ORIGINAL ARTICLE

Topical Delivery of Emulsomal Gel for the Management of Skin Cancer

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Abstract

Aim: The aim of the present study was to develop and characterize lipid vesicular gel for managing skin cancer through topical route, because on the delivery of intact drug molecule, it distributed between normal cell and cancerous cell, leading to unacceptable side effects and also require high dose of drug to treat. **Materials and Methods:** Developed gel base with varying concentration of polymers and prepared emulsomes was incorporated in gel base, and then, emulsomal gel was evaluated for different parameters such as pH determination, viscosity, spreadability, homogeneity, and stability studies. The optimized emulsomal gel was done for their *in vitro* and *in vivo* drug release study. **Results:** The optimization of gel base was performed for different concentrations of Carbopol 934, methyl paraben sodium, glycerine, polyethylene glycol, and PVP. Emulsomal gel was prepared by distribution of emulsomes into the gel base. Physical analysis demonstrated that the resulting emulsomal gel had a light yellow color and was uniform and smooth when applied. The optimized emulsomal gel formulations were shown to be appropriate for all other characterization parameters, such as pH, viscosity, spreadability, and homogeneity. The *in vitro* drug release study investigation was revealed an improved emulsomal gel, showed a sustained profile over a 12 h period. Stability studies of emulsomal gel in terms of residual drug content and particle size indicated that at 25 ± 2°C temperature formulation, <2% degradation was observed over the 45 days. Skin irritation study and *in vivo* study were performed for developed emulsomal gel formulation. The emulsomal gel composition has been shown to be non-irritant to the skin. Hence, emulsomal gel represents promising result for improving the bioavailability of anti-cancerous drugs; moreover, emulsomal gel produces sustained drug release. Thus, the presented system explores the favorable alternative to conventional chemotherapy for the management of skin cancer through topical delivery. **Conclusion:** From the overall study, it was concluded that the methotrexate emulsomal gel was successfully formulated and evaluated.

Key words: Emulsomal gel, methotrexate, skin cancer, topical delivery

INTRODUCTION

For local and systemic treatment, topical administration is an attractive route. It is accepted as a successful method of treating local dermatologic illnesses to provide medications directly to the skin.[1] It can enter the skin more deeply and provide excellent absorption as a result. In order to maximize the local effects and reduce the systemic ones, or to ensure appropriate percutaneous absorption, attempts are being made to use topical drug carriers that assure adequate localization or penetration of the drug within or through the skin.[2] Topical preparation avoids the GI irritation, avoids the metabolism of drug in the liver, and increases the bioavailability of the drug. Topical preparations act directly at the site of action.[3] Topical gel preparation has remains one of the most popular and important pharmaceutical dosage forms. As a result, the therapeutic effects of the drugs are achieved effectively whereas the systemic side effects can be avoided or reduced. Topical gel formulations provide a suitable delivery system for drugs because they are less greasy and can be easily removed from the skin.[4]

The skin is an organ that separates the human body and the environment. It acts as a barrier that protects the body

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against UV radiation, toxic substances, and infections. The epidermis is the outermost layer of skin. Keratinocytes, dendritic melanocytes, and Merkel and Langerhans cells are different types of cells present in the epidermis. The underlying dermis contains connective tissue with antigen-presenting dermal dendritic cells, mast cells, and memory T-cells.[5] Skin cancer is the most common malignant disease found, particularly in Caucasians. More than a million new cases are reported worldwide each year. The various types of skin cancer are named after the cells they originate from and their clinical behavior. The most common types are basal cell carcinoma, squamous cell carcinoma (together referred to as non-melanocytic skin cancers), and malignant melanoma.[6,7] The color of skin in humans is primarily determined by the presence of melanin and dark skin has larger melanocytes that produce more melanin which protects the deeper layers of the skin from the harmful effects of the sun.[8] Human skin is repeatedly exposed to ultraviolet radiation that influences the function and survival of many cell types and is regarded as the main causative factor in the induction of skin cancer.[6] Ionizing radiation, pollutants, chemicals, and occupational exposures are also linked with skin cancers.[9] A higher amount of epidermal melanin in people of color filters at least twice as much UV radiation as the epidermis of fair color. This is the reason that the White population is the primary victims of skin cancer and the incidences of skin cancer are lower in POC when compared with Whites.[10,11] The main reason for skin cancer is due to UV exposure because large amounts of UV radiation reach earth's surface due to depletion of ozone layer.

