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Original Article

DESIGN, DEVELOPMENT AND IMPROVEMENT OF AN EMULGEL CONTAINING SILVER NANOPARTICLES AND VITAMIN D-3 FOR ITS POTENTIAL TO ACCELERATE THE HEALING OF WOUND

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ABSTRACT

Objective: The aim of this research work was to prepare a topical emulgel based dosage form incorporated with vitamin D-3 and silver nanoparticles to reduce the wound healing time in any kind of wound.

Methods: Central Composite Design (CCD) was applied for the optimization of emulgel by using *Design expert software*. Three responses (pH, viscosity, and *in vitro* drug release) and two factors (Carbopol concentration and stirring duration) were chosen, and Statistical Analysis of Variance (ANOVA) revealed that all the factors were significantly affecting the responses. Silver Nanoparticles (SNPs) was prepared with Green Tea Extract (GTE) and evaluated for particle size, Poly Dispersity Index (PDI), zeta potential and Fourier Transform Infra-red (FTIR) spectroscopy and revealed that SNPs of desired range and stability have been synthesized. Here excision wound model was used to evaluate the wound healing activity of formulation *in vivo.*

Results: Maximum *in vitro* release 88.2±2.1 has shown by the optimized formulation F13, pH and viscosity were also found in optimum range i.e., 6.2±0.4 and 1672±33 respectively, followed by Korsmeyer and Peppas model. Total eight groups were designed for animal study and silver sulphadiazine was used as marketed formulation. F13 formulation was further evaluated for *in vivo* data, it was revealed that emulgel loaded with high dose of vitamin D-3 along with silver nanoparticles has shown 100.5±1.7% wound contraction, while marketed formulation has shown 103.7±1.1% wound contraction, which was much similar with test formulation. Cytotoxic cell study was done using assay on chicken egg, formulation has not shown any cytotoxic behaviour like haemolysis and cell damage on chick embryo's blood vessels. Accelerated stability study of the optimized formulation was also performed to check whether the formulation was stable or not and it was revealed that optimized formulation was found stable for the period of six months.

Conclusion: It was revealed that emulgel loaded with high dose of vitamin D-3 and SNPs found suitable to accelerate wound healing and showed almost similar response in wound contraction on comparison with marketed formulation. This emulgel promised to controlled the delivery of the drug for the longer duration.

Keywords: Silver nanoparticles, Vitamin D-3, Emulgel, Green tea extract, Central composite design

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INTRODUCTION

When gels and emulsions are mixed together this forms a new drug delivery system known as emulgel. These are the mixture of emulsion and gel in one formulation and basically used to enhance the solubility of lipophilic drug as it is the rate-limiting parameters for all the lipophilic drugs [1]. Both oil-in-water and water-in-oil emulsions are used as vehicles to deliver various drugs to the skin [2]. Emulgel is good for dermatological purpose contains several favourable properties such as being water soluble, non-greasy, good spreadability, removes easily, emollient, non-staining property and carrying longer shelf life [3]. Emulgel is eco-friendly, transparent, having pleasing appearance. Penetration enhancers are also used in the formulation of emulgel [4]. These enhancers alter the chemical nature of skin layer and penetrate the drug in desired amount. Emulgel is promising agent for topical delivery of several lipophilic drugs and also enhance the therapeutic effect as well retention time to the skin [5]. Vitamin D-3 has an antimicrobial as well as anti-inflammatory activity in tuberculosis and HIV. This vitamin helps by inhibiting several inflammatory mediators [6]. Vitamin D-3 has approved by USFDA for the off label topical use in different cutaneous disease. Green tea extract is an antioxidant and an excellent reducing agent. Preparing silver nanoparticles with green tea extract is a cost-effective, green, and environmentally friendly one-step synthesis [7]. Vitamin D-3 promotes wound healing and can easily be included into the oil phase of an emulgel due to its lipophilic nature [8]. When combined with silver nanoparticles, it is thought to have a stronger therapeutic impact due to antibacterial effect of silver [9, 10]. Emulgel-based topical medication delivery is needed in this work because a silver nanoparticles and vitamin D-3 coupled emulgel is not yet available. The aim of this study is to synthesize vitamin D-3 and silver nanoparticles loaded emulgel using central composite design in Design Expert Software® to achieve prolong drug release as well as suitable topical formulation for healing of wound.

MATERIALS AND METHODS

Materials

Silver nitrate, vitamin D-3, carbopol 940, tween 80, span 20, glutaraldehyde, liquid paraffin, methyl paraben, propyl paraben was purchased from Loba Chemicals, Colaba, Mumbai. Green tea Extract was purchased from Fermenta Biotech Ltd; H. P. marketed formulation (Silver sulphadiazine) was purchased from local chemist shop.

Synthesis of silver nanoparticles

0.5M silver nitrate solution was prepared in distilled water on other hand green tea extract was prepared by adding the tea bag in 100 ml boiling water. In a separate beaker, the required amount of silver nitrate solution was added and then 1 ml of green tea extract was also added, kept the solution for 5 min [11]. Silver nitrate solution started to change the colour from white to brown this indicated that silver nitrate has been reduced by green tea antioxidant and it has converted into nanoparticles. Presence of nanoparticles was confirmed by FTIR followed by particle size and zeta potential [12].

