Development of Novel Topical Formulations for Anti-aging Effect

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Abstract:-**There is increase in demand for cosmetics which have anti-ageing properties as majority of the population is concentrating on personal care and maintenance of skin . Different strategies are used to enhance the efficacy of dermatological delivery systems. Several anti-ageing therapies are used by people, but these therapies are associated with several side effects which limit their applicability in the market. The present article describes the novel trends in treatment of ageing. This article focuses on the multiple uses of DL α Tocopheryl acetate in the treatment of ageing, wrinkles and its effect on moisture retention, elasticity and fatigue of the skin.**

Key Words**:- Anti-ageing, Nanoemulsions, Nanoemulsion based sprays and Nanogels, Novel delivery systems for cosmetics.**

Key Message: **Novel Drug Delivery Systems are emerging to provide more beneficial results than the conventional formulations This research highlights the use of novel delivery systems in cosmetics, analyzing new approaches for obtaining sophisticated cosmetic products and examining the same. Advances in basic research on the antioxidant activity, mechanism of action, penetration and photoprotection of vitamin E in human skin have led to the development of numerous new formulations for use in cosmetics and skin care products.**

I. INTRODUCTION

Topical drug delivery systems have the major advantages as they bypass the first pass metabolism and avoid the risks and inconveniencies of intravenous therapy. Topical formulations increase the patient compliance. Topical drug delivery systems include two basic types of products i.e. external topicals which are spread on cutaneous tissue or sprayed (gels, emulgels, lotions, etc) and internal topicals are those which are applied to mucous membrane orally, vaginally or for rectal use (solutions, drops, etc) .

As there is an increase in the awareness about skin care and its maintenance, people today are more focused on their health and appearance .With an advent of retirement age, there has been an increased interest in anti-aging cosmetics. As a result there is a considerable rise in the demand for anti-aging agent containing cosmetic formulations (Sharma, 2012). Aging is a progressive failure of metabolic processes. A key reason for aging is the activity of free radicals on skin. Free radical reactions can be divided into three stages: Initiation, Propagation and Termination. The defense mechanisms against these are inactivating them within the cells soon after production; remove them by scavenging antioxidants and increasing the elimination of material already damaged by free radicals. The Free radicals damage the cell membrane which is composed of lipids and proteins. Their interaction results in the production of the chemical melano-dialdehyde which is very harmful. Hence, free radical scavenging compounds are incorporated in anti-aging formulations. The two distinct types of aging are intrinsic (internal) and extrinsic (external) aging (Leone, 2001). Skin aging is a complex biological process influenced by combination of intrinsic (genetics, cellular metabolism) and exogenous (chronic light exposure, pollution) factors. Causes of aging include continuous exposure of skin to solar UV radiations, cell damage by free radicals, metabolic processes, high level of collagen degrading enzymes due to collapsed fibroblasts and depletion of collagen in skin. Symptoms of aging include appearance of fine lines, thinning of skin, dryness of skin , appearance of wrinkles , loss of skin elasticity, dullness and roughness, thickened epidermis, mottled discoloration (Baschong, 2012) . All these have been put forth into various theories like Free radical theory, Neuro-endocrine theory , Telomerase theory of aging, Wear and tear theory ,Rate of living theory, Waste product accumulation theory, Cross-linking theory, Immune theory, Order to disorder theory, Errors and Repairs theories REF. Novel trends in cosmetic Delivery systems include development of prolonged acting, controlled release cosmeceutical formulations.

Several delivery systems like emulsion based delivery systems, particulate based systems, & vesicular systems, have been developed to enhance the diffusion of active components through the skin for cosmetic applications. A cosmetic delivery system is a product that can enhance the perceptual performance of cosmeticeuticals.REF

Conventional and alternative medical disciplines are used in an integrated approach to achieve the best possible results for the patient. Primary structural components of the dermis i.e collagen, elastin have been the subjects of majority of anti-aging research and efforts for aesthetic-anti-aging strategies pertaining to the skin, from ”anti-wrinkle creams” to various filling agents. Due to customer benefits and better results than conventional dosage forms, Novel Delivery systems are transforming the new product development in the cosmetics sector (Black., 2001).Hence there is a need to develop novel anti-aging cosmetics which can provide moisturization, protection against free radicals and anti wrinkle effect (V Gallandro ., 2005) . Prevention of skin

aging and its treatment is an emerging field for development of new formulations in cosmetics. Anti-aging formulations are predominantly moisturizer based cosmeceuticals and skin care products marketed with claims of reducing wrinkles, fine lines, pigmentation, discolouration, sagging of skin. Anti-aging formulations maintain the elasticity of skin , provide moisturization to skin and enhance the collagen levels of skin (M. Schafer-Korting.,2012).Clinical studies were performed to estimate the efficacy of anti-aging formulations in enhancing collagen levels (Robinson,2005) , anti-wrinkle activity (Mukherjee , 2006), Free radical scavenging activity (Algin Yapar 2013) and Spots reduction (Bissett , 2004). The current study mainly focuses on enhancing the permeation of DL alpha tocopheryl acetate through skin. Hence attempts were made to develop stable and easily manufacturable emulgel formulations containing DL alpha tocopheryl acetate to slow down aging process and for increasing hydration of the skin.

