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NANOSPONGES BASED HYDROGEL CONTAINING ESSENTIAL OIL FOR WOUND HEALING ACTIVITY

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Keywords:

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ABSTRACT: The present study was aimed at incorporation of Frankinsense oil in nanosponges based hydrogel for wound healing activity. The oil was incorporated in nanosponges by the emulsion solvent diffusion method using different combinations of hydrophilic polymers like HPMC K15M, Poly vinyl alcohol, agar, hydrophobic polymers like ethyl cellulose, eudragit S100 and dichloromethane as crosslinking agent. Prototypes A3, D1 and E were selected based upon the yield Further screening was done based on morphology (SEM images), particle size (8.829µm, 0.567µm,17.416µm resp.) and entrapment efficiency (49.01%, 81.75%, 34.71%) and Prototype E1 was selected for optimization. Optimization was done by using 3^2 factorial design with design expert software and formulations F1 to F9 were developed. Using entrapment efficiency as the response, contour plot and surface response curve were plotted. GC analysis of dried sample of nanosponge formulation reflected all major constituents present in the oil concluding that the constituents of the oil were unaffected by the formulation process. Optimized nanosponges were incorporated in hydrogels and tested for viscosity (67,666 Centipoise), spreadability (0.57g/cm^2/sec), pH (4.84) and *in-vitro* release study (around 100% in 8 hours). From wound healing study using albino Wistar rats for 14 days, it was seen that there was sufficient contraction in the wound area in test group (77%) as compared to control group (50%). From this is concluded that Frankincense oil can be effectively used for wound healing property in the form of nanosponge based hydrogels.

INTRODUCTION: Skin being the largest organ suffers from various fungal, bacterial infections, allergies, gets damaged due to sun exposure or wounds as a result of injuries or any other conditions [1-7].

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Frankincense oil is obtained from *Boswellia serrata* species and is found to possess wound healing and anti-inflammatory properties. This research focuses on exploring wound healing activity of Frankincense oil [8-11].

Many essential oils topically benefit the skin but are very potent, and cause irritation in some people. Also owing to volatile nature, they do not remain on the skin for longer duration resulting in reduced therapeutic effect. To overcome all these problems and to provide an effective drug delivery system, this research was focused upon development of an

hydrogel formulation incorporated with nanosponges of Frankincense oil. Nanosponges were chosen for incorporation of oil so as to control the release and maintain stability of essential oil. Nanosponges are tiny porous polymeric drug delivery systems, having a three-dimensional scaffold that degrade in the body naturally. These can be used to carry hydrophilic and lipophilic drugs. Nanosponges are a type of nanostructures comprising of hyper crosslinked biocompatible and biodegradable polymers [8, 13].

Hydrogels were selected as dosage form because they provide moist environment, enhance cell proliferation, help in oxygen diffusion, easy spreadability and help in controlled release.

MATERIALS AND METHODS:

Materials: Frankincense oil and Carbopol 940 were obtained as a gift samples from Amsar Goa Pvt. Ltd. Frankinsence oil was provided with certificate of analysis from Avra synthesis private limited, containing β citronellol as major constituent being 95%. Polyvinyl alcohol was obtained from Sd-fine chem. Limited, Mumbai. Ethyl cellulose and dichloromethane were obtained from Molychem Mumbai, HPMCK15M from Kamphasol Mumbai, Agar from Genuine Chemical Co. Mumbai, Eudragit S100 from Molychem Mumbai, Ethanol from Changshu Hongsheng Fine Chemical Co. Ltd.

Methods:

GC Analysis of Frankicense Oil: The purity of the given Frankincense oil was confirmed by performing GC analysis. Analysis was done using Gas Chromatography Mass Spectrometer (Thermo Scientific U.S.) Model: Trace 1300 G.C coupled with Thermo TSQ8000 Triple Quadrupole MS

Conditions used for GC analysis were as follows:

- Column used: 25m fused capillary column with CPSil 5CB
- Sample insertion method: Liquid sample
- MS scan range: 50- 600
- Injection volume: 1µl

The list of components present were obtained from GC-MS library [14-16].

Development of Analytical Method [17]:

Analytical Method Development for Drug Content Determination: Frankincense essential oil represents a complex mixture of volatile terpenoids and oxygenated diterpenes; therefore, direct quantification of individual constituents using UV spectrophotometry is inherently challenging due to the absence of chemical specificity for multicomponent systems. Therefore a marker-based approach was used was adopted to enable reproducible quantitative estimation during formulation evaluation. Analytical method was developed in methanol by UV–Visible spectrophotometry by preparing standard stock solution of 1000µg/ml which was scanned from 200-400nm and λmax was found to 264.7nm attributed to conjugated chromophoric functional groups present in oxygenated terpenoid compounds.

Linearity: Standard stock solution was further diluted with methanol to obtain series of solutions having concentration in the range of 100-800mcg/ml. The absorbance was measured at 264.7nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equation was obtained.

Analytical Method Development for *In-vitro* Release Study: Analytical method for finding the release of Frankincense oil was developed by HPTLC method using pet ether: chloroform: formic acid: 5:4.5:0.1v/v/v as mobile phase. The stock solution of concentration 5000 µg/ml was prepared in chloroform and λmax found to be 254nm. With further dilution of stock solution a series of solutions were prepared in the range of 15-75µg/ml. The calibration curve and regression equation was obtained.

Preparation of Nanosponges: Combinations of various hydrophilic and a hydrophobic polymers like HPMC K15M, ethyl cellulose, eudragit S100, polyvinyl alcohol and agar were tried for prototype formation.

Procedure for Preparation of Nanosponges [8, 18]: Hydrophilic polymer was dissolved in 20ml of water and kept on magnetic stirrer at room temperature (aqueous phase). Hydrophobic polymer and Frankincense oil were dissolved in

10ml of dichloromethane (disperse phase). The disperse phase was added slowly, in drop wise manner using syringe in aqueous phase kept on magnetic stirrer at 1000rpm for 2 hrs. At the end of 2 hrs the formed nanosponges were separated by filtration and air dried. The following proto type systems (PS) were developed by varying the combinations and ratio of polymers

TABLE 1: DEVELOPMENT OF PROTOTYPE SYSTEMS

Prototype system	Combination of hydrophilic and hydrophobic polymers	Ratio of polymers				
P1	HPMC K15 M and ethyl cellulose	(A1)0.5:1		(A2)1:1		(A3)1:2
P2	HPMC K15 M and Eudragit S100	(B1)0.5:1		(B2)1:1		(B3)1:2
P3	PVA and Eudragit S100	(C1)0.5:1		(C2)1:1		(C3)1:2
P4	Agar and ethyl cellulose	(D1)0.5:1		(D2)1:1		(D3)1:2
P5	PVA and ethyl cellulose	(E1)1:1	(E2)1:2	(E3)2:1	(E4)2:3	(E5)3:2

Based upon the yield and the type of product formed few combinations were selected from the above mentioned prototypes and were further evaluated.