Preparation of emulgel

Carbopol 940 polymer was used for the preparation of gel base. In previously heated distilled water at 60 °C carbopol 940 was added and

made a thick gel. In the gel base, a silver nanoparticle prepared with green tea extract was added and adjusted the pH with triethanolamine from the range 5.5-6.5. Oil phase was prepared by mixing liquid paraffin and span 20, on other hand in water phase tween 80 and distilled water was added [13]. 25000 IU of vitamin D-3 was added in ethanol to prepare drug solution and then this solution was added in oil phase with constant stirring at magnetic stirrer. Methylparaben was added in propylene glycol and then mixed with aqueous phase. Both phases were heated separately at 70-80 °C for 10 min and then it was to cooled at room temperature. After cooling both phases were mixed at room temperature. It indicated that emulsion has been prepared [14]. Prepared emulsion and gel base was mixed together in ratio1:1 followed by addition of glutaraldehyde as a cross-linking agent [15]. The composition of emulgel is described below in table 1.

Table 1: Composition of various emulgel batches (%w/w)

S. No.	Ingredients	F1 Quantity(%w/w)
1	Vitamin D-3	0.1
2	Carbopol 940	1-2
3	Tween 80	0.8
4	Span 20	0.92
5	Liquid paraffin	7.5
6	Propylene glycol	30
7	Methylparaben	0.01
8	Distilled water	q. s to prepare100 gm of emulgel

Evaluation of silver nanoparticles

Particle size

A zetasizer is a tool often utilized to examine the dimensions and zeta potential of microscopic substances suspended in fluid. It employs a procedure called dynamic light scattering (DLS), also termed photon correlation spectroscopy, to decide particle size. Dynamic light scattering gauges how suspended particles influence light smaller particles cause more erratic light scattering than larger ones. By analysing light scattering fluctuations over time, the zetasizer can calculate the particle size distribution profile and average size of structures in suspension. This technique is non-invasive and suitable for analysing samples without needing to dilute or modify them. SNPs suspension was placed in cuvette and then the sample was kept in zetasizer for analysis [16].

Zeta potential

Zeta potential defines the stability of nanoparticles. Malvern Panalytical zetasizer was used for the analysis of zeta potential. Sample was placed in zetasizer using quartz cuvette for identification of zeta potential [17].

PDI (Poly dispersity index)

This data indicates the similarity and equality to the size of nanoparticles. If value is equal to 1 then it indicates that all the particles in the suspension are in the equal size range. For identification of PDI, sample was placed in Malvern zetasizer using quartz cuvette and allowed to scan the sample for measuring the particle size distribution [18].

FTIR (Fourier transform infrared)

This spectrum was used for the confirmation of the nanoparticles synthesis. This spectrum helps to identify functional groups associated with synthesized nanoparticles [19].

Optimization of emulgel

pH

pH was measured with electronic pH meter first of all pH meter was calibrated with different buffer solution, then sample was taken in a beaker and glass electrode was dipped in sample to check the pH at room temperature [20].

Viscosity

The texture of the formulation is a crucial aspect of topical products, as they are applied in thin layers on the skin. The viscosity of the emulgel plays a vital role in regulating the drug's ability to penetrate the skin. Brookfield viscometer (LVDE-5) was used to measure the viscosity of emulgel by using 6 number spindles at 100 rpm at room temperature [21].

***In vitro* release kinetics study**

The release of vitamin D-3 from topical emulgel was determined by using Franz diffusion cell through dialysis membrane. Receptor compartment was filled with phosphate buffer having 5.5 pH was filled. The fixed amount (100 mg) of emulgel was placed in donor compartment of Franz diffusion assembly. The buffer media in the receptor compartment was continues stirred with magnetic stirrer at 50 rpm and 37 °C±2 temperature and the inlet-outlet water supply were maintained to keep the temperature constant. Then up to 12 h at different time interval the sample was withdrawn from receptor compartment and maintained the sink condition. All the sample were examined using UV spectrophotometer (Labtronics Model LT-2010) at wavelength of 264 nm in triplicates calculated the amount of drug release from formulation by using previously prepared calibration curve [22].

Design of experiment

Three level two factors Central Composite Design (CCD) model was applied using Design Expert Software® and it was also utilized for statistical optimization of emulgel formulation. The statistical optimization was performed to achieve suitable pH, viscosity and controlled *in vitro* release of vitamin D-3 from formulation. For this purpose, several variables were used to prepare formulations; some factors were giving significant results some were not on that basis only two independent variables such as concentration of carbopol 940 another is stirring time were selected and three dependant variables, such as % cumulative release, pH and viscosity were selected [23]**.** Central composite design is shown in below mentioned table 2.

Table 2: Central composite design for optimization of emulgel

Composite	Level		
Factors (independent variables)	Low	Medium	High
Conc. of carbopol (940)	1 (%w/w)	1.5 (%w/w)	2 (%w/w)
Stirring time	10 (min)	20 (min)	30 (min)
Responses (dependant variables)	Constraints		
% release	Highest		
pH	Optimize		
Viscosity	Optimize		

***In vivo* excision wound model**

Animals was placed in the animal house, temperature and humidity was maintained at 26±2 °C and 44–56%, respectively, followed by dark and light cycles of 14 and 10 h, respectively, specified atmosphere was maintained to one week before and during the experiments. Standard rodent pellet diet was fed to the animals and kept in large hygienic cage. All the animals were allowed to take free access in cage during the experiment for getting their food and water. Required ventilation and diet was followed for proper nutrition requirements through the experiment. "Principles of laboratory animal care" (NIH publication no. 82-23, revised 1985) guidelines was followed throughout the experiment.