II. MATERIALS AND METHODS

DL α Tocopheryl acetate was procured as a gift sample from BASF , Mumbai . Carbopol Ultrez 10 was obtained from Lubrizol Pvt. Ltd, while Humectants were procured as gift samples from SD Fine chemicals . Permeation enhancers were obtained from Loba chemicals.

Experimental work

The present research has described the formulation and evaluation DL α Tocopheryl acetate emulsions and emulgels . The developed formulations were evaluated for parameters like, in-vitro diffusion profiles, viscosity, homogeneity, spreadability, compatibility, stability, and efficacy studies .

Formulation of DL α tocopheryl acetate emulsion:

Emulsion comprised of DL α tocopheryl acetate solubilized in oil i.e Capryliccapric triglyceride (CCTG), this forms the oil phase of the emulsion. Aqueous phase composed of humectants like propylene glycol and glycerine, surfactants like Tween 80 and Polyethylene glycol dispersed in water . Preservatives like methyl paraben and propyl paraben were dispersed in ethanol . Both the oily and aqueous phases were heated separately in water bath at 70 to 75 ℃ and were allowed to stand for some time. These phases were then mixed at room temperature under continuous stirring using over head stirrer for 2 hours to obtain a stable emulsion.

Fig 1. DL α tocopheryl acetate emulsion

Prototype formulations (PF): Three emulsions were developed using three different Carrier oils i.e CCTG, sunflower oil and almond oil to observe the effects of carrier oil on formulation development.

Table 1.Composition of developed Emulgel Formulations

Prototype formulations

Sr. no	Ingredients	PF 1	PF 2	PF 3	PF4	PF5	PF6	Pf7	PF8	PF9
1	DL-aplhaTocopheryl acetate	1%	1%	1%	1%	1%	1%	1%	1%	1%
2	CCTG	1%	2.5 %	5%	-	-	-	-	-	-
3	Sunflower oil	-	-	-	1%	2.5%	5%	-	-	-
4	Almond oil	-	-	-	-	-	-	1%	2.5%	5%
5	Purified Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
6	Propylene Glycol	3%	3%	3%	3%	3%	3%	3%	3%	3%
7	Glycerine	3%	3%	3%	3%	3%	3%	3%	3%	3%
8	Methyl Paraben	0.18%	0.18%	0.18%	0.18%	0.18%	0.18%	0.18%	0.18%	0.18%
9	Propyl Paraben	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
10	Ethanol	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%
11	Tween 80	4.5%	4.5%	4.5%	4.5%	4.5%	4.5%	4.5%	4.5%	4.5%
12	PEG 600+200	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%

Evaluation of the prototype formulations:

Sr .no	Evaluation Parameters	PF 1 to PF 3	PF 4 to PF 6	PF 7 to PF 9
1	Visual Examination	Homogenous , no traces of undissolved matter	Nonhomogenous	Non homogenous .
2	Viscosity (m pas)	2.7 to 2.9	3.1 to 3.3	2.8 to 3.1
3	Phase separation	Emulsions were stable. No phase separation observed	Phase separation observed after 2-3 hours	Phase separation observed after 2 hours
4	pH	5.5 to 5.7	5.5 to 5.9	5.4 to 5.6

Developed emulsions were compared and it was found that emulsion formulated using sunflower oil separated in two phases after a period of 40- 45 minutes. The drug, 12 mg, was dispersed in 5 ml of sunflower oil. Due to less solubility of drug in sunflower oil, solution was not homogenous. When almond oil was used similar results were observed and the emulsion separated within 25- 30 minutes after formulation of emulsion. The drug, 12 mg was dispersed in 5 ml almond oil but, the solution separated in two phases after it was kept steady for 30 minutes. When CCTG was used as a carrier oil, it was stable with good solubility of drug in it .The drug, 12 mg, was dispersed in 5 ml CCTG . Oil globules of drug were uniformly dispersed in the carrier oil. Hence CCTG was selected as carrier oil for the formulation of DL α tocopheryl acetate emulsion.

Preparation of DL α Tocopheryl acetate emulgel:

Initially the gel base was formulated using Carbopol Ultrez 10 as a gelling polymer. Carbopol Ultrez 10 was weighed and soaked in the measured amount of distilled water for 30-45minutes. Triethanol amine was added drop wise with continued stirring to adjust the pH to neutral. Developed emulsion and the gel base were mixed in 1:1 ratio to get the emulgel formulation. Fig.2.