Evaluation and characterization of nanosponges [8, 18-22]:

Drug Content: Nanosponges were accurately weighed and dissolved in methanol to obtain a final concentration of 10 µg/ml and analysed using a UV–Visible spectrophotometer **at 264.7 nm**.

Entrapment Efficiency:

Direct Method: In the direct method, nanosponges are lysed or dissolved completely and the entrapped drug is quantified.

% Entrapment Efficiency = Amount of drug entrapped in nonosponges / Total amount of drug added × 100

Indirect Method: Unentrapped (free) drug present in the supernatant after centrifugation is quantified.

Entrapped Drug:

Entrapped Drug = Total drug added – Unentrapped drug

Entrapment Efficiency (%):

% Entrapment Efficiency = Total drug added – Unentrapped drug / Total drug added × 100

Percentage = Practical yield / Theoretical yield × 100

FESEM Analysis of Nanosponges: The surface and structure of following samples of dried nanosponges A3, D1, and E1 were investigated using Field Emission Scanning Electron Microscope (Quanta FEG- 250) (at Central Sophisticated Instrumentation Facility, BITS Pilani Goa).

Particle size Analysis of Nanosponges: The particle size, polydispersity index and zeta potential of the following samples of nanosponges A3, D1, and E1 were determined using NANO PLUS-Zeta/ Nano particle analyser (at Central Sophisticated Instrumentation Facility, BITS Pilani Goa). The samples were provided as dry powders and then they were diluted with distilled water before analysis to form aqueous suspension and then analysed.

GC Analysis of Dried Nanosponge Sample: Analysis was done using Gas Chromatography Mass Spectrometer (Thermo Scientific U.S.) Model: Trace 1300 G.C coupled with Thermo TSQ8000 Triple Quadrupole MS

Conditions used for GC analysis were as follows:

- Column used: 25m fused capillary column with CPSil 5CB
- Sample insertion method: Liquid sample
- MS scan range: 50- 600
- Injection volume: 1µl

Solid dried sample was dissolved in methanol and injected. The sample was run for 20 mins and then the GC graph was obtained. The list of components present were obtained from GC-MS library.

Optimization of Nanosponges:

Optimization of PVA and ethyl Cellulose Nanosponges [8, 18, 20]: For optimization of nanosponges 3^2 full factorial design was applied. 9 runs were involved. The formulations were optimized based on their entrapment efficiency and the best or the optimized formulation was further selected for preparation of hydrogels.

TABLE 2: FORMULATION OF PVA AND ETHYL CELLULOSE NANOSPONGES FOR OPTIMIZATION

Ingredients	Formulation								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Frankincense oil	100mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg
Polyvinyl alcohol: Ethyl cellulose	0.5:0.5	1:0.5	1.5:0.5	0.5:1	1:1	1.5:1	0.5:1.5	1:1.5	1.5:1.5
Dichloromethane	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml
Distilled water	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml

TABLE 3: OPTIMIZATION TABLE

		Factor 1	Factor 2
Std	Run	A:PVA	B:EC
1	2	-1	-1
2	3	0	-1
3	6	+1	-1
4	7	-1	0
5	5	0	0
6	9	+1	0
7	1	-1	+1
8	8	0	+1
9	4	+1	+1

Preparation of Hydrogels: The optimized formulation was incorporated in hydrogel containing 1 % carbopol 940. Dried nanosponges containing oil equivalent to 100mg were weighed and added to the gel kept on vortex. This was further stirred was 20mins so that the nanosponges are uniformly dispersed and then the gel was neutralized using Triethanolamine.

Evaluation of Hydrogels [8, 18, 20-23]:

Visual Examination: The prepared formulation was examined for its colour, odour, consistency, homogeneity and existence of lumps by visual check after they were set in the container.

Assay of Hydrogels: 0.5g of the 1% hydrogel was weighed and dissolved in small amount of methanol and filtered. The filtered solution added to 10ml volumetric flask and volume was made up with methanol and the absorbance was determined at 264.7nm. The content was determined using the regression equation.

Determination of the pH: The pH of the 10% solution of hydrogel was determined using calibrated pH meter and found to be 4.84.

Determination of Viscosity: The viscosity of the prepared gel was determined using Brookfield LV viscometer. The prepared 40g of gel was taken in a beaker and the viscosity was determined by selecting the spindle number 64, at 6 rpm and at 25 °C.

Spreadability Measurement: 1g of hydrogel was added on the glass slide having dimentions 7.4 × 2.5cm. The second slide was kept of the top of first slide and the diameter of the gel was measured. Then 50g of standard weight was put and left for 5mins, after that the weight was removed and diameter of spread circles was measured again in cm and were taken as comparative values for spreadability. The experiment was carried out in triplicate and the average value was determined.

***In-vitro* Drug Release:** 0.5 g of 1% hydrogel was applied to parchment paper attached to one end of the diffusion tube. 100ml of acetate buffer pH 5.5 was added in a beaker which was placed on magnetic stirrer at 200rpm. One end of the diffusion tube was attached to the stand and the second end with parchment paper was placed in beaker containing the acetate buffer pH 5.5 at 37 °C at 200rpm. The 1ml of sample was withdrawn at the end of every 1 hour and replaced with buffer solution to maintain the sink condition. The diffusion studies were carried out for 8 hrs and the samples were analyzed by HPTLC method.

Wound Healing Study [24- 27]: The animal study was carried out with approved protocol number PESRTBCOP/IAEC; Clear 2022 R-96. Wound healing activity was carried out by making excision wound on rat. The study was carried out as per CPCSEA guidelines.

Procedure: The study was carried out on Wistar albino rats. The rats were divided in two groups that is test and control. The animals were anesthetized using anesthetic ether. The dorsal back of the rat was shaved with electric clipper and then methylated spirit was applied to the shaved portion as antiseptic before wound creation. A excision wound was made by removing full thick pieces of skin using sterilized surgical blade or scalpel. Rats were left undressed to the open environment. In test group, hydrogel was applied regularly and in control group no treatment was given. The wounds were observed regularly and the wound diameter was measured. This study was carried out for 14 days. At the end of 14 days the animals were again anesthetized and the skin samples were removed and histological examinations were done.

RESULTS AND DISCUSSION:

GC Analysis: The purity of the oil was determined by GC analysis.