This animal study was performed at Pinnacle Biomedical Research Institute, Indore with protocol number PBRI/IAEC/PN-2304. Total eight groups and six animals in each group were selected and described below in table 3.

Table 3: Number of groups for animal study

Groups	Route	Dose (per g of emulgel)
I. Normal Control	--	--
II. Experimental control	Topical	--
III. Blank gel	Topical	--
IV. Standard (Silver sulphadiazine)	Topical	500 mg
V. ESVDL	Topical	3000IU
VI. ESVDH	Topical	25000IU
VII. SNPs Low dose	Topical	0.3 mg
VIII. SNPs High dose	Topical	1.0 mg

Procedure for excision wound

Rats were anaesthetized using ketamine (30 mg/kg). Firstly, shaven the back dorsal thoracic region of the skin of the art with epilator and an area of about 200 mm^2 was marked with depth of 1 mm on the back of the rat using a standard ring. The entire thickness of the marked skin was cut carefully and wound was allowed to leave open. Wounds and its surrounded area were observed for any type of microbial infection if any symptoms related to infection was found then the rat was separated and replaced with the healthy rat. Then test and standard formulation was used to apply on wound in specific dose in specified groups of animals for 16 d. Wound was traced on the transparent sheet by using marker on the day of wounding and then every 4 d until the 16th day, then on alternate days until complete wound healing. Changes in the dimension of wound area were measured on definite interval, and the wound contraction rate was calculated using the standard formula. The significance of the test groups as well as standard treated group, was determined by comparing healed wound area test and standard with respective days to healed wound area of the control group [24].

% Wound contraction was calculated using below mentioned formula:

$$\%\,Wound\ contraction = \frac{Wound\ \ area\ on\ initial\ day - Wound\ area\ \ on\ test\ day}{Wound\ Area\ on\ Initial\ day} * 100$$

Epithelialization period

It is the time duration in days required for the eschar to fall down from the surface of partially filled wound.

Histopathology

For histopathology of the tissue, rat skin specimens were separated from sacrificed animals and all the tissue was properly kept in formalin solution (10%). Distilled water was used to wash the skin tissues and then water content was removed using different grades of alcohol, followed by addition of toluene to clear all the tissue, and then placed in molten paraffin wax for specific periods. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Micro sections of tissue were cut and dried on a hot plate for 15 min and then stained with 1% aqueous eosin and haematoxylin to visualize general characteristics of tissue structure. Stained tissue was dehydrated in various alcohols, again washed with xylene and mounted using canada balsam. Sections were viewed microscopically using 10X lenses [25].

Statistical analysis of excision wound model

Statistical analysis was performed using Prism software. Results of all the *in vivo* data was analysed statistically using variance ANOVA (two-way) followed by Dunnett Post test and P value less than 0.0001 was considered as level of significance while comparison between different groups.

HET-CAM assay for toxicity testing

A HET-CAM assay can be used to determine the toxicity or skin irritation of formulation in place of Draize test (Rabbit eye test), it is cost-effective and quick test. In this test, fertilized egg were purchased from a poultry farm and kept in an incubator at 37 °C for proper incubation insuring that air sac is upright. Using sterile method albumin of the egg was removed out at 3rd day of expermint and egg opening was closed by using parafilm. The development of the chorioallantoic memebrane(CAM) was allowed. At 10th day when the membrane developed completely, made an casement of 2*2 cm^2 and 0.5 ml of formulation inserted over the CAM, after 20 sec rinsed the membrane with 5 ml of warm saline. Examined the membrane vascular drainage, haemorrhagic damge and coagulation for upto 300 sec. After getting all the data placed in below mentioned formula and calculated the score. Then evaluate the score from standard data and revelaed that either formulion is irritant or not [26].

$$\text{Scores} = [301 - H/300] * 5 + [301 - L/300] * 7 + [301 - C/300] * 9$$

Where H= Haemorrhage (Sec), L=Lysis time (Sec), C= Coagulation time (Sec)

Table 4: Scoring chart for HET-CAM test

Score range	Interfrence
0-0.9	Non irritant
1-4.9	Slighly irritant
5-8.9 or 5-9.9	Moderate irritant
9-21or 10-21	Strong irritant

Accelerated stability study (ICH Q1 R2)

Stability studies of the emulgel was conducted following ICH guideline for stability studies Q1 R2, the formulation was kept for 6 mo at 45 °C±5 °C and 75% RH. At the different time interval, the formulation was evaluated for pH, viscosity, and cumulative drug release till six months [27].

RESULTS AND DISCUSSION

Particles size, zeta potential and PDI

Particle size, zeta potential and PDI of optimized nanoparticles was measured 200±34 nm, 12.9±1.1 mV and 0.98±0.1 respectively. This indicated that synthesizes nanoparticles are stable and found in desired particle range [28]. Data of zeta potential shown in below mentioned fig. 1.