Fig 2 . DL α tocopheryl acetate emulgel

Optimization of prototype formulations using 3^2 Factorial design: From developed prototype formulations, it was observed that the concentration of Caprylic capric triglyceride (CCTG) and Carbopol Ultrez 10 affected the formulation characteristics. Thus these two parameters were selected for developing optimization batches as per 3^2 factorial design. According to this design nine formulations were prepared containing varying concentrations of CCTG (1-5 %) & Carbopol Ultrez 10 (0.5- 1%)

Following Dependent & Independent variables were selected for Optimization of the formulation & various developed formulations are mentioned in Table3:

Table 2

Independent variables		Dependent variables
CCTG (oil Phase)	Carbopol Ultrez 10 (gelling polymer)	
Low (1%)	Low (0.5%)	Diffusion
Medium (2.5%)	Medium (0.7%)	Viscosity
High (5%)	High (1%)	

Table3: Formulation of nine batches using 3^2 Factorial design

Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
Drug	1	1	1	1	1	1	1	1	1
CCTG	1	2.5	5	1	2.5	5	1	2.5	5
Propylene Glycol	3	3	3	3	3	3	3	3	3
Glycerine	3	3	3	3	3	3	3	3	3
Methyl Paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ethanol	2	2	2	2	2	2	2	2	2
Polysorbate 80	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
PEG 600+200	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
CarbopolUltrez 10	0.5	0.5	0.5	0.7	0.7	0.7	1	1	1
Triethanol amine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Oleic acid	20	20	20	20	20	20	20	20	20

Developed formulations were evaluated for the respective parameters and based on the results of parameters of dependent variables i.e diffusion coefficient and viscosity of the developed emulgels, Formulation F5, was selected as optimized batch, as the diffusion and rheological behavior was better than other batches .Explain this part in details ADD a Sentence/Paragraph.

Evaluation of Developed Formulations

Optimized batch of developed formulations was further characterized and evaluated for following parameters.

DL α tocopheryl acetate emulsions

1) Visual Examination:

Developed emulsions were inspected for clarity, homogeneity and presence of any foreign particles.

2) pH:

The pH of DL alpha tocopheryl acetate emulsion was measured by Eutech pH tutors Cyberscan pH meter.

3) Drug content: DL α tocopheryl acetate content in emulsion was measured by dissolving 0.5 gm of emulsion in the solvent (Ethanol) and recording the absorbance at 281 nm using UV visible spectrophotometer.

4) Phase separation evaluation: Developed emulsion was subjected to centrifugation at 8400 rpm using Microspin centrifuge TC 4815 D and separation of emulsions in aqueous and oil phase was observed at time intervals of 15, 30, 60 minutes and 5 hours . Due to centrifugation unstable emulsions separated in to two distinct phases but stable emulsions remained homogenous and single phase systems.

5) Rheological study: The Rheological behavior and viscosity of DL alpha to copheryl acetate emulsion were determined by Brookfield viscometer (DV III Ultra Model No. D 220) at 25 ± 1°C using spindle 18 at 50 rpm .

6) In-vitro diffusion studies: In-vitro drug diffusion profile of developed emulsions was determined on Franz diffusion cell using dialysis membrane (150 Da) and diffusion medium comprising of 1:1 phosphate buffer pH 6.4 : ethanol + 0.5 % Tween 80 . Percentage of drug diffused was determined spectrophotometrically at λmax of 281 nm. Permeability and flux were calculated . **RESULTS IN TABLE & FIG.**

DL α Tocopheryl acetate Emulgels

a) Physical appearance:

Developed formulations were inspected for colour, consistency, homogeneity and pH.

b) Spreadability measurement:

Spreadability of emulgel was evaluated using spreadability testing apparatus. The apparatus was made of wooden block with scale and two glass slides having a pan mounted on a pulley. Excess formulation was placed between two glass slides and desired weight was placed on the upper glass slide for 5 min to compress the formulation to uniform thickness. Weight of 2gm was added to the pan. Time in seconds required to separate the two slides was noted .The spreadability was calculated by using the formula, S = M L/T. Where, S is the Spreadability, M is the weight tied to upper slide, L is the length of glass slide, T is time in seconds.

c) Extrudability measurement:

It is the test to measure the force required to extrude the formulation from the collapsible tube. The method adopted to evaluate the extrudability was based on the quantity in percentage of gel extruded from aluminium collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of emulgel in 10 second.

d) Rheological study:

The apparent viscosity of emulgel was determined at 25 ± 1° C by Brookfield viscometer (DV III ultra model D 220) using spindle 18. Results in Table----

e) Drug content determination:

DL α Tocopheryl acetate content in emulgel was measured by dissolving 0.5 gm of emulgel in solvent (Ethanol) and recording the absorbance at 281 nm using UV visible spectrophotometer after suitable dilutions..Results in Table----

f) In-vitro drug diffusion studies:

In-vitro diffusion profile of DL α-Tocopheryl acetate from emulgel formulations was determined using Franz diffusion apparatus .Combination of phosphate buffer pH 6.5 : Ethanol (1:1) + 0.5 % Tween 80 was used as a diffusion medium. Dialysis membrane of 150 dalton was mounted on Franz diffusion cell in between the donor and receptor compartments .The emulgel was placed on the dialysis membrane. Temperature of the diffusion medium was maintained at 37 ± 1 °C by a thermostatic arrangement . Sink conditions were maintained by magnetic stirring at 500-600 rpm. Aliquots of 1 ml were withdrawn at predetermined time intervals and replenished by equal volumes of fresh diffusion medium. Sample aliquots were withdrawn for a period of 24 hours. The drug concentration in the aliquots was determined spectrometrically and was calculated using standard calibration curve.(Results in Table & Fig ..)

g) Ex-vivo skin permeation and retention studies:

Ex-vivo diffusion studies were carried out using pig ear skin .The pig ear was procured from the local slaughter house. Pig ear was cleaned by scraping the fat layers and washed thoroughly with distilled water, then it was kept in tyrode solution. Pig ear skin was mounted between donor and receptor compartment on the Franz diffusion cell. Diffusion rate of drug through pig skin was evaluated by assessing the drug diffused through the skin at varying time intervals. Appropriate quantity of emulgel was placed in the donor compartment. The receptor compartment consisted of the

diffusion medium used during in-vitro studies. Aliquots were withdrawn at 5, 10, 15, 30, 45 minutes, 1 hour and then after every successive hour. Cumulative Drug release up to 24 hours was determined by the developed Analytical method.(results in Table /Fig >>>)

h) Bioadhesion Studies:

This test was for the measurement of bioadhesive force of the developed emulgels. Fresh pig ear skin was cut into pieces and washed properly. Two pieces were tied with two glass slides separately. From that one glass slide was fixed on wooden piece and the other piece was tied with the balance on the right hand side. Emulgel was placed (1 gm) between two slides containing pig ear skin, and the extra weight from the left pan was removed to sandwich the two pieces of skin and some pressure was applied to remove the presence of air. A definite Weight was added slowly to the left pan. The weight required to detach the emulgel from the skin surface gave the measure of bioadhesive strength. It was calculated using the formula :Bioadhesive strength = weight required (in gm) / area (cm^2). (Fig 3)

Fig 3. Bioadhesion testing apparatus for developed emulgels.

i) Skin irritation studies:

This test is for the estimation of irritation that may be caused after application of the developed product. Emulgel was applied on the properly shaven skin of rat (4.5 X 3.9 cm) and the changes in skin colour, morphology or inflammation were checked up to 24 hours . Set of 8 rats was used for this study. If no irritation persisted then the test was passed.

j) Determination of anti-aging efficacy

1) Moisture retention capacity :

Anti-aging products enhance the moisturization of skin thus help in reducing the signs of aging and improve the skin texture .This test determined moisturization provided by the anti-aging product to the skin. In this test we have determined the moisture retention capacity of the developed formulations on 6 volunteers. Volunteers were acclimatized for 1 hour after face washing at the test site i. e C.L.A.I.M.S Pvt Ltd. The volunteers were then taken in the study room and a spot was marked on the face of the volunteer by taking 2 measurements of the distance between the crow's feet area and the cheeck bone , the other measurement is the distance between corner of the nose to the same cheek bone . Then the moisture content of the volunteer on the marked spot was determined by moisture meter Make. These readings formed the baseline readings. The developed anti-aging emulgel(20 gm) and nanoemulsion based spray (15 gm) , were dispensed to the volunteers 1 and volunteer 6 .They were given instructions to apply 0.5 gm of the developed emulgel on the right side of the face and 0.5 gm of the developed nanoemulsion based spray formulation on the left side of the face . The anti-aging efficacy of the developed formulations was compared by this study. Rest four volunteers were also given the instructions of product application on both sides of the face i.e twice a day 0.5 to 0.7 gm at each time without using any other cosmetics on the face . After fifteen days of the product application, the volunteers were called at the site. The collapsible tubes containing the developed formulation which were dispensed to the volunteers were weighed to determine quantity of the formulation used by the respective volunteers .Moisture content of the skin those volunteers was determined again on the same marked spot . The difference between the readings noted prior to the product application and after its applications measures the total moisture retaining efficacy of the developed formulation .Results inTable…

Fig 4 Moisture meter (Make)

2) Trans Epidermal Water Loss (TEWL) :

Usage of Anti-aging formulations reduces the trans- epidermal water loss. Thus it was of prime importance to estimate the amount of trans- epidermal water loss. TEWL determines the moisture retaining efficacy of anti-aging products .All the six volunteers employed for the above study were also subjected to determination of TEWL through skin. After measuring the baseline moisture content of skin, the TEWL readings were noted on the same spot of all the volunteers by Vapometer (MAKE). Then the developed product was dispensed to the volunteers. After fifteen days of the product applications, TEWL was measured on each volunteer . Difference in the baseline readings and the final readings determines the

efficacy of the developed formulation to minimize the water loss through the skin**. Results in TABLE**