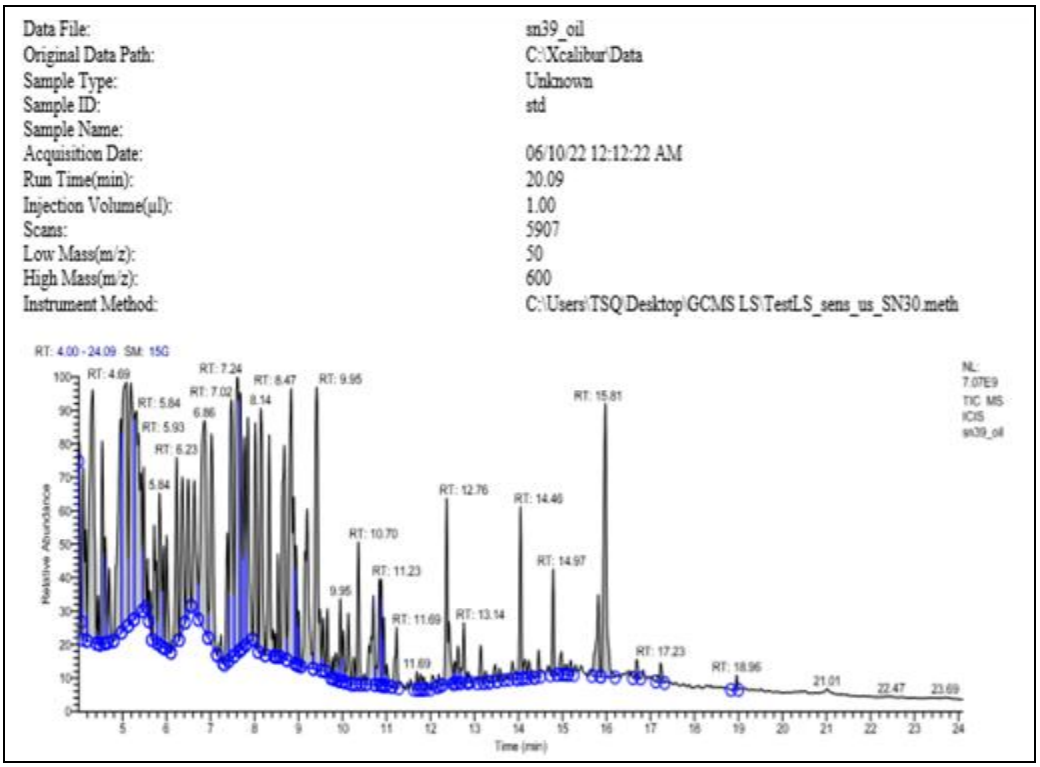


FIG. 1: IMPORTANT CONSTITUENTS PRESENT IN FRANKINCENSE OIL SAMPLE DETERMINED BY GC ANALYSIS

TABLE 4: LIST OF THE COMPOUNDS DETECTED IN THE GC ANALYSIS OF THE OIL SAMPLE

Sr. no.	Molecular formula	Name of the compound	CAS No.	% Area
1	$C_{10}H_{16}O$	Thujone	546-80-5	4.75
2	$C_{10}H_{18}O$	Terpinen-4-ol	562-74-3	4.75
3	$C_{10}H_{12}O$	Estragole	140-67-0	4.42
4	$C_{10}H_{12}O$	Anethole	104-46-1	4.42
5	$C_{10}H_{18}O_2$	2,6,6-trimethyl(1S,2S,3R,5S)-(+)-Pinanediol	53404-49-2	4.32
6	$C_{10}H_{18}O_2$	Bicyclo(3.1.1)heptanes-2,3-diol	18680-27-8	4.32
7	$C_{15}H_{24}$	Isocaryophillene	NA	4.29
8	$C_{10}H_{16}O$	Bicyclo[3.1.0] hexan-3-ol	3310-02-9	3.13
9	$C_{15}H_{24}$	(-)-α Bourbonene	5208-59-3	3.97
10	$C_{15}H_{24}$	Bicyclo[3.1.1] hept-2-ene	17699-05-7	2.62
11	$C_{12}H_{20}O_2$	Bornyl acetate	76-49-3	1.66
12	$C_{11}H_{20}O$	Methyleugenol	78996-11-9	1.92
13	$C_{10}H_{16}$	α –phellandrene	99-83-2	1.84
14	$C_{10}H_{16}$	c- terpinene	99-85-4	1.84
15	$C_{10}H_{20}O$	3,7 dimethyl citronellol	40607-48-5	0.95
16	$C_{10}H_{20}O$	2-octane-1-ol	106-22-9	0.95

The important constituents reported to be present in the Frankincense oil were found to be present in the analyzed oil sample. Hence the oil sample was found to be pure.

Development of Analytical Method:

For Determination of Drug Content: Analytical method was developed for finding the content of Frankincense oil in methanol by UV-VIS spectrophotometry and the λmax was found to be 264.7nm.

Further a standard calibration curve was plotted of absorbance verses concentration (µg/ml) and the regression equation was obtained.

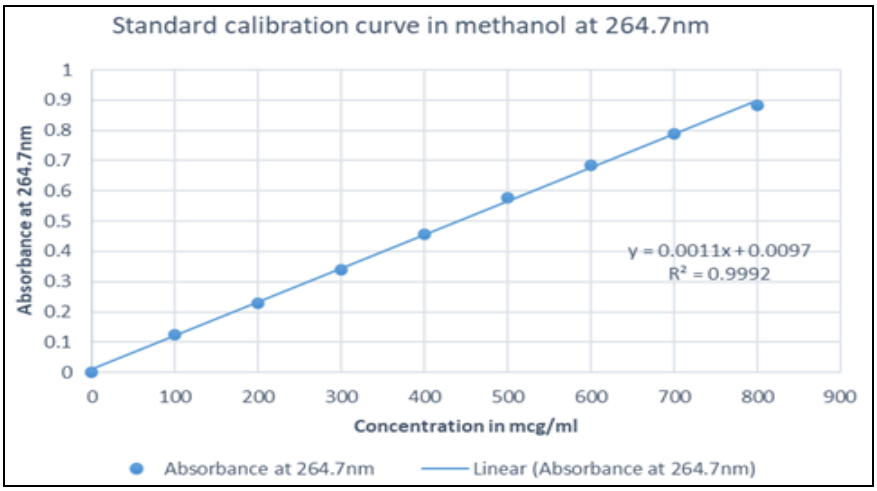


FIG. 2: STANDARD CALIBRATION CURVE OF FRANKINCENSE OIL IN METHANOL

For Determination of *In-vitro* Release: Analytical method was developed for finding the *in-vitro* release of Frankincense oil by HPTLC method using Pet-ether: Chloroform: Formic acid mixture in 5:4.5:0.1 v/v/v as mobile phase. The λmax was found to be 254 nm and further a linearity curve of AUC verses concentration (µg /ml) was plotted and regression equation was obtained.

