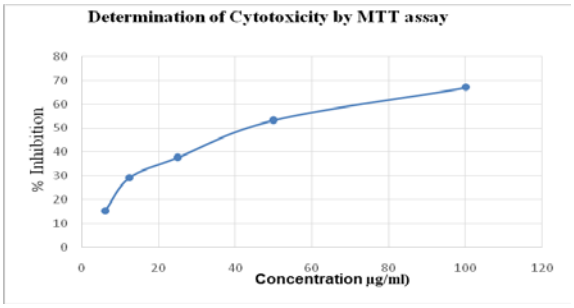


Fig. 11: cytotoxicity for drug

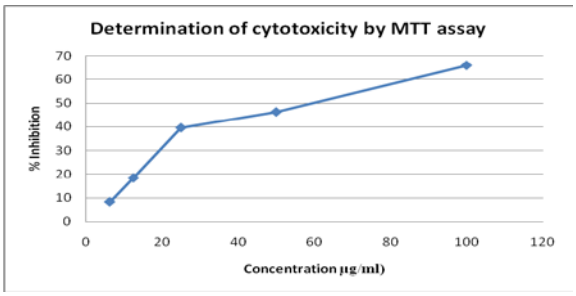


Fig. 12: Cytotoxicity for formulation F8

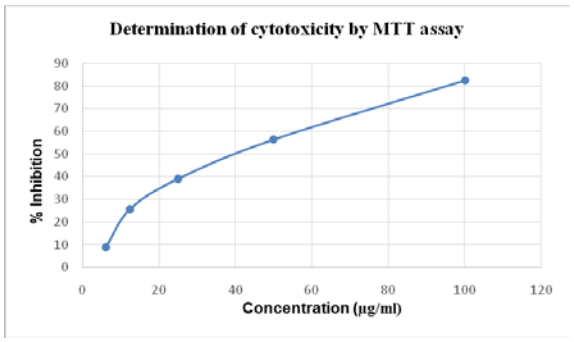


Fig. 13: Cytotoxicity for formulation F8 placebo

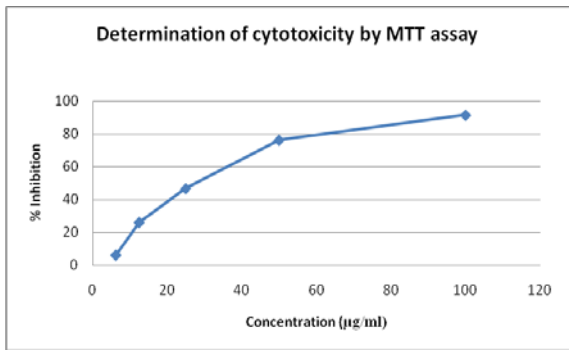


Fig. 14: Cytotoxicity of the formulation F6

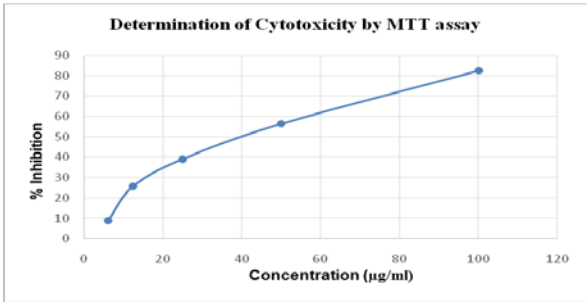


Fig. 15: Cytotoxicity of the formulation F6 placebo

The %inhibition of 50% of cells of the formulation F8 was found to be more than the formulation F6, so this formulation was considered as more toxic as compared to F6 and Drug.

3.7.10 Thermodynamic studies

a) Heating cooling cycle: Six cycles among refrigerator temperature 4°C and 45°C with storage at every temperature of not less than 48h was calculated. Those formulations, steady at these temperatures were subjected to centrifugation.

b) Centrifugation: approved formulations were centrifuged at 3500rpm for 30min. Those formulations that have not been shown any phase separation were taken for freeze-thaw cycle check.

c) Freeze-thaw cycle: Three freeze-thaw cycles among – 21°C and +25°C with storage at every temperature for not less than 48 h was done for the formulations. The example is examined for notable changes as the period partition. This finishes one cycle. The emulsion was rehashed this test through 3 cycles. No significant changes have seen in the all three formulation at this storage condition.

4. DISCUSSION

Capmul CMC showed maximum solubility of Curcumin 90 ± 0.18mg/mL. Thus 1:2 and 1:3 ratio of SCoS having formulation (F8 and F6) was chosen for incorporation in the final formulation of nanoemulsion gel. The UV absorbance of curcumin standard solutions was in the range of 10-50µg/ml of the drug in ethanol showed linearity at 425nm. SEM results exposed the nanoparticles were in the range of the 120-135nm. The percentage growth inhibition was determined using the following formula and concentration of drug or test samples required to inhibit cell growth by 50% values were created from the dose-response curve for each cell line. The %inhibition of 50% cells for the formulation F8 was found to be more than the formulation F6, so this formulation was considered as less toxic as compared to F6 and drug used.

5. CONCLUSION

All the above investigations brought out numerous facts which lead to following conclusions:

The study on various process and formulation variables exposed that all the variables are vital in the formulation of nanoemulsion gel.

By considering the evaluation parameters, the nanoemulsion gel with oil: smix ratio of 1:8 has shown the superior results than that of nanoemulsion gel having 1:6 ratios.

Nanoemulsion gel with oil: smix ratio 1:8(F8) was observed to release the drug slower when compared to 1:6(F6) ratios, at the end of the 24th hour. We found F8 released 66.11% and F6 59.41% of the drug. By this, it can be concluded that F8 was ideal for the treatment and has excellent enough permeation.

Spontaneous technique was found appropriate for formulation of nanoemulsion gel. Lipophilic drugs like curcumin can be successfully used in the formulation of nanoemulsion carbopol gel. The sustained release of the drug and nanoemulsion gel systems were most excellent chosen dosage for permeation enhancement and less toxic for patient compliance.

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