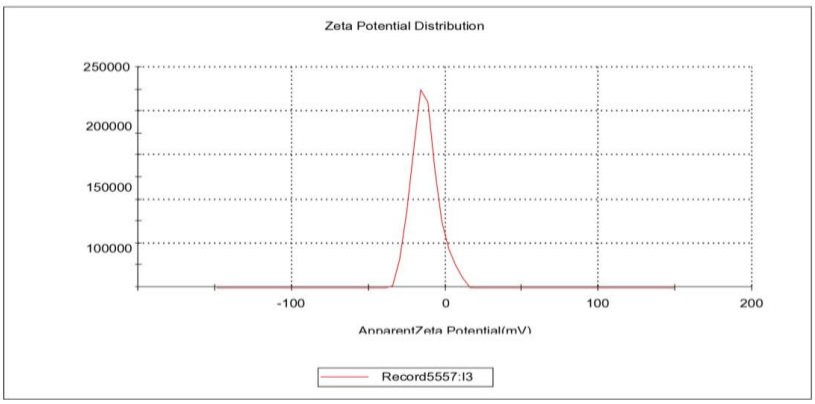

Fig. 1: Zeta potential of silver nanoparticles

FTIR spectra

The probable moieties were involved and acted as reducing agents for the silver nanoparticles synthesized from green tea extract. FTIR spectrum was used to confirmation of synthesis of nanoparticles. There were four intense peaks was found, it confirms the presence of SNPs and shown in below mentioned fig. 2 and table 5 [29].

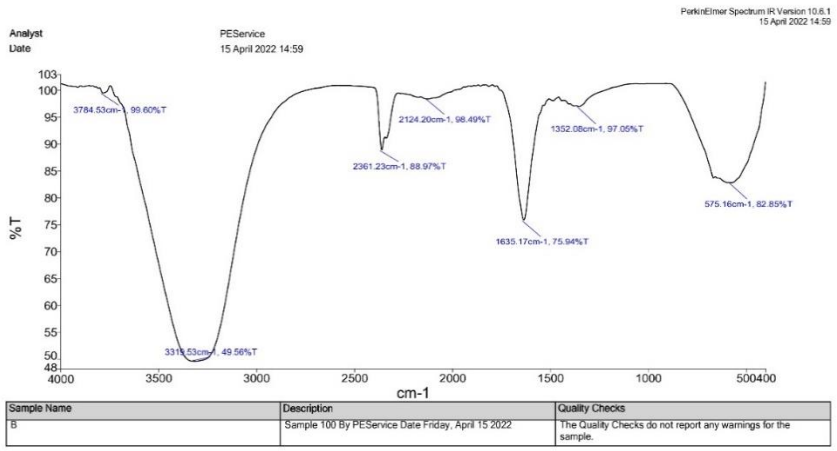

Fig. 2: FTIR spectra of silver nanoparticles containing significant peak

Table 5: FTIR data of silver nanoparticles indicating significance peak

% Transmittance	Functional group
3319 cm^{-1}	Vibration of O-H stretching
2124.20 cm^{-1}	C≡N stretching
1635.17 cm^{-1}	Stretching C=O in amide group, existence of protein as capping agent
575 cm^{-1}	Aromatic stretching vibration

Table 6: Various formulation of emulgel with responses

Std	Run	Factor 1	Factor 2	Response 1	Response 2	Response 3
		Conc. of carbopol (%w/w)	Stirring time (min)	% Release*	pH*	Viscosity (cps)*
4	F1	2	30	87.5±4.1	6.1±0.2	1661±31
9	F2	1.5	20	81.3±5.2	5.8±0.1	1339±34
11	F3	1.5	20	81.3±3.5	5.8±0.4	1339±45
5	F4	0.7	20	61.1±2.7	5.1±0.2	1034±36
3	F5	1	30	68.2±3.6	5.4±0.3	1223±41
2	F6	2	10	79.1±2.3	5.5±0.5	1521±39
13	F7	1.5	20	81.3±4.5	5.8±0.4	1339±40
12	F8	1.5	20	81.3±4.5	5.8±0.1	1339±40
10	F9	1.5	20	81.3±4.5	5.8±0.3	1339±40
8	F10	1.5	34	83.2±4.2	5.9±0.2	1338±42
1	F11	1	10	65.3±4.3	5.5±0.3	1225±35
7	F12	1.5	5.8	55.2±4.8	4.7±0.2	1340±38
6	F13	2.2	20	88.2±2.1	6.2±0.4	1672±33

*Values are expressed as mean±SD, n=3

Optimization of emulgel by using design expert® software

Design expert software suggested 13 formulations for further evaluation upon application of the central composite design on the basis of the dependent and independent variables the data of 13 formulations was mentioned in table 6.

***In vitro* cumulative % release**

The data was estimated by Design Expert® software. *In vitro* release of vitamin D-3 from emulgel was found between the range 55±1.7-94.5±1.6%. The contour plot revealed that independent variables showed effect in release profile of API from emulgel. It was revealed upon increasing the concentration of carbopol % release time was also increasing. P value was found 0.016 which is less than 0.0500 indicates model is significant [30]. Response surface plot effect of concentration of Carbopol and mixing time on % release, 3D surfaces plot effect of conc. of Carbopol and mixing time on % release and perturbation plot for effect on all by all two factors is mentioned below in fig. 3.

pH of formulation

The data was estimated by Design Expert® software. pH of emulgel was measured between the range 4.4±0.7-6.1±0.5% The contour plot revealed that independent variables showed effect in pH of emulgel. It was revealed upon increasing the concentration of carbopol, pH of the formulation was also increasing. P value was found 0.0275 in study of pH which is less than 0.0500 indicates model is significant [31]. 2D response surface plot effect of conc. of Carbopol and mixing time on pH of formulation,3D surfaces plot effect of conc. of carbopol and mixing time on pH and perturbation plot for effect on formulation by all two factors is mentioned below in fig. 4.