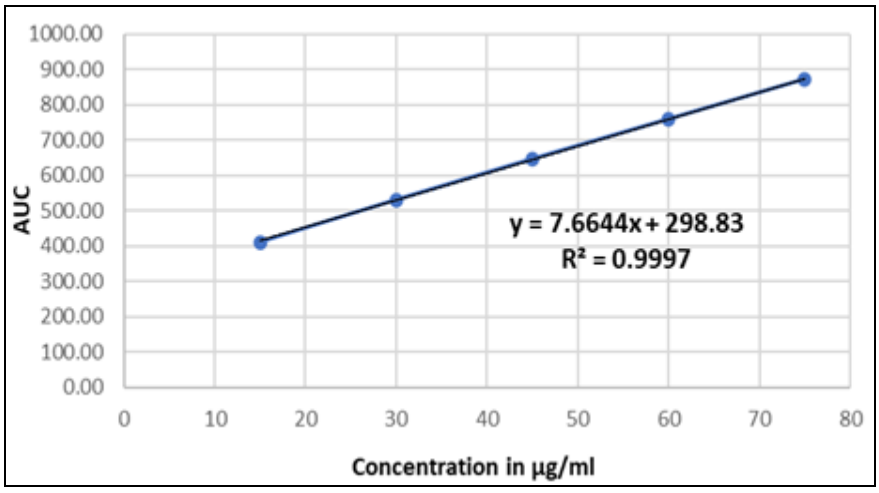


FIG. 3: LINEARITY CURVE OF FRANKINCENSE OIL

Preparation of Nanosponges:

Prototype Systems: Based upon the yield and type of the product formed the following three prototype systems were selected.

- HPMC K15M : EC 1:2 (A3)
- PVA: EC 1:1(E1)
- Agar: EC 0.5:1(D1)

These were further evaluated for FESEM, particle size analysis and entrapment efficiency.

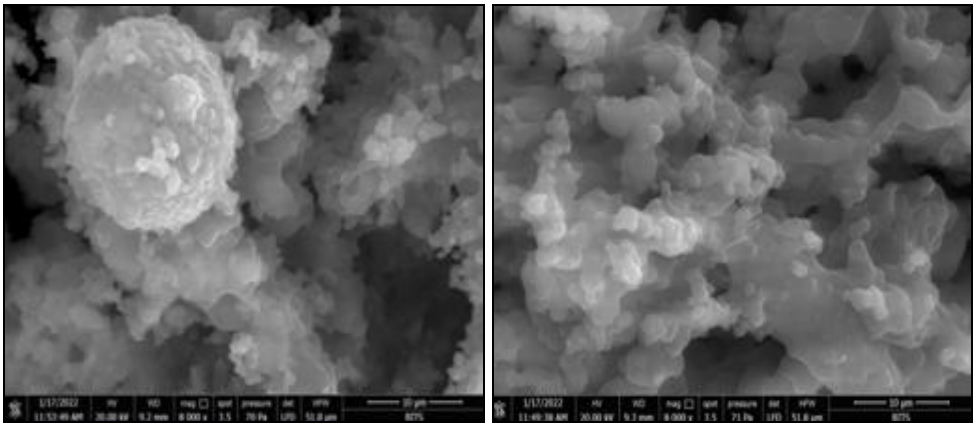
Evaluation of Nanosponges: Determination of drug content, entrapment efficiency and percentage yield Equation used from standard calibration curve: y = 0.0011 x + 0.0097

TABLE 5: EVALUATION OF NANOSPONGES

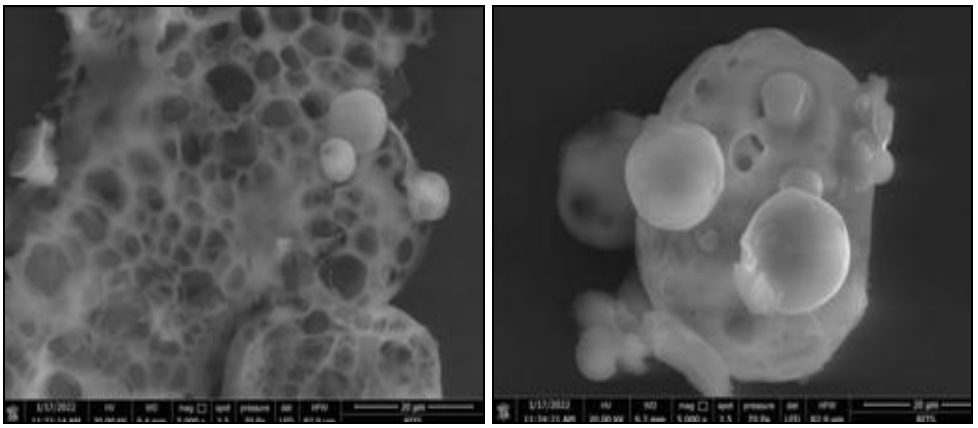
Formulation	Drug content	Entrapment efficiency (direct method)	Percentage yield
HPMC: Ethyl cellulose 1:2 (A3)	53.915mg	49.01%	38.53%
Polyvinyl alcohol: Ethyl cellulose 1:1(E1)	81.75mg	81.75%	8.9%
Agar: Ethyl cellulose (D1)	34.71mg	34.71%	14%

Formulation	Drug Content (mg) Mean ± SD (n=3)	Entrapment Efficiency (%) Mean ± SD (n=3)	Percentage Yield (%) Mean ± SD (n=3)
HPMC : Ethyl Cellulose (1:2) (A3)	53.91 ± 1.24	49.01 ± 1.08	38.53 ± 0.96
Polyvinyl Alcohol : Ethyl Cellulose (1:1) (E1)	81.75 ± 0.87	81.75 ± 1.15	8.90 ± 0.42
Agar : Ethyl Cellulose (D1)	34.71 ± 0.98	34.71 ± 1.21	14.00 ± 0.63

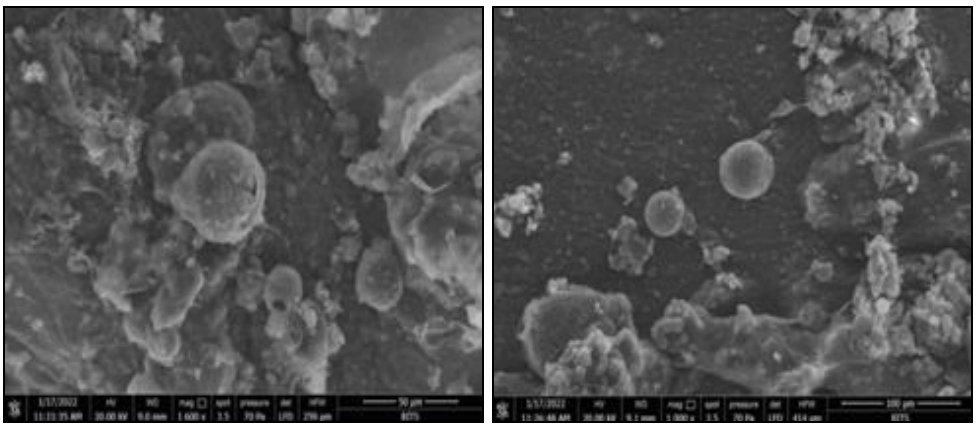
FESEM Analysis of Nanosponges:



FESEM IMAGES OF HPMC K15M: ETHYL CELLULOSE (1:2) (A3)



FESEM IMAGES OF POLYVINYL ALCOHOL: ETHYL CELLULOSE (1:1) (E1)



FESEM IMAGES OF AGAR: ETHYL CELLULOSE (0.5:1) (D1)

FIG. 4: FESEM IMAGES OF NANOSPONGES

Particle Size Analysis of Nanosponges: From the above evaluation studies it was found that formulation E1 that is PVA and EC nanosponges (1:1) gave the best results. Therefore nanosponges prepared using Polyvinyl alcohol and ethyl cellulose were further selected for optimization study.