Vesicular drug delivery systems define as "Vesicles have become the vehicle of choice in drug delivery system." Vesicular drug delivery systems are highly organized assemblies made up of one or more concentric bilayers that arise when amphiphilic building blocks self-assemble in the presence of water.

Vesicular drug delivery system provides alternative to traditional therapy. Encapsulation of anti-cancerous drug into vesicles provides some advantages such as high stability, reduction in dose of drug, and minimizing the side effects and significantly reduces the cost of treatment. Vesicular drug delivery systems are particularly important for targeted delivery of drugs because of their ability to localize the activity of drug at the cancerous cells of skin, thereby lowering its concentration at the normal cell of skin. Emulsomes are a new generation colloidal carrier system in which internal core is made of fats and triglycerides which is stabilized by high concentration of lecithin in the form of o/w emulsion.[12] Emulsomes are a novel lipoidal vesicular system with an internal solid fat core surrounded by a phospholipid bilayer. Emulsome is an advance nanocarrier technology for poorly aqueous soluble drugs. Emulsomes consist of microscopic lipid assembly with apolar core, which contains water-insoluble drugs in the solution form without requiring any surface active agent or cosolvent. Besides the other vesicular formulations, emulsomes are much stabilized and nano range vesicles. It is a new emerging delivery system and, therefore, could play a fundamental function in the effective treatment of life-threatening ailment.[13]

The antimetabolite drug methotrexate is well known for having a wide range of cytotoxic effects against various cancers. It is a cancer prevention drug.[14] It works by competitively inhibiting the dihydrofolate reductase enzyme, which then prevents cancer cells from synthesizing DNA.[15] Moreover, it also exhibits many other properties, such as anti-inflammatory,[16] anti-neoplastic,[17] anti-rheumatic,[18] antibacterial,[19] and anti-viral.[20]

Emulsome-based system showed excellent potential for targeting. The formulations could significantly modify providing sustained release action at comparatively low drug doses, thereby reduction in the toxicity problem due to complimentary localization of the drug in target cells.

MATERIALS AND METHODS

Methotrexate obtained as a gift sample from Neon Pharmaceutical Ltd., Mumbai, India. Phospholipid (Soya Lecithin), Tristearin, and Sephadex G-50 (medium grade) were purchased from Sigma-Aldrich Chemicals Co., St. Louis, MO. Tween 80 and others chemical and reagents were of analytical grade and were used as they were procured. Distilled water used in all the experiments.

Preparation of emulsomal gel

The emulsomal gel formulation was prepared with incorporation of different formulations of methotrexate-loaded emulsomes (prepared by the cast film method[12] with slightly modification and their development and characterization also communicated for publication) with optimized gel base.

Preparation and characterization of gel base

Gel base was prepared by dissolving required quantity of methyl paraben, glycerine, and polyethylene glycol in 30 ml of water in a beaker with stirring at high speed using mechanical stirrer. Then, Carbopol 934, sodium CMC, and PVP were added slowly in given amount to the beaker containing liquid during continuous stirring. The triethanolamine (act as gelling agents) was added slowly during stirring to attain gel structure. The different composition of base gel formulation is mentioned in Table 1.

The different gel bases were characterized for: Feel of application, spreadability (g.cm/sec), consistency, pH, viscosity (cps), and extrudability.