Fig 5. Vapometer (MAKE)

3) Determination of Elasticity :

The baseline elasticity was determined on all the six volunteers on the same marked spot which was used for the previous studies on day one. Developed product was dispensed to all the volunteers . After fifteen days of product application, elasticity of the skin was measured by Cutometer (Make). Difference in the baseline readings and the final readings determines the efficacy of the developed product to maintain the elasticity of the skin. **Results in Table…**

Fig 6. Cutometer (Make)

k) Stability studies:

Optimized formulations were subjected to stability studies as per ICH Guidelines (Q1A) R2 for a period of 3 months. Long term : 25 ± 2˚C/ 60 ± 5% RH , Intermediate : 30 ± 2˚C / 65 ± 5% RH , Accelerated : 40˚C ± 2˚C / 75 ± 5% RH .The stability of the optimized formulations was investigated based on appearance, viscosity, pH, drug diffusion and drug content of DL α tocopheryl acetate. **RESULTS in Table…**

III. RESULTS & DISCUSSION

All the developed formulations were evaluated for the parameters like pH, spreadability, extrudability, viscosity, drug content and in-vitro drug diffusion as mentioned in Table …

Table Number Evaluation of the developed emulgel formulations

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
pH	5.58	5.82	5.77	5.70	5.76	5.57	5.82	5.75	5.72
Spreadability gm cm / sec	23	22.5	27.9	24	26.5	23.1	25	23.5	24.2
Extrudability gm/ cm²	19.7	18.58	19.01	20.78	22.12	19.86	23.65	20.35	24.58
Viscosity m. pascals	4.8	4.72	4.5	6.02	6.89	6.67	7.01	7.11	6.71
Drug content	98%	98.5%	98.3%	97.8%	97.9%	98.3	98.3%	98.4%	97.8%
% In-vitro drug diffusion after 4 hours	26.45	26.72	27.29	26.93	27.91	25.89	25.48	24.57	25.39

Optimization of Formulations using Factorial Design:

The dependent variables of the factorial design were viscosity and drug diffusion. Hence for the optimization of the formulation, we have compared the results of viscosity and in-vitro drug diffusion of all the nine batches . Formulation F5 was optimized due to the highest in-vitro drug diffusion and better rheology as compared to the other formulations. Formulation F5 was selected as it has optimum viscocity i.e 6.5 to 6.9 mpas due to the incorporation of medium concentration (2.5 %) CCTG in the emulsion. As the concentration of CCTG increased, viscosity of the emulgel decreased. The in-vitro drug diffusion of F5 emulgel was better i.e 11.34 % in four hours due to the use of medium concentration of gelling agent. Drug diffusion was inversely proportional to the concentration of gelling agent used . The highest drug diffusion was recorded for F5 emulgel , among all the nine batches . WHY

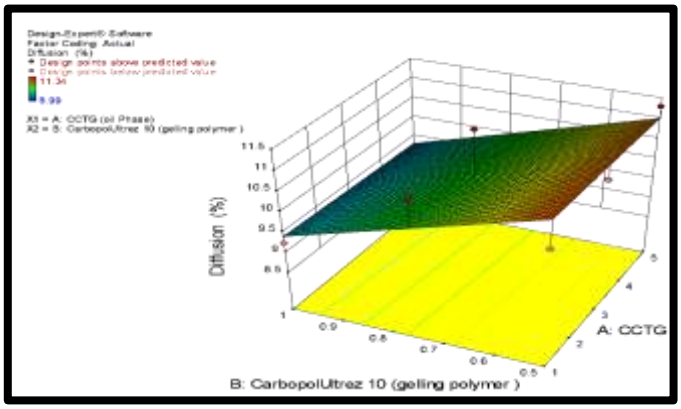


Fig 7 . Optimization of the batch using 3 [2] factorial design .

Describe in Experimental How the Plot was Made

This plot FIG 7 described the relation between the concentration of dependent variables and the rate of drug diffusion . As the concentration of CCTG was increased, the in-vitro diffusion of drug increased. As the concentration of gelling agent was increased, the in-vitro diffusion of drug has been reduced.