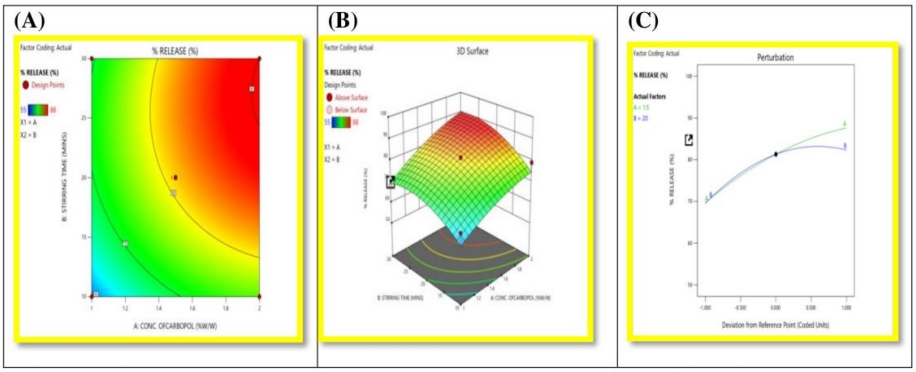

Fig. 3: (A) Response surface plot effect of conc. of carbopol and mixing time on % release, (B) 3D surface plot effect of conc. of carbopol and mixing time on % release, (C) Perturbation plot for effect on formulation by all two factors

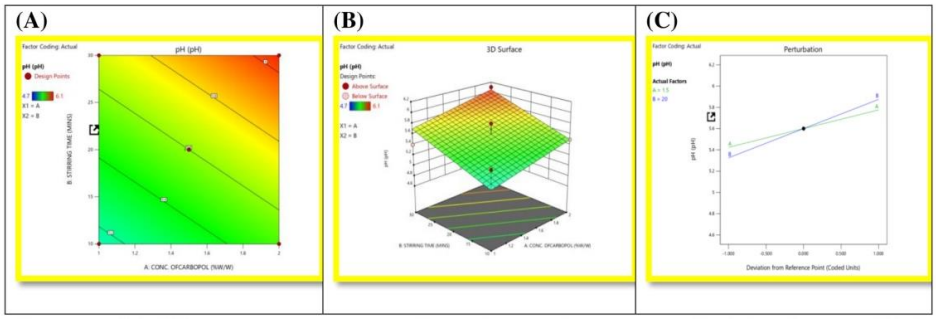

Fig. 4: (A) 2D response surface plot effect of conc. of carbopol and mixing time on pH of formulation, (B) 3D surface plot effect of conc. of carbopol and mixing time on pH, (C) perturbation plot for effect on formulation by all two factors

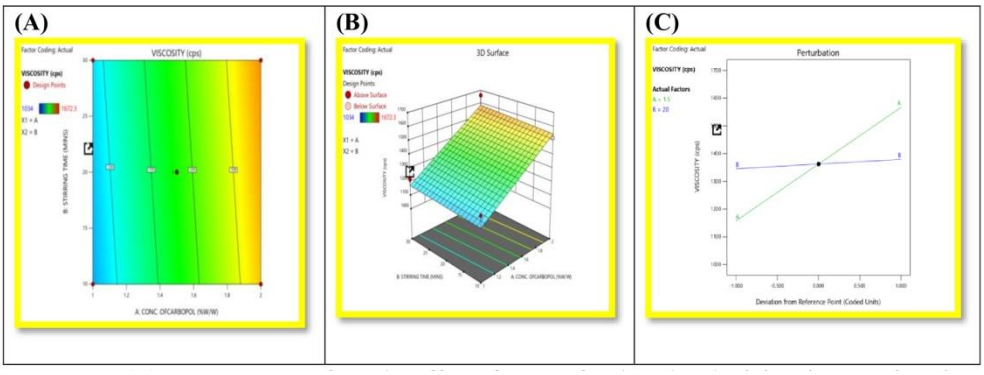

Fig. 5: (A) 2D response surface plot effect of conc. of carbopol and mixing time on viscosity of formulation, (B) 3D surface plot effect of conc. of carbopol and mixing time on viscosity, (C) Perturbation plot for effect on formulation by all two factors

Viscosity

The data was estimated by Design Expert® software. Viscosity of emulgel was measured between the range 1034±6.6-1674±8cps**.** The contour plot revealed that independent variables showed effect in viscosity of emulgel. It was revealed upon increasing the concentration of carbopol, viscosity was also increasing. P value was found 0.0349 in study of viscosity which is less than 0.0500 indicates model is significant [32]. 2D response surface plot effect of conc. of Carbopol and mixing time on viscosity of formulation, 3Dsurfaces plot effect of conc. of Carbopol and mixing time on viscosity and perturbation plot for effect on formulation by all two factors was mentioned in fig 5.

Optimization and selection of suitable formulation

As per above data F13 formulation showing highest release, pH and viscosity were also found in optimized range. For the further evaluation F13 formulation was selected.

Evaluation of emulgel

pH

pH of the optimized formulation (F13) was adjusted with triethanolamine and measured between 6.2±0.4. It was revealed that formulation has an optimum pH which is required for topical delivery of drug from emulgel (5.5-6.5) [33].

Viscosity

Viscosity of the optimized formulation (F13) was measured between 1672±33 cps. It was observed that on increasing the concentration of gelling polymer (Carbopol), viscosity of formulation was increased as the gelling polymer was responsible to reduce interfacial as well as surface tension the flow of gel and thereby increases the viscosity. Optimized formulation has shown desired viscosity for topical delivery of drug [34].