Table 1: Different composition of base gel formulation

Formulation	Carbopol 934 (g)	Sodium CMC (g)	PVP (mg)	Methyl paraben sodium (mg)	Glycerine (ml)	PEG (ml)	Triethanolamine (ml)
G1	0.5	0	50	75	5	5	1
G2	1	0	50	75	5	5	1
G3	1.5	0	50	75	5	5	1
G4	2	0	50	75	5	5	1
G5	0	0.5	50	75	5	5	1
G6	0	1	50	75	5	5	1
G7	0	1.5	50	75	5	5	1
G8	0	2	50	75	5	5	1

Characterization of prepared emulsomal gel formulation

Physical appearance

The physical appearance of prepared gel was visually checked as parameters, that is, color, appearance, and feel on application.

pH determination

The pH of emulsomal gel was determined using the digital pH meter. A 1 g of gel was dissolved in 100 ml distilled water and stored for 2 h. pH electrodes were completely dipped into the formulations and pH was noted. The measurement of pH of each formulation was done in triplicate manner and average values were calculated.[21]

Extrudability determination

About 20 g of gel were placed within a closed collapsible tube, which was then tightly clamped to prevent any rollback. The gel was ejected after the cap was taken off. Weighing was done once the extruded gel was collected. The extruded gel % was calculated.[22]

Viscosity determination

The viscosity of the prepared emulsomal gel was measured by Brookfield viscometer. The sufficient quantity of gel base was filled in wide mouth jar separately and it should sufficiently allow dipping the spindle. The RPM of the spindle was adjusted to 2.5 RPM. The viscosities of the formulations were recorded.[23]

Spreadability

Spreadability means the extent of area to which emulsomal gel readily spreads on application to skin or affected part of skin. The therapeutic potency of a formulation also depends on its spreading value. Spreadability of formulation is expressed in terms of time in seconds taken by two slides to slip off from gel base, which is placed in between the slides under the direction of certain load.[24,25] The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for 3 times and the mean time was taken for calculation[26] (Jain *et al*., 2007).

Lesser the time taken for the separation of two slides, better the spreadability. It is calculated using the following formula

$$S = M * L/T$$

Where, M = Weight tied to upper slide; L = Length of glass slide; and T = Time taken to separate the slides.

Homogeneity

The emulsomal gel has been set in the container; all prepared gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any lumps, flocculates, or aggregates.

Grittiness

The emulsomal gel was evaluated microscopically for the presence of any appreciable particulate matter under light microscope. The preparation should free from particles and the grittiness of any topical preparation can check.

Determination of entrapment efficiency

The entrapment efficiency was determined after separation of the unentrapped drug by the use of mini-column centrifugation method.[27,28] Sephadex G-50 (1.2 g) was swelled in 20 ml of 0.9% NaCl solution for 5 h at room temperature with occasional shaking. The Sephadex G-50 gel was formed and it was stored at 4°C. To prepare the mini-column, the hydrated gel was filled up to top in the barrel of 1 ml disposable syringe, plugged with Whatman filter pad. Then, barrel placed in the centrifuge and centrifuged at 2000 RPM for 3 min to remove saline solution. Eluted

volume was removed from the centrifuged tubes and in the center of Sephadex G-50 gel bed, emulsomal gel was placed. Columns were again centrifuged at 2000 RPM for 3 min to expel and remove void volume containing emulsomal gel to the centrifuge tubes. Elute was remove and 0.25 ml of saline was applied to each column and centrifuge as previously. The amount of drug entrapped in the vesicle was then determined by disrupting the vesicle using 1 ml of 0.1% v/v triton-X 100, filtering it and the drug content was determined using UV–Vis spectroscopy at 304 nm. The percentage efficiency was determined by the following equation:

$$\text{Percentage Drug Entrapment Efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Total amount of drug}} \times 100$$

In vitro drug release studies of emulsomal gel

In vitro drug release studies of emulsomal gel were performed using dialysis method with cellophane membrane of 10 kDa molecular weight (Sigma, MO, USA). The formulation (2 ml) was placed in the donor compartment and the receiver compartment was filled with 35 ml dialysis medium (saline phosphate buffer pH 7.4) stirred continuously at 100 RPM using magnetic stirrer at 37 ± 1°C. At 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h time intervals, 5 ml of solution from the receptor compartment was pipette out and immediately replaced with fresh 5 ml phosphate buffer solution so as to maintain equal volume level each time. All samples were withdrawn in triplicate. Samples were analyzed for amount of methotrexate release by disrupting the vesicle using 1 ml of 0.1%v/v Triton-X 100, filtering it and the drug content was determined using UV–Vis spectroscopy at 304 nm. The obtained values were fitted into zero-ordered, first-ordered, Higuchi matrix, and Korsmeyer–Peppas model. The results of *in vitro* release profile obtained for all the formulations were plotted in models of the data treatment as follows:

- Zero-ordered kinetics model – Cumulative % drug released versus time
- First-ordered kinetics model – Log cumulative percent drug remaining versus time
- Korsemeyer–Peppas model (Log cumulative % drug release versus log time)
- Higuchi's model – Cumulative percent drug released versus square root of time.