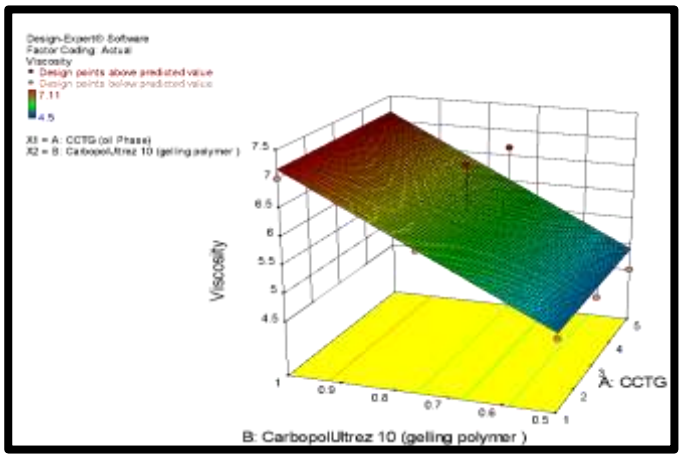


Fig 8. Optimization of the batch using 3 ^{2} factorial design

This plot FIG 8 described the relation between the concentration of dependent variables and the rheology of the developed formulations. As the concentration of CCTG was increased the viscosity of the emulgels decreased. When the concentration of gelling polymer was increased , the viscosity also increased .

Characterization of DL α tocopheryl acetate emulsion E, the optimized formulation (F5):

a) *Visual Examination:* The Emulsion was homogenous, when observed for visual clarity against black background. No traces of undissolved drug or other ingredients were observed in the emulsion.
b) *pH :* The pH of developed emulsion was in the range of 5.8 suitable for topical application
c) *Drug content:* It was determined by UV visible spectrophotometry. Drug content of developed emulsions was in the range of 97-98 %.
d) *Phase separation:* Emulsion was uniform and there was no phase separation seen in the developed product.
e) *Rheological behaviour:* The viscosity of the optimized emulsions was determined by Brookfield viscometer (DV III ultra model no. D 220) and it was in the range of 2.9- 3.2 m pas. What was the Flow Behaviour
f) *In-vitro diffusion studies:* In-vitro diffusion study indicated that more than 83 % of drug was diffused within 24 hours from emulsion of DL α tocopheryl acetate .

Characterization of DL α tocopheryl acetate emulgels

1) *Visual Examination:* The repared formulations were white, odourless and viscous with a smooth and homogeneous appearance.
2) *pH:* The pH of the developed emulgel was in the range of 5.5 to 6.8.
3) *Spreadability:* Spreadability is one of the important parameters for topical formulations . Spreadability indicatesr ease of application and improved patient compliance. Spreadability of the optimized formulation was in the range of 21- 28 gm cm/sec indicating good feel after application.
4) *Viscosity:* Rheological behaviour of emulgels indicated that the systems were Non-Newtonian & pseudoplastic in nature showing decrease in viscosity as shear rate increases . The apparent viscosity of the optimized emulgel at 50 rpm was found to be in the range of 4.50 to 7.12 m pas.
5) *Drug content:* Drug content of developed emulgels was 96-101 % which indicates good drug loading capacity of the formulation and required dose of drug was available for the pharmacological action.
6) *Extrudability:* It is important test for semisolid topical formulations. Pressure required to extrude semisolid preparation is dependend on its viscosity and consistency of the formulation. More quantity of emulgel was extruded at a little applied pressure on the tube which may result in better patient compliance. The extrudability of emulgel was in the range of 19 to 25 g/cm ^{2} indicating ease of extrusion.
7) *In-vitro drug diffusion study:* The study indicated that from emulgel formulations, 100% drug was diffused in 16 hours as compared to reference vitamin E formulation of which up to 100 % drug was diffused in 17 hours. This indicates comparatively better performance of the developed emulgel formulations for prolonged use.
8) *Ex-vivo skin permeation and retention studies:* Ex-vivo studies through pig ear skin on for the developed emulgel formulations exhibited 100% drug release in 18 hours ,whereas 90 % drug release was recorded from the reference formulation in 24 hours .
9) *Bioadhesion studies:* Up to 6 gm weight was required to separate both the pieces of skin over an area of 0.785 cm^2. Bioadhesive strength calculated for the developed emulgel was 7.643 gm/cm^2.
10) *Skin irritation studies:* No skin redness or irritation were noted after 24 hours of the application of the developed emulgel to the albino rats .

Fig 9. Skin irritation and redness studies on albino rats

Comparison of the Characterization Results for the reference vitamin E cream Mention Marketed Name & Supplier even in Text earlier) and the results of developed emulgel

Formulation	Reference product	Developed Emulgel
pH	6.29	5.76
Spreadability gm cm / sec	23.5	25.64
Extrudability gm/ cm²	18.53	21.91
Viscosity m. pascals	5.19	6.79
Drug content (%)	98.2	97.9
In-vitro drug diffusion (% drug release after 4 hours)	23.64	27.91
In-vitro drug diffusion (% drug release after 16 hours)	95.52	100
Ex-vivo drug diffusion studies (% drug diffusion after 18 hours)	100	100

Comparison of the results obtained for reference product and for developed emulgel showed that the developed emulgel formulation exhibited better spreadability due to the emollient action of humectants, the extrudability was good due to the use of suitable carrier oil at optimum concentration. % cumulative release for In-vitro drug diffusion of the developed emulgel was 27.91 % in 4 hours. Drug permeation was 4 % higher as compared to the results for the reference product due to the incorporation of 20 % oleic acid as permeation enhancer in the developed emulgel .Almost 100 % drug diffusion was noted within 16 hours from the developed emulgel, 95.52 % of drug diffusion was recorded from the reference product within the same time. Drug diffusion was 5% higher at 16 hours for the developed emulgel due to the enhancement of in-vitro drug diffusion by the permeation the enhancer.