***In vitro* cumulative % release**

Drug release was performed for 12 h and there were four models was applied for best kinetic release profile like zero order, first order, Higuchi plot as well as Korsmeyer and Peppas model. Here drug cumulative release was correlated with linearity. R^2 value 1 represents model is linear and drug is releasing from the formulation in constant manner as the time is increasing. It was revealed that optimized formulation has shown zero order kinetics followed by Korsmeyer and Peppas model as these two models was found closest R^2 value. That represents the drug delivery from polymeric network and delivered 88.2±2.1% of vitamin D-3 from the formulations [35]. Release kinetics profile of emulgel is mentioned below in fig. 6 and fig. 7.

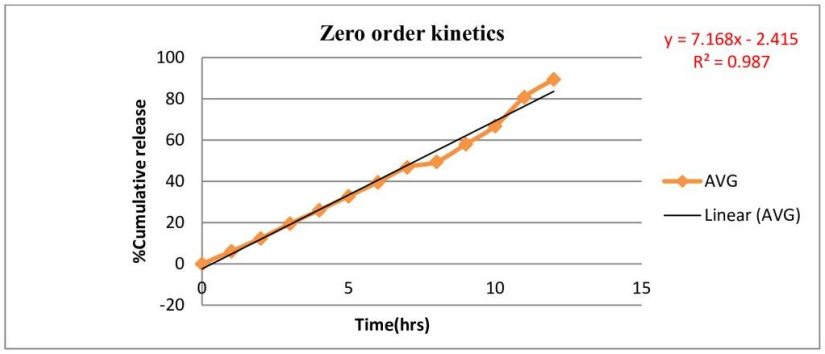

Fig. 6: Zero order kinetics release of vitamin D-3 from emulgel

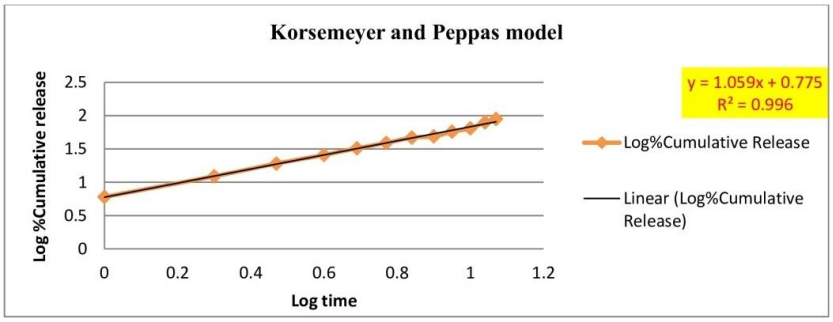

Fig. 7: Korsemeyer and peppas model for release of vitamin D-3 from emulgel

Table 7: Release kinetics profile

Zero order	First order	Higuchi	Korsmeyer and peppas
K= 7.16	K= 0.122	K=26.23	K=1.05
$R^2$0.987	$R^2$0.782	$R^2$0.883	$R^2$0.996

Excision wound model

The maximum % wound contraction was found in marketed formulation from 29.29±3.22% to 103.725±1.12% from 4th to 16th days. Upon comparison with other test formulations, it was revealed that emulgel loaded with high dose of vitamin D-3 has shown 100.5± 1.7% wound contraction on the 16th day of experiment. It was observed that % wound contraction was more in marketed and ESVDH (emulgel of silver nanoparticles-vitamin D-3 high dose) formulation in comparison to experimental control control group. Two-way ANOVA was used for statistical analysis followed by Dunnett post-test and it was revealed that over all P value for marketed and test was found<0.0001 this indicated that model is significant [36]. % wound contraction of all the groups is mentioned below in table 8.

Table 8: % Wound contraction in mm²

Groups	Route	Wound contraction area in mm²			
I. Normal control	--	--	--	--	--
II. Experimental control	--	9.3±2.6	21.5±2.4	24.3±3.5	29±1.8
III. Blank gel	Topical	8.2±2.9	20.5±1.2	24.3±1.9	28.9±2.3
IV. Standard	Topical	29.2±3.2	58.9±2.8	85.1±2.9	103.7±1.1
V. ESVDL	Topical	14.5±3.6	40.3±3.9	52.4±2.5	62.9±2.5
VI. ESVDH	Topical	22.7±1.9	67±1.9	84.5±1.9	100.5±1.7
VII. SNPs Low dose	Topical	14.5±1.7	23.9±3.7	28.9±4.2	34.8±2.4
VIII. SNPs High dose	Topical	17.5±2.7	26.4±2.4	31.4±1.4	40.3±1.1

Values are expressed as mean±SD, n=6. ESVDL-Emulgel of Silver Nanoparticles-Vitamin D-3 Low Dose, ESVDH-Emulgel of Silver Nanoparticles-Vitamin D-3 High Dose.