TABLE 6: PARTICLE SIZE ANALYSIS OF NANOSPONGES

Formulation	Average Particle Size (µm) Mean ± SD (n = 3)
HPMC : Ethyl Cellulose (1:2) (A3)	8.83 ± 0.42 µm
Polyvinyl Alcohol : Ethyl Cellulose (1:1) (E1)	0.57 ± 0.05 µm
Agar : Ethyl Cellulose (D1)	17.42 ± 1.18 µm

GC Analysis of Dried Nanosponges: The GC analysis was done for optimized formulation of nanosponges F5.

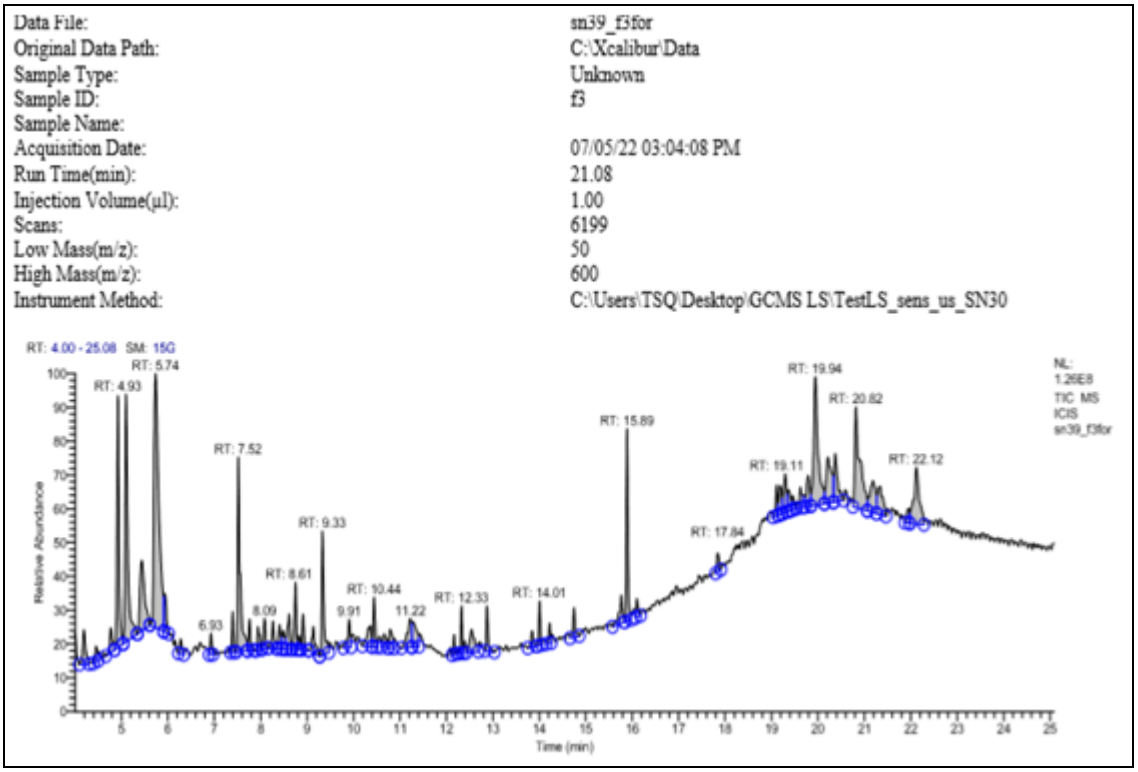


FIG. 5: GC ANALYSIS GRAPH FOR OPTIMIZED (F5) NANOSPONGE FORMULATION

The constituents found to be present in the nanosponge formulation were: Thujone, terpinen-4-ol, estragole, anethole, bicycle (3.1.0) heaxan 3-one, 2 octen-1-ol, α-phellandrene.

From the GC analysis of dried sample of nanosponge formulation it was found that the major constituents present in the oil were also found to be present in the formulation, hence it was concluded that the constituents of the oil were unaffected by the formulation process and further no traces of the solvent dichloromethane were seen in gas chromatography ensuring efficacy of method of preparation.

Optimization of PVA and Ethyl Cellulose Nanosponges:

TABLE 7: OPTIMIZATION TABLE

		Factor 1	Factor 2	Response
Std	Run	A:PVA	B:EC	%Entrapment efficiency
		mg	mg	%
1	2	-1	-1	70.12
2	3	0	-1	40.31

3	6	1	-1	45.6
4	7	-1	0	38.54
5	5	0	0	76.44
6	9	1	0	64.337
7	1	-1	1	35.16
8	8	0	1	67.72
9	4	1	1	72.4