Storage stability studies

The developed emulsomes gel (EM4F11G4) stored at temperature 2 ± 2°C, 25 ± 2°C and 60% RH and 40 ± 2°C and 75% RH for a period of 45 days as per the ICH Q1A guidelines. These formulations were examined by any alteration in particle size and residual drug content at regular time interval, that is, 0, 15, 30, and 45 days.

In vivo study

All the experimental procedures followed during studies were prior approved and were conducted following guidelines for care and use of laboratory animals of IAEC (Indian Animal Ethical Committee) of Vedica College of B. Pharmacy, RKDF University, Bhopal (M.P.), and certificate number is IAEC/VCP/2021/003/02.

Swiss albino male/female mice (6–8 weeks old and 20–25 g in weight) were taken for the *in vivo* study. They housed in a well-ventilated room at 25 ± 2°C with a relative humidity of 30–60% and animals were subjected to 12:12 h light and dark cycle for at least 7 days before the experiment.

Animals were divided into four groups, each group having four animals. Group I was taken as control group (without any treatment). All other groups were treated with carcinogen to develop skin cancer. Group II served as carcinogen control. Group III was treated by the methotrexate gel. Methotrexate gel was applied to cover lesion b.i.d. for 6 weeks. Group IV was treated by 1% methotrexate-loaded emulsomal gel. Emulsomal gel was applied to cover lesion b.i.d. for 6 weeks.

Specific hairs of back portion of abdomen of animals were depleted by shaving before 2 days of treatment. Control group was kept without treatment and another group was treated with carcinogens at 2 times in a day with rubbing on skin. Skin tumorigenesis was initiated by topical application of 7,12-dimethylbenz (a) anthracene at an interval of 72 h, at a dose of 0.05 g/kg body weight in acetone (100 ml/mouse), followed by croton oil (1% w/v) in acetone (100 ml/mouse), twice in a week up to ninth week starting from day 8 of first 7,12-dimethylbenz(a)anthracene application.[29,30] After applying gel containing methotrexate and developing emulsomal gel, mice's skin tumors were evaluated for alterations. At the conclusion of the 2nd, 4th, and 6th weeks after administering the appropriate treatments, visual examination of the tumor site was performed. The size of the tumor was used to grade the skin, with 0 being normal skin, 1 being a mild tumor, 2 being a moderate tumor, and 3 being a large tumor. For the entire group, an average is presented.

Skin irritation study

Protocol for general procedures and use of animals for conducting this study has been reviewed and approved by the Institutional Animal Ethics Committee. Skin irritation test was carried on Swiss albino mice, weighing 25–30 g. Mice were grouped into two groups, each containing two mice. The animals were kept under standard laboratory conditions, at a temperature of 25 ± 2°C. The animals were housed in polypropylene cages, two per cage, with free access to standard laboratory diet and water. This test was carried out to confirm the skin compatibility of the emulsomal gel formulation. A single dose was applied to the left ear of

the mouse with right ear as control. The development of erythema, which is a manifestation of cutaneous vascular dilatation, was monitored daily for 7 days.[31] The average score obtained according to Uttley and Van Abbe (1973) scoring scale was reported each day. The data collected were interpreted according to the score obtained as follows:

- 0–9: Probably not perceptibly irritant to human skin
- 10–15: May be slightly irritant to some users
- Over 15: Likely to prove sufficiently irritant to some users so that level of complaints might be unacceptable.