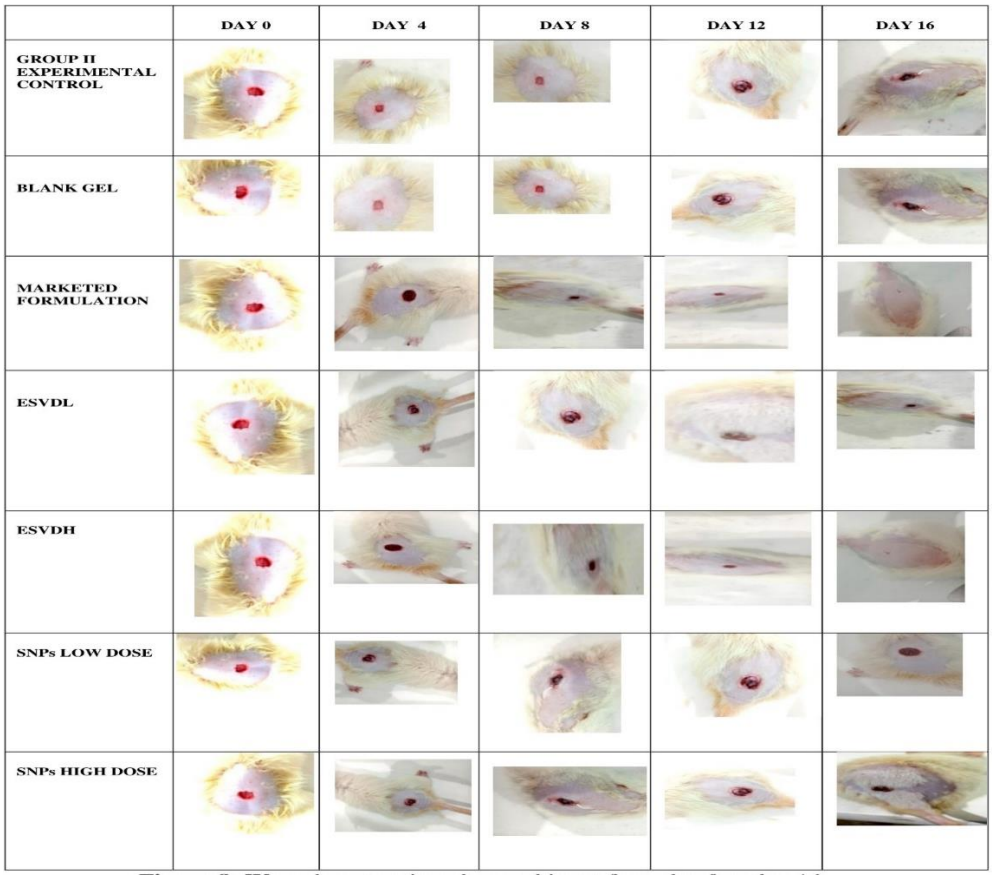

Fig. 8: Wound contraction in particular day, ESVDL-Emulgel of silver nanoparticles-Vitamin D-3 low dose, ESVDH-Emulgel of silver, Nanoparticles-vitamin D-3 high dose

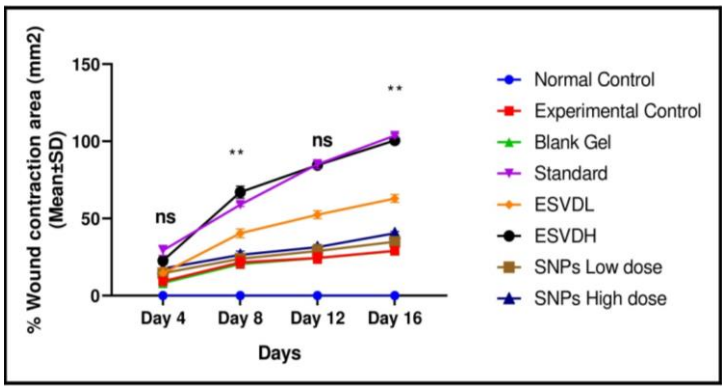

Fig. 9: % Wound contraction area in mm², denotes statistically significant values (Two Way ANOVA followed by dunnett post test) relative to control group (ns=non-significant; ****= p<0.0001, ***=p<0.001,= p<0.01 and *= p<0.1). ESVDH(emulgel of silver nanoparticles-Vitamin D-3 high dose); ESVDL(emulgel of silver nanoparticles-Vitamin D-3 low dose); SNPs (Silver nanoparticles). values are expressed as n=3, mean±SD**

Epithelialization

It is the time required by wound to form a new epithelial tissue. Only in marketed treated group and formulation ESVDH (emulgel of silver nanoparticles-vitamin D-3 high dose) treated group has shown the epithelialization on the 15th day of experiment. It was revealed that optimized formulation has good wound healing property approximately equal to marketed [37].

Histopathology

Histopathology of the animals was performed on the 16th day of animal study and evaluated for several parameters which are responsible for wound healing. Total 8 groups were evaluated for histopathology. Group II (experimental control group) has shown inflammatory cells, reduced amount of collagen fibre, non-viable cells, blood vessels and tissue with visible scar. Group III (Blank gel) has also shown inflammatory cells. Group IV (Standard treated) and Group VI (ESVDH) has shown viable cells, complete epithelialization, increased number of fibroblast cells, higher amount of collagen fibre, blood vessels and reduced number of inflammatory cells and remaining group has shown dead cells and visible scar and blood vessels [38].

Het-CAM study

In the HET-CAM test system, three reactions are determined, namely, haemorrhage, lysis and coagulation of the chorio-allantoic membrane of the fertilized chicken egg. There were no significant changes observed in the chicken egg after 300 seconds of formulation application and irritation score was found in the range 0.69±0.05. It is indicated that the formulation has not created any cytotoxic effect and found non-irritant [39].