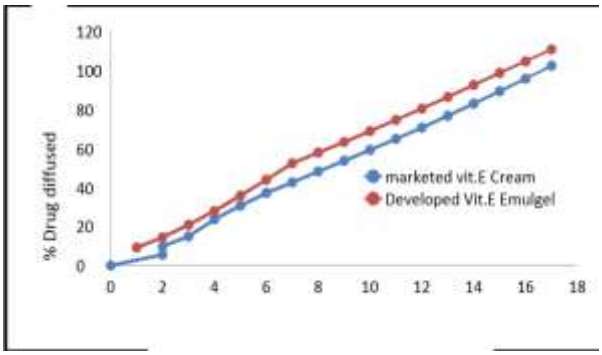


Fig 10. Comparison of the in-vitro drug diffusion for reference vitamin E cream and developed emulgel (graph must have SD Bars)

Anti-aging efficacy evaluation: Anti-aging efficacy was assessed based on the parmeters like measurement of trans epidermal water loss, moisture retention in skin and elasticity.

Trans epidermal water loss: TEWL is the amount of water loss through skin layers. Higher percent of TEWL makes the skin dry, generates fine lines and decreases the elasticity of the skin. Moisturizing agents are used to reduce trans-epidermal water loss and maintain the elasticity of the skin. The potential of the developed formulations to reduce TEWL was assessed by using Vapometer.

TEWL readings for developed emulge formulation:

Volunteer no.	Baseline TEWL % ANY UNITS	Final TEWL after 15 days %
1	11.4 ± 0.953	9.5 ±0.3
2	11.0 ±0.152	9.6 ±0.45
3	12.6 ±0.208	11.33 ±0.15
4	11 ±0.264	9.66 ±0.47
5	11 ±0.721	10.13 ±0.40
6	13 ±0.173	11.8 ±0.26

Moisture Content: Moisture retention in the skin is essential to maintain skin elasticity and reduce the formation of wrinkles on the skin . Reduction in moisture content due to chronic exposure to sun and other harmful radiations may lead to several dermatological problems. The developed anti-aging formulations have moisture retaining property which was assessed using moisture meter.

Moisture content readings for developed emulgel formulation:

Volunteer no.	Baseline Moisture content UNITS	Final moisture content after 15 days Units
1	30.9 ±0.36	31.7 ± 0.2
2	71.3 ±0.41	78.6 ± 0.36
3	33.7 ±0.92	35.16 ± 0.6
4	52.4 ± 0.47	52.5 ± 0.95
5	67.8 ±0.20	68.76 ± 0.11
6	81.1 ±1.42	81.66 ± 0.20

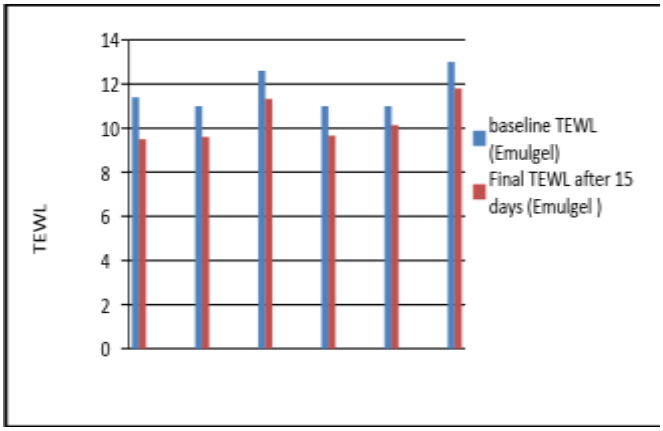
Elasticity: It is an important property of the skin to stretch and then return to its normal state . Dry skin is more prone to lose elasticity due to low moisture content in the skin and lack of essential nutrients. Elasticity was measured by Cutometer . It works on the principle of negative pressure suction method i.e the pressure required to pull the skin to measure its elasticity .This pressure is inversely proportional to the elasticity of the skin. Lesser the force required to pull the skin, more is the elasticity of the skin .