RESULTS AND DISCUSSION

Preparation of emulsomal gel

Preparation of gel base

G1–G8 gel base formulations were prepared by dissolving required quantity of different polymers and all were stirred at high speed using mechanical stirrer. The different compositions of base gel formulation were prepared and evaluated for different parameters such as pH, viscosity, spreadability, homogeneity, and grittiness [Table 2]. The prepared gel bases G1 and G2 containing 0.5% and 1% of Carbopol 934 form a very thin gel that liquefies within 4 and 5 h of after preparation, respectively. The prepared gel base G3 containing 1.5% Carbopol 934 gelling agent to some extent better gel was obtained but the problem of liquefaction after 24 h was observed. The gel base G4 was formulated with 2% of Carbopol 934 and it showed uniform smooth gel and does not liquefy upon keeping long time duration. The pH of the formulation was determined to be sure that the formulation can be used without the risk of irritancy to the skin. The pH was found to be 7.54 for G4 gel base formulation, which was very nearer to the neutral skin pH, thus the formulation G4 can be used without the risk of irritancy to the skin. The spreadability of formulations was found to decrease with increasing the concentration of gelling agent. The value of spreadability for optimized gel base was found to be 10.1 cm, indicating that the gel is easily spreadable by small amount of shear stress. As the result of all evaluation parameters, 2% of Carbopol 934 was selected as the optimized concentration of gelling agent and this gel formulation is used for further gel preparations.

G4 gel base

Methyl paraben sodium (75 mg), glycerine (5 ml), and polyethylene glycol (5 ml) were dissolved in about 30 ml of water in beaker. The dissolved mucilaginous materials were stirred at high speed using mechanical stirrer. Then, Carbopol 934 (2g) and PVP (50 mg) were added slowly to the beaker containing above mixture and getting liquid dispersion while stirring. A 1 ml triethanolamine (act as gelling agents) was added slowly while stirring to attain translucent gel structure with maximum viscosity.

Preparation of emulsomal gel formulation

Optimized three emulsomes preparations, EM4F8, EM4F11, and EM4F18, were selected for incorporated into gel base (G4) to formed emulsomal gel formulation. These emulsomal gels were evaluated as gel bases [Table 3]. The physical evaluation as the color of prepared emulsomal gels was light yellow to pale yellow in color and appearance of emulsomal gels was transparent and translucent in nature and smooth on application at skin. The individual properties such as consistency of formulations were good and texture of prepared emulsomal gel was found to be smooth. The pH value of the prepared gel formulation was observed at room temperature and valued range at 7.03–8.21. The literature informed that from epidermis to dermis, pH of the skin increases and attained the neutral value. The pH ranges 7.0–7.6 are desirable to skin, so the emulsomal gel formulation does not interfere with the physiology of skin. The prepared emulsomal gel formulations were evaluated for good extrudability due to having good viscous properties.

In vitro drug release kinetics of emulsomal gel

Different kinetics models were utilized to analyze the *in vitro* release data and define the release kinetics. The system where the drug release rate is unaffected by its concentration is known as a zero-order rate. In a system where the drug release rate is concentration dependent, the first-order explains the release.

R^2 emulsomal gel formulation EM4F11G4 among different models [Table 4] was found to be highest for the Huguchi's model. This indicated that the drug release from

Table 2: Evaluation parameter of gel base formulation

Parameters	Formulations							
	G1	G2	G3	G4	G5	G6	G7	G8
Feel of application	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Spreadability (g.cm/sec)	13.2	12.5	10.7	10.1	8.9	7.4	6.9	5.7
Consistency	Poor	Good	Good	Excellent uniform	Poor	Poor	Fairly good	Good
pH	6.22	6.43	7.01	7.54	6.88	7.88	7.98	8.01
Viscosity (cps)	1088	2091	3195	3763	1099	2394	4388	2576
Extrudability	Good	Good	Excellent	Excellent	Poor	Poor	Good	Good

Table 3: Evaluation of emulsomal gel formulation

Parameters	Formulations		
	EM4F8G4	EM4F11G4	EM4F18G4
Colors	Pale yellow color	Light yellow color	Pale yellow color
Appearance	Transparent	Transparent	Translucent
Odor	Define odor	Define odor	Pleasant odor
Feel of application	Smooth	Smooth	Smooth
Spreadability (g.cm/sec)	10.1	10.23	13.2
Consistency	Excellent uniform	Excellent uniform	Good
pH	7.54	7.03	6.22
Viscosity (cps)	3693	3832	3713
Drug content (%)	99.81	99.87	99.84