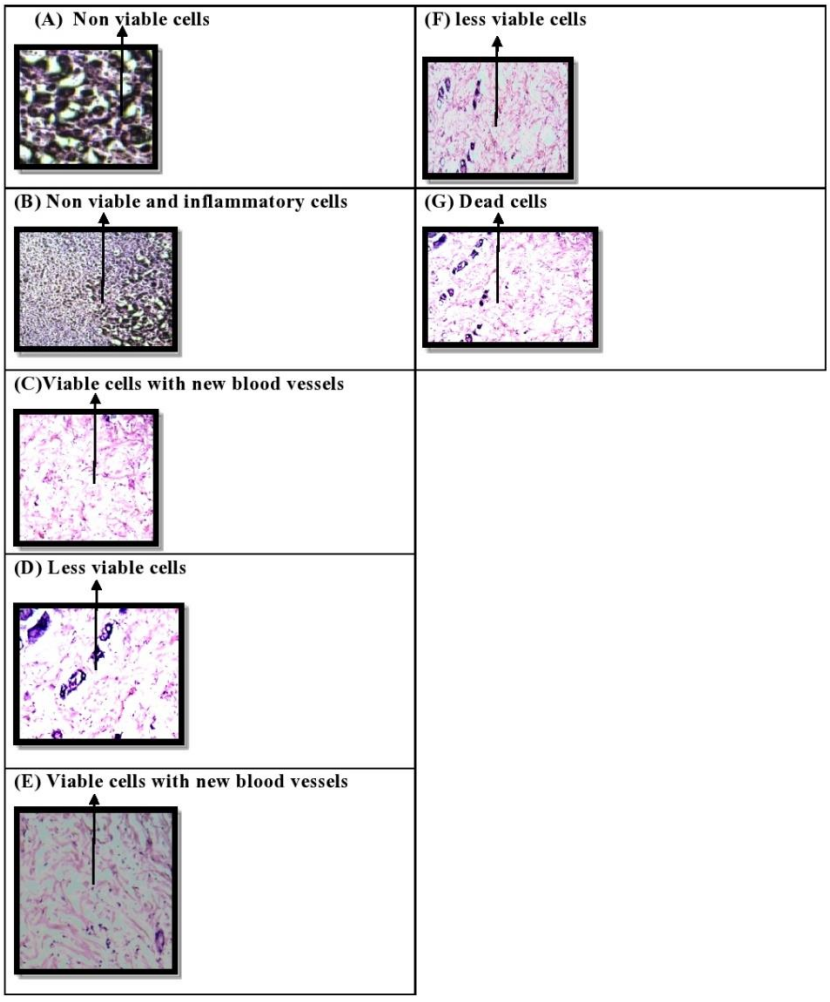

Fig. 10: (A) Experimental control group, (B) Blank gel, (C) Standard formulation, (D) ESVDL, (E) ESVDH, (F) SNP s Low dose, (G) SNPs high dose

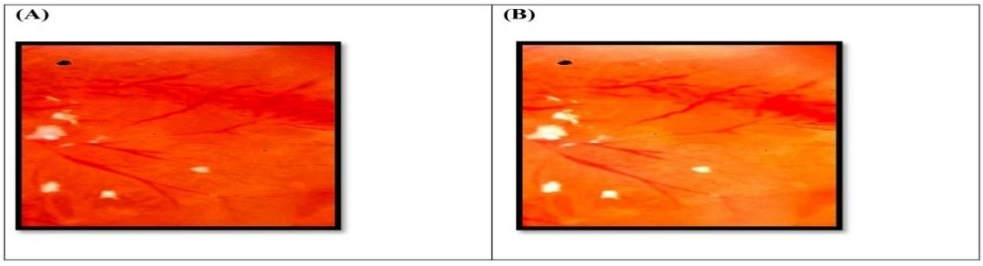

Fig. 11: (A) After 30 sec of formulation application, (B) After 300 sec of formulation application

Accelerated stability study

Optimized formulation was found stable till the period of six months at different parameter of evaluation and shown pH range 5.79±0.3. It was revealed that formulation has an optimum pH required for topical delivery (5.5-6.5). Viscosity of the formulation was measured between 1371.9±19 cps till the study period. It was discovered that the formulation has maintained viscosity for topical drug administration during the stability research, and no significant changes in cumulative drug release were found during the investigation as cumulative drug release was observed 88±4%. It was determined that the formulation was stable throughout the duration [40].

CONCLUSION

Vitamin D-3 and silver nanoparticles in emulgel may aid in wound healing due to their antibacterial properties and ability to boost cathelicidin synthesis. Emulgel is a novel drug delivery system that can deliver lipophilic and hydrophilic ions of drugs in two phases. The optimized formulation (F13) has the highest cumulative drug release, pH 6.2±0.4%, and viscosity 1672.5±33cps. Silver nanoparticles are useful for the treatment of wounds, skin infection, and burn. Combination of SNPs with vitamin D-3 may enhance wound healing potential. *In vitro* data recommends favourable topical delivery, and *in vitro* parameters like particle size, zeta potential, PDI, and FTIR spectra have confirmed the presence of nanoparticles. *In vivo* data for optimized formulation has shown desired result on wound contraction. An accelerated stability analysis of the formulation was conducted, and the optimized formulation was found stable up to the period of six months. This combination may increase patient compliance and found effective in treating wounds *in vivo*.

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AUTHORS CONTRIBUTIONS

Rishu Yadav-Literature review, method selection and writing original draft; Narendra Kumar Pandey-Review, compilation of data, editing, and supervision; Rajiv Kukkar-Review, compilation of data, editing, and supervision.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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