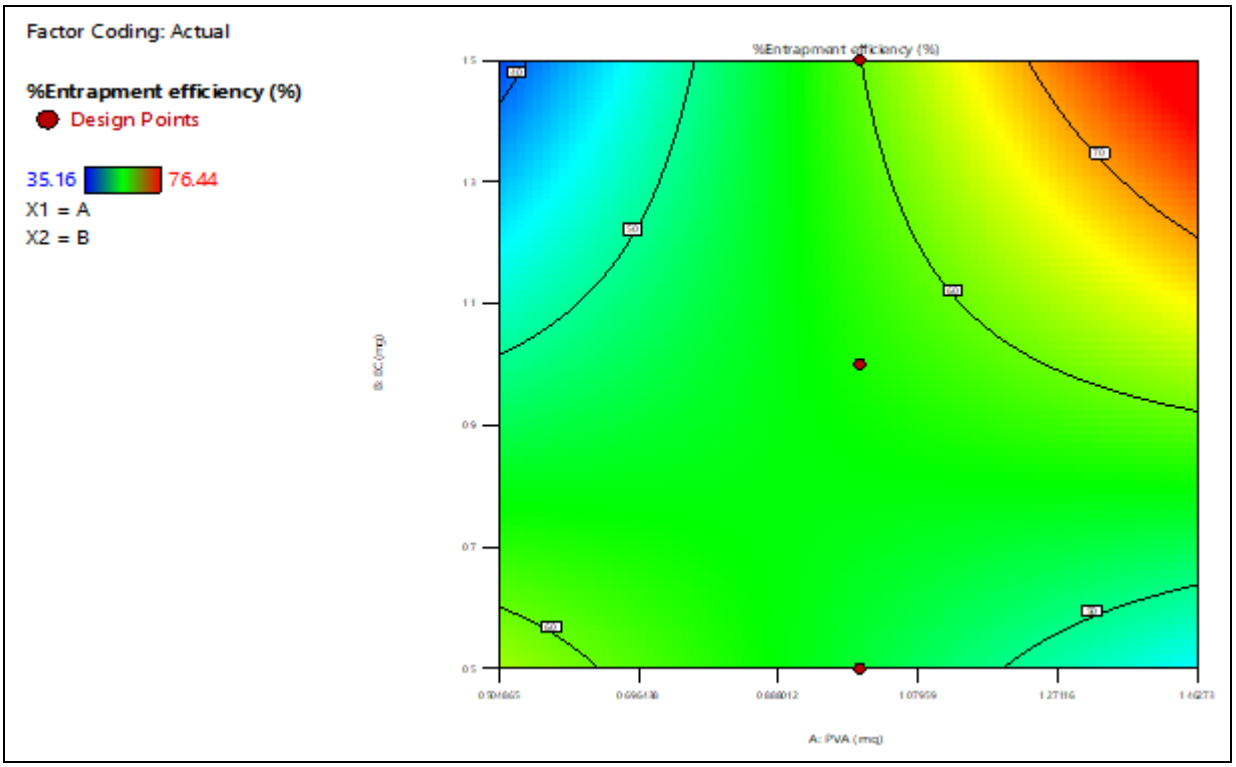


FIG. 6: CONTOUR PLOT

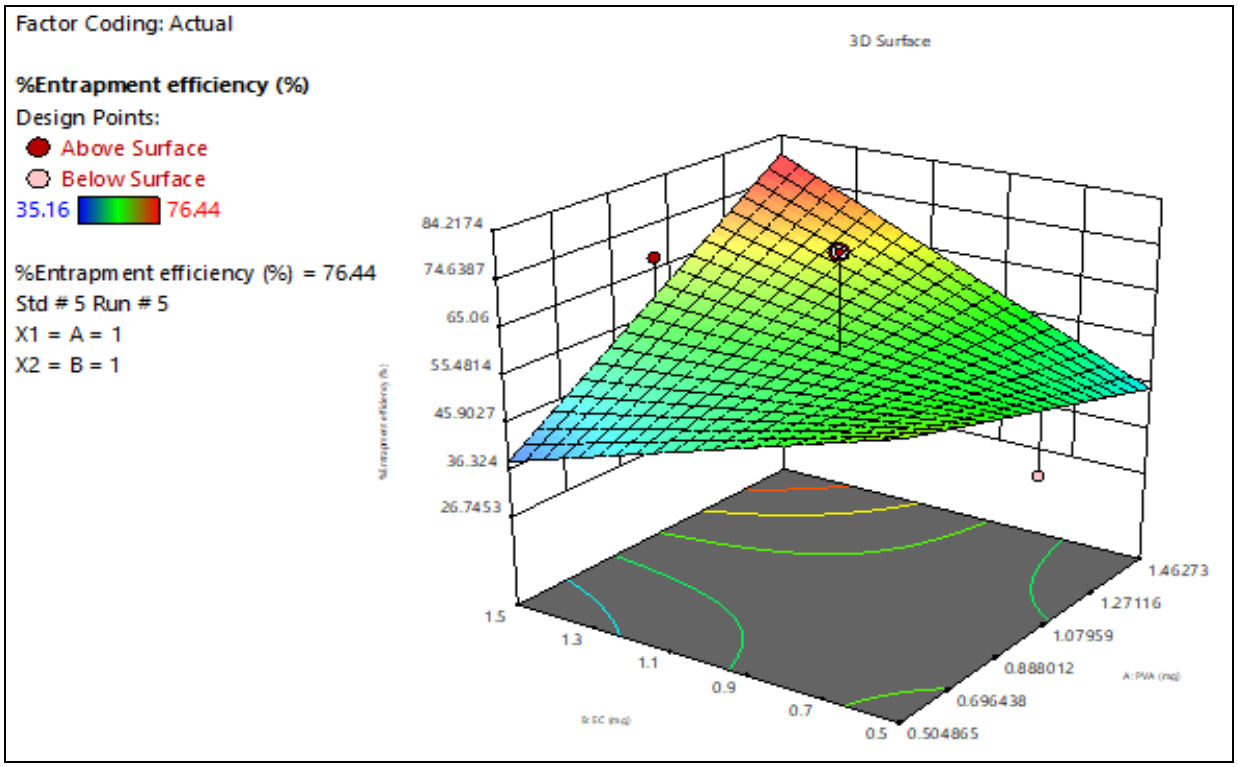


FIG 7: 3D SURFACE RESPONSE CURVE

For optimization of PVA and ethyl cellulose nanosponges 3^2 factorial design was used. To decide the best ratio of PVA and ethyl cellulose, total 9 formulations were developed using PVA and ethyl cellulose as two factors their ratio was varied at three levels and the entrapment efficiency was decided as response factor. Based upon the entrapment efficiency value the best polymer ratio was selected.

The contour plot shows concentration of PVA (factor A) on x-axis and the concentration of EC (ethyl cellulose) (factor B) on y-axis. From the contour plot it was seen that the ratio of PVA to ethyl cellulose of the optimized formulation should be 1:1 and the same can be concluded from surface response curve [36].

Evaluation of Hydrogels:

TABLE 8: PHYSICOCHEMICAL CHARACTERISTICS OF TOPICAL GELS

Formulation	Appearance	Colour	Homogeneity	pH	Viscosity (cps)	Spredability (g cm/s)
Hydrogel	Smooth gel with slight gloss	White	Good	4.84	67,666cps	0.57g/cm^2/sec

1. Assay of hydrogels: From the calculations it was seen that % drug content of the hydrogel was found to be 97.84%

2. *In-vitro* drug release:

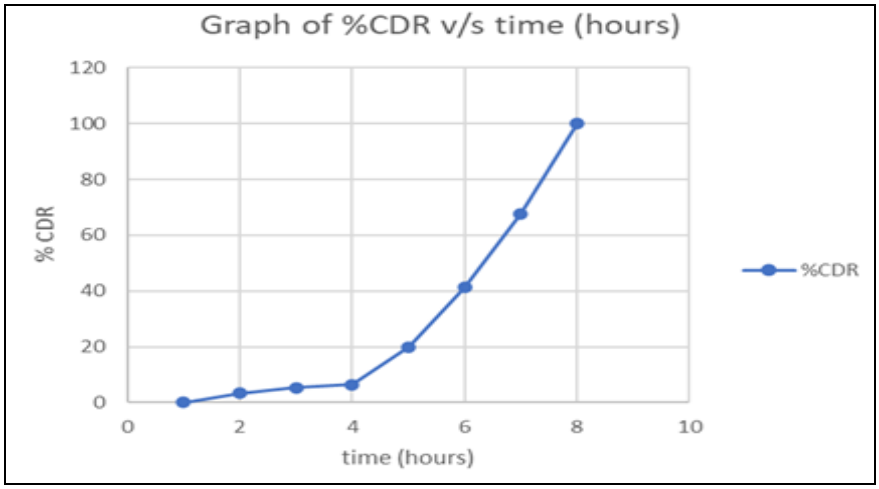
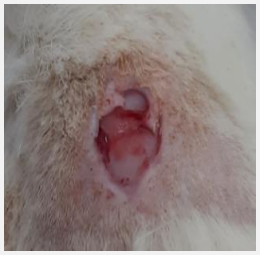
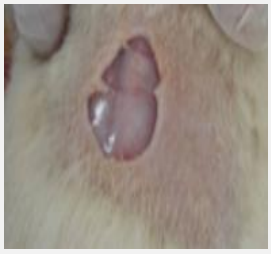
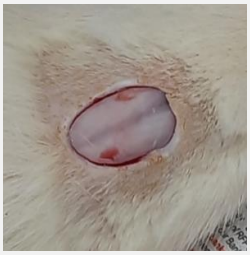
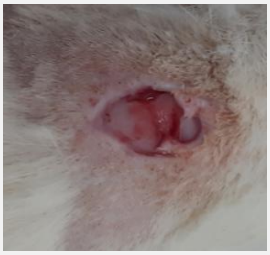


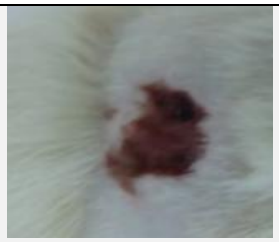
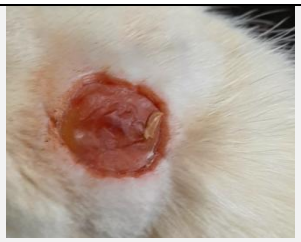
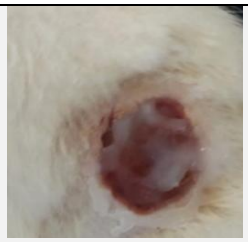
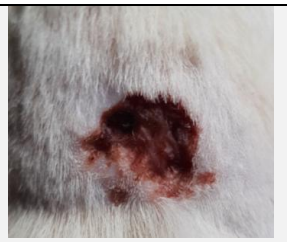
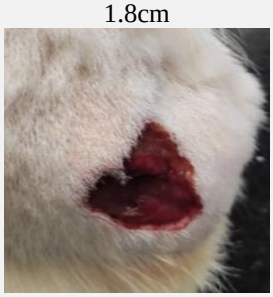
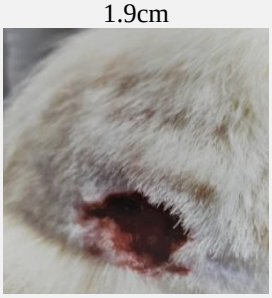
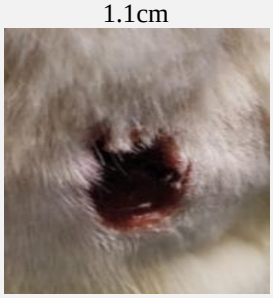
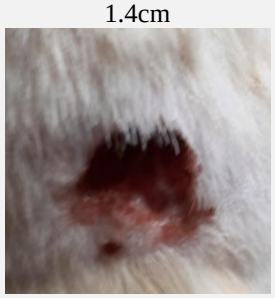
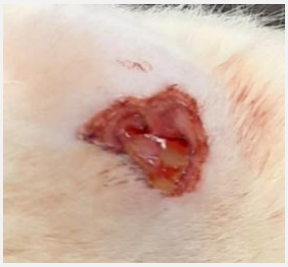
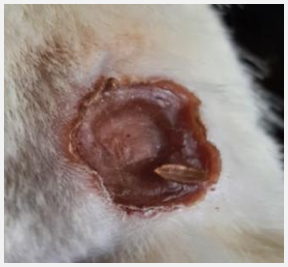
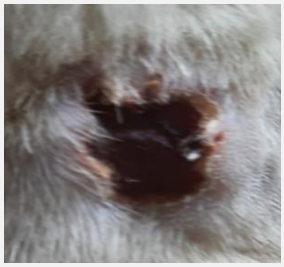
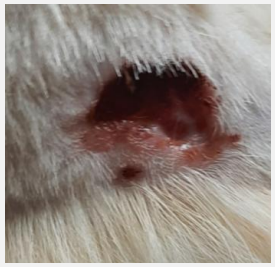
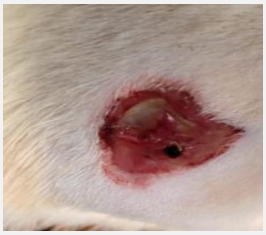
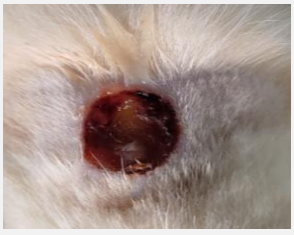
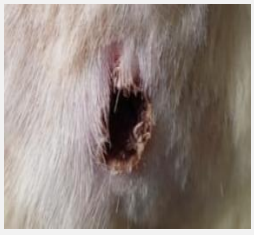
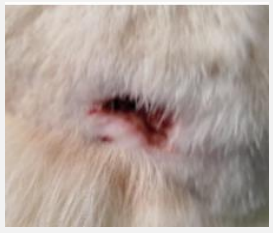
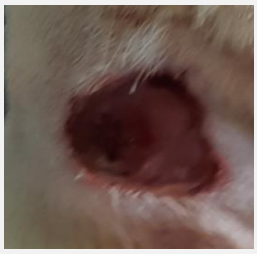
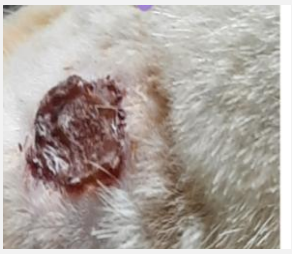
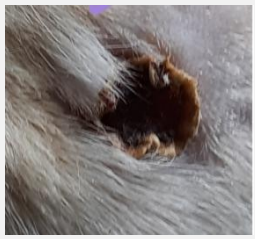
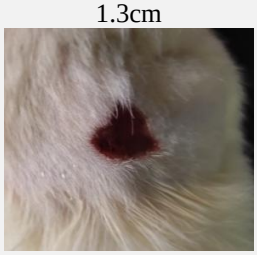
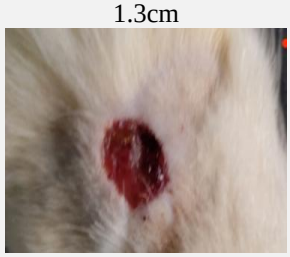
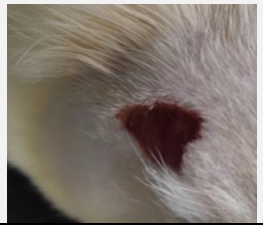
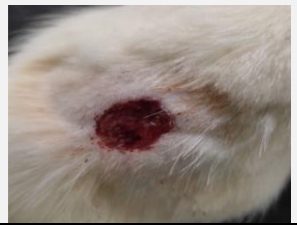
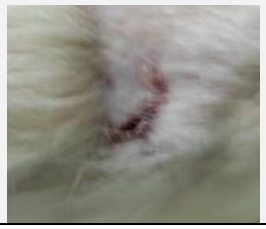
FIG. 8: GRAPH OF %CUMULATIVE DRUG RELEASE V/S TIME (HRS) FOR HYDROGEL FORMULATION