Table 4: Release kinetics for emulsomal gel formulations

Plot	Zero		First		Higuchi		Peppas	
x/y axis	%CDR versus T		Log % remain versus T		% CDR versus square root of T		Log C versus Log T	
Formulation	r^2	K_0	r^2	k_1	r^2	K ($min^{-1/2}$)	r^2	n
EM4F8G4	0.6675	0.6318	0.3871	0.0079	0.8642	9.1999	0.8665	0.8676
EM4F11G4	0.8151	0.6543	0.4811	0.0087	0.9611	8.2181	0.8902	0.8819
EM4F18G4	0.6915	0.6344	0.4991	0.0099	0.9114	8.1113	0.8412	0.8513

Table 5: Skin tumor produced on the exposure of skin of mice to DMBA+1% croton oil

Group	Weeks			
	Zero	Second	Fourth	Sixth
I: Control group	0	0	0	0
II: Carcinogen control	3	3	3	3
III: Methotrexate gel	3	2.95	1.89	1.67
IV: Methotrexate-loaded emulsomal gel (EM4F11G4)	3	2.34	1.07	0.42

Mean (±SD) of skin tumor size in mice

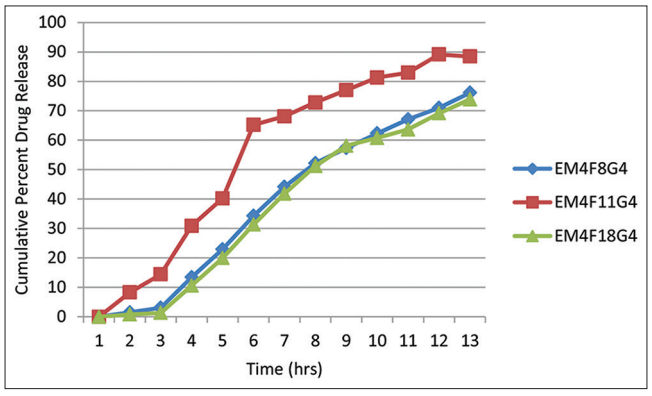

Figure 1: Zero-order plots of emulsomal gel formulation

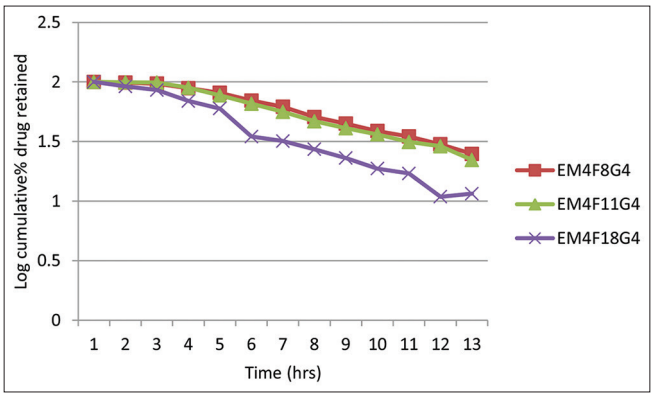

Figure 2: First-order plots of emulsomal gel formulation

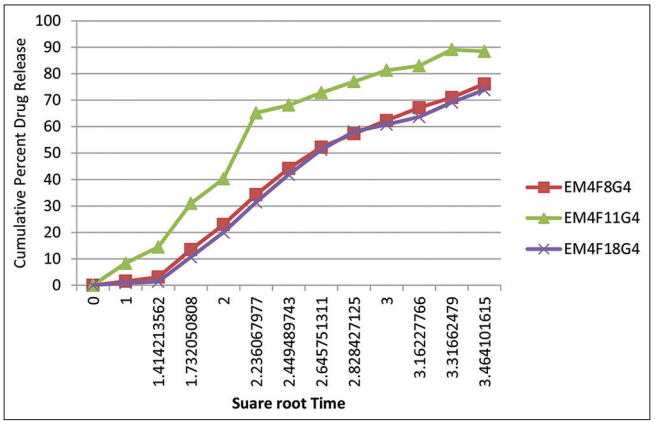

Figure 3: Higuchi plots of emulsomal gel formulation

all the formulations followed diffusion controlled release mechanism shown in Figures 1-3. Higuchi's model describes the release of drugs from the insoluble matrix as a square root of time-dependent process based on Fickian diffusion equation.