Baseline elasticity (R0) and fatigue (R7) readings for developed emulgel:

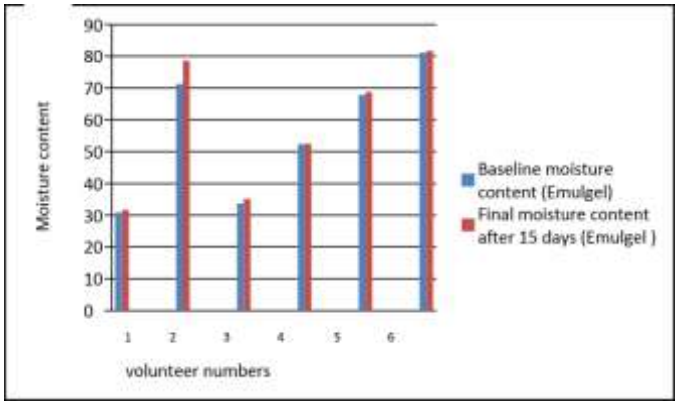
Volunteer no.	Units R0	R7	SD (±) R0	R7
1	0.3723	0.2183	0.016	0.0041
2	0.3560	0.2522	0.014	0.056
3	0.379	0.439	0.010	0.023
4	0.2	0.315	0.012	0.026
5	0.426	0.417	0.009	0.020
6	0.258	0.317	0.019	0.030

Final elasticity (R0) and fatigue (R7) readings for developed emulgel

Volunteer no.	Average R0	R7	SD (±) R0	R7
1	0.3654	0.2279	0.018	0.011
2	0.3495	0.2591	0.015	0.058
3	0.3362	0.4541	0.049	0.066
4	0.1877	0.3210	0.011	0.031
5	0.4128	0.4204	0.010	0.017
6	0.2504	0.3223	0.016	0.029



Effect of developed emulgel on increasing TEWL of skin



Effect of developed emulgel on increasing the Moisture content of skin

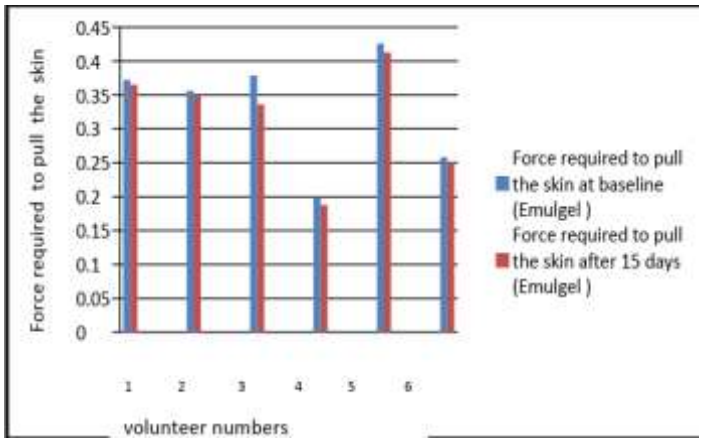


Fig Nos. at the base Table nos on top of Table

Effect of developed emulgel on the force required to pull the skin -increase in elasticity

The results indicated the influence of developed formulations on TEWL, Moisture content and Elasticity of skin .The developed formulations exhibited a decrease in transepidermal water loss .The TEWL was found to be reduced to the extent of 11.4 % after application of emulgel to the volunteers . This indicates protective effect of the developed emulgel formulations on aging skin of selected volunteers .

Moisture content of the skin was found to be increased after application of developed emulgel formulations to elderly volunteers .This indicates moisture retaining efficacy of DL αtocopheryl acetate emulgels .On an average 3.2 % increase in moisture retention on the skin was observed over a period of 15 days of application.

Elasticity of skin was also found to be enhanced by 4.46 % using the formulations as indicated by the reduction in the force required to pull the skin as measured through the Cutometer.

Present research work described the formulation development and evaluation of Novel topical dosage form like emulgel with anti-aging efficacy. Comparative study of developed emulgel and reference product was performed. The results obtained for the developed emulgel were comparatively superior to those of the reference product. Diffusion profiles and anti-aging efficacy of DLαtocopheryl acetate emulgel was compared with reference product. Drug permeation was found to be enhanced for developed emulgel formulations due to incorporation of permeation enhancer like oleic acid. Greater efficacy was observed for the developed emulgel even during anti-aging efficacy evaluation. Hence we can conclude that the use of optimum concentration of permeation enhancer enhanced the penetration of active component through skin and thus may increase the efficacy of the developed product .

New techniques are creeping in the field of cosmeceuticals to improve topical drug delivery, although a lot of research and human studies in this field are required to obtain a real life data.

IV. CONCLUSION

In the present study, an attempt was made to increase the efficacy of developed cosmeceutical antiaging product. The developed emulgels were characterized for parameters like spreadability, permeability, bioadhesion and the results were superior compared to the reference formulation..

As emulgel systems are used to incorporate hydrophobic drugs in water soluble gel bases, it may be a promising approach for novel cosmetic products. Also emulgel formulations possess an edge in terms of spreadability, permeability and extrusion as compared to conventional topical formulations .Due to the emerging techniques in the formulation development , several emulgel based products have been formulated which have more beneficial results and minimal side effects .There is advancement of new techniques in the field of dermatology to enhance the efficacy of the active component and minimizing the dermal side effects of the topical products. Several preclinical and clinical studies in this field are in progress to obtain more data in this field of research.

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