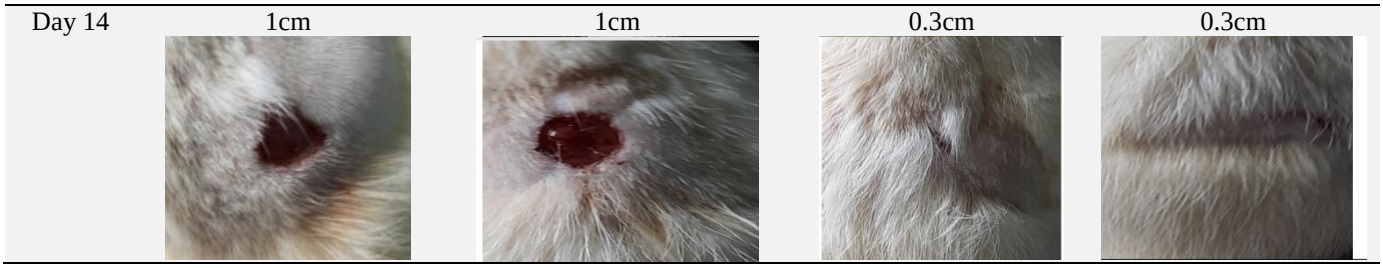
Initially the release was found to be very less may be due to insufficient hydration and slow diffusion of the drug from gel. But after 3 hours the release was found to increase reaching to 100% at the end of 8 hrs.

Wound healing study: For hydrogel

TABLE 9: WOUND HEALING STUDY OF THE HYDROGEL

Day	Rat C1	Rat C2	Rat T1	Rat T2
Day 0	2cm	2cm	1.5cm	1.5cm
				
Day 2	2cm	2cm	1.2cm	1.4cm

	 1.8cm	 1.9cm	 1.1cm	 1.4cm
Day 4	 1.7cm	 1.8cm	 1cm	 1.3cm
Day 5	 1.4cm	 1.5cm	 0.9cm	 1.2cm
Day 7	 1.4cm	 1.3cm	 0.8cm	 1cm
Day 9	 1.3cm	 1.3cm	 0.7cm	 0.9cm
Day 11	 1.2c	 1.1cm	 0.4cm	 0.6cm
Day 13				



The wounds were wet on day 0. From day one it started drying in test group. The wounds started contracting from day 3 in test group, however this process was found to be much slower in control group. There was decrease in release of wound exudates in treatment group. But the control group showed development of sum puss and redness with inflammation and wounds were found to be wet.

In test group the wound were found to be dried and epithelization stated from day 5 and the wounds in the test group completely healed in 14 days while the wounds in control group did not heal. It was also found that the formulation did not cause any irritation in the test group on the other hand it was found to decrease the inflammation associated with wound.

Histopathology Study:

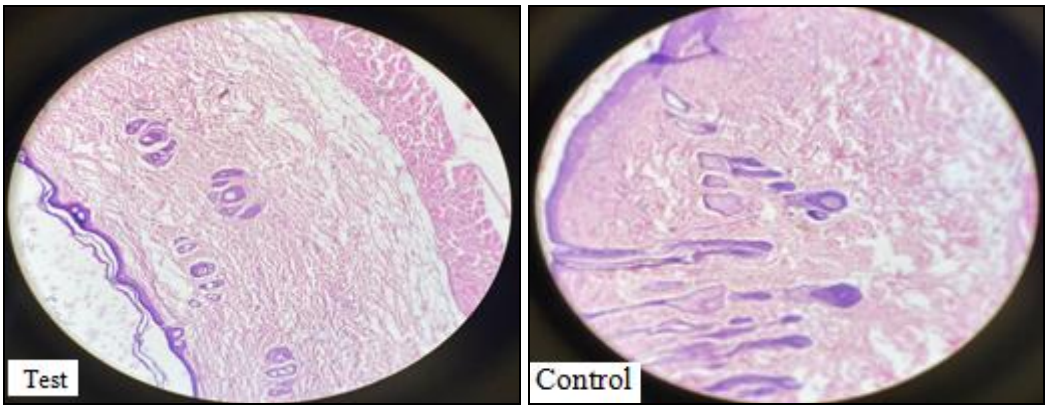


FIG. 9: HISTOPATHOLOGY SLIDE OF THE TEST AND CONTROL RAT SKIN SAMPLE

In Test group:

Epidermis: All 5 layers of epidermis are distinctly visible. Stratum corneum is reformed and well distinguished from other layers of epidermis. Stratum corneum shows multiple layers. Stratum courneum is found as colourless layer of flat cells without nucleus. Stratum lucidum visible as single dark blue colour thin layer. Stratum granulosum and Stratum spinosum are visible as single layer, not distinctly visible. Stratum basale layer is well distinguished from dermis layer.

Dermis is consisting of two layers papillary dermis and reticular dermis. The layer just below stratum basale is papillary dermis. Papillary ridges are visible. Below this is reticular dermis. Dermis shows presence of elastin, visible as red colour bundles along with considerable amount of collagen accumulation. Dermis layer also shows presence of some blood vessels.

Hypodermis: Below the dermis is the subcutaneous tissue called hypodermis shows the presence of well formed adipose tissue visible as colourless layer made of bundles of fat cells.

In Control Group:

Epidermis: Stratum corneum is not well formed, visible as slight colourless thin layer. Different cells layers of it are not visible. Stratum lucidum not well formed at some places, at some places seen as fine dark blue line. Stratum granulosum and statum spinosum seen as purple colour layer, but not distinctly visible. Stratum basale is not distinctly visible.

Dermis Layer: Papillary dermis is not well defined. Papillary ridges are not visible. Reticular dermis, the cells are not well formed, not well arranged. Elastin and collagen visible as red bundles but not well formed and blood vessels not

clearly visible. Hypodermis was found to be damaged, not visible.

CONCLUSION: From this research results it can be concluded that nanosponges based hydrogels containing frankincense oil is an effective topical drug delivery system and can be used for wound healing activity.

From this research results it can be concluded that nanosponges are effective for entrapment of frankincense oil and also to decrease it's volatility and irritancy potential. From the pharmacological studies it can be concluded that nanosponge based hydrogels containing frankincense oil is an effective topical drug delivery system and can be used for wound healing activity.

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