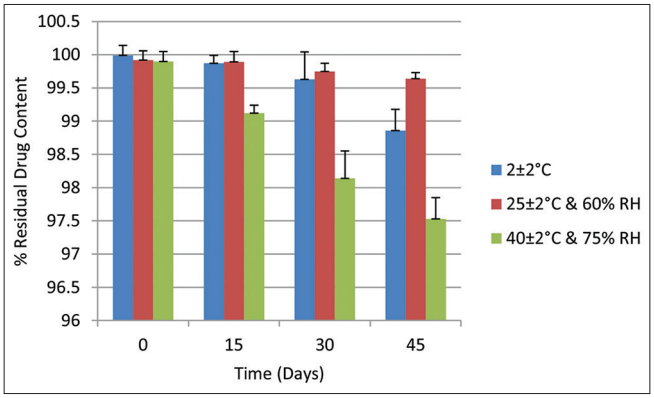

Figure 4: Residual drug content of emulsomal gel (EM4F11G4)

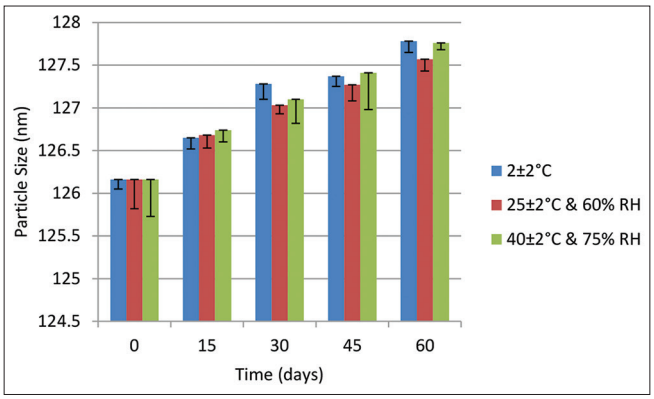

Figure 5: Particle size of emulsomal gel (EM4F11G4)

$$Q = KH\ t1/2$$

Storage stability studies

The stability of a formulation is known as the power of the materials to stay on inside definite restrictions over a fixed phase of time and known as shelf life of the product. The developed emulsomes gel (EM4F11G4) stored at temperature 2 ± 2°C, 25 ± 2°C and 60% RH and 40 ± 2°C and 75% RH for a period of 45 days as per the ICH guidelines. These formulations were examined by any alteration in particle size and residual drug content at regular time interval, that is, 0, 15, 30, and 45 days. The data of stability studies are presented graphically in Figures 4 and 5 and results revealed that the emulsomal formulation EM4F11G4 stored at temperatures of 25 ± 2°C and 60% RH, informed that it was <2% degradation at the end of 45 days.

Histopathological study

After sacrifice, the skin from the skin tumor site was taken. Skin samples were embedded in paraffin, fixed in 10% buffered formalin, and sectioned at 6–10 mm. After that, sections were stained with hematoxylin and eosin stain (H&E stain) and examined under a light microscope at ×40.[32,33]

Histopathological examination of mice skin from control (Group I), carcinogen control (Group II), methotrexate gel-treated (Group III), and methotrexate loaded emulsomal gel-treated (Group IV) groups was performed after the 3rd and

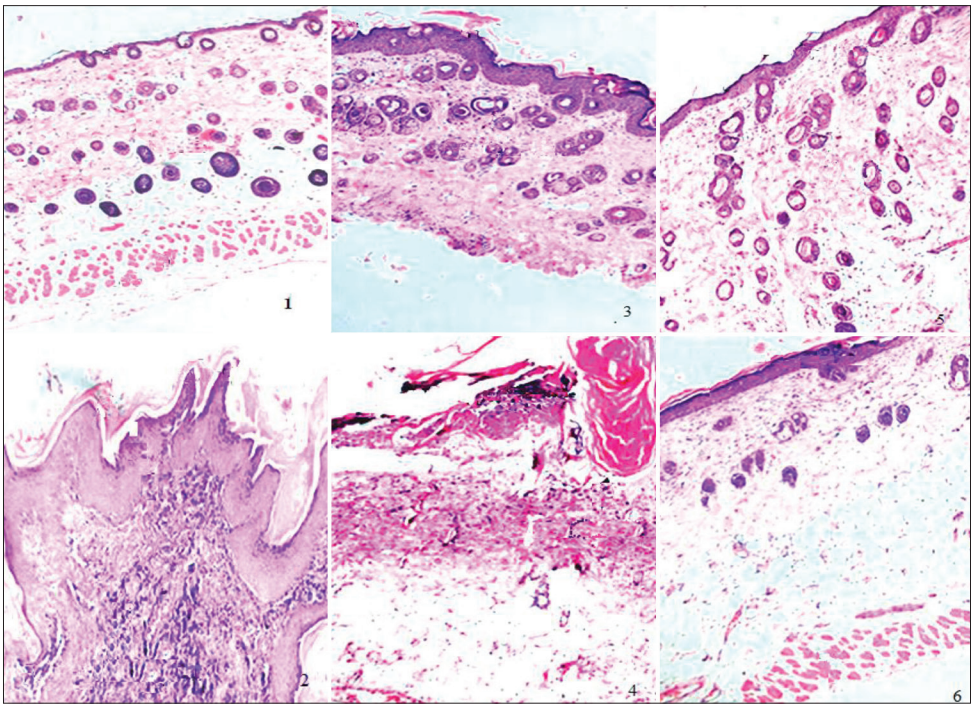

Figure 6: Hematoxylin and eosin stained cross-sections of mice skin

Table 6: Skin irritation scores for emulsomal gel

Formulations code	Group	Score after (days)							Mean irritation score
		1	2	3	4	5	6	7	
Methotrexate gel	I	10	9	9	8	8	6	6	8.0
Methotrexate-loaded emulsomal gel	II	6	6	7	8	9	10	6	7.29

6th weeks. It was found that the skin of the control group [Figure 6(1)] has thin epidermis and normal skin layers. The carcinogen control group Figure 6(2)shows moderate hyperkeratosis in epidermis, leukocyte infiltration in middle layers, and moderate acanthosis. Figure 6(3) shows the skin treated with the methotrexate gel after 3 weeks; mild hyperkeratosis in epidermal layer and moderate acanthosis in middle layers were found. Therefore, it shows that the methotrexate gel was effective in treating skin cancer. Figure 6(4) shows the skin sections after the 3rd week of methotrexate-loaded emulsomal gel application; the skin showed less hyperkeratosis and mild acanthosis compared to the skin treated with the methotrexate gel. Therefore, it shows that the methotrexate-loaded emulsomal gel was more effective than the methotrexate gel. Figure 6(5) shows the skin after 6 weeks treatment with the methotrexate gel. The skin shows mild hyperkeratosis and mild acanthosis. After receiving treatment for 6 weeks with the methotrexate-loaded emulsomal gel, the skin in Figure 6(6) shows minimal hyperkeratosis and no acanthosis. Normal skin layers are seen. As a result, the results of the histopathology of the skin following different treatments show that the methotrexate-loaded emulsomal gel was more successful in managing skin tumors [Table 5].

Skin irritation study

It was found that the mean value of the skin irritation scores for the methotrexate bearing Methotrexate gel and Methotrexate loaded emulsomal gel were 8.0 and 7.29, respectively [Table 6]. The applied formulation was reported to be non-irritating to the skin if indices are between 0 and 9.[31] This aims to ensure that the cancer cells, not the healthy ones, will be irritated and killed when the methotrexate contained in the formulation is released.

CONCLUSION

Methotrexate-loaded emulsomal gel was able in reducing the side effect of methotrexate such as reduce skin irritation on application, increase time of contact with the target cell, increase bioavailability of drug at the site of action, and it provides cancer cell specific interaction, easy to apply. Minimum size of emulsomes in gel base accommodating in good skin penetration and provides sustain release of drug at the target cell. Topical delivery of methotrexate for the management of skin cancer through emulsomal gel was effective alternative over the conventional route of administration.

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