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A RESEARCH ON PIPERINE LOADED NANO SIZED-PARTICLES NANO GEL FOR THE TREATMENT OF SKIN CANCER: DEVELOPMENT AND PROSPECTS

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Abstract : Background

In this present investigation piperine loaded nanogel for skin cancer was formulated by using piperine, carbopol, sodium tripolyphosphate

Method

Piperine loaded Nanogel is formulated by Ion gelation method using stpp, chitosan and it is evaluated by Entrapment efficiency, particle size analyzer, zeta potential, effect of particle size, Fourier- transformed infrared spectroscopy, screening electron microscopy, ex Vivo permeation study, box behnken design, regression analysis, formulation of nanoparticles, incorporation into transdermal nanogel and evaluated by physical examination drug content, extrudability, spreadibility, in Vivo study, washablity, stability study of gel, in Vivo dissolution, drug diffusion study

Result

Stiring time, sodium sulfate concentration, stiring speed, effect influence the particle size and instrument efficiency formulation percentage drug release particle size in a range of 0 to 200 NM where obtained data potential is 3.2 mv which can show good storage time for nanoparticles approx 10 months poly dispersity index was 0.152 which is below 1 was acceptable range Entrapment efficiency was 95% training electron microscopy show spherical shape of nanoparticles obtained Nanogel so 87% of drug release no skin irritation for physical examination consistency, wash ability, spread ability, drug content, stability study, in vitro drug release, in Vivo drug release, ex Vivo drug release where in acceptable range

Conclusion

Clear nanogel is obtained by physical examination for topical drug delivery system and drug bioavailability enhanced for 30% in market it was enhanced up to 85% current work can be useful for successful preparation and evaluation of Nanogel for treatment of skin cancer

IndexTerms - Nanoparticles, Nanogel, box behnken design, skin irritation, study in vitro, ex Vivo drug content

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Introduction

The skin is viewed as the biggest organ of the body and has various capacities. The skin capacities in thermoregulation, assurance, metabolic capacities and sensation. The skin is partitioned into two principle districts,

the epidermis, and the dermis, each giving an unmistakable job in the general capacity of the skin. The dermis is joined to a basic hypodermis, additionally called subcutaneous connective tissue, which stores fat tissue and is perceived as the shallow belt of gross life systems.

The epidermis, the furthest layer of skin, gives a waterproof boundary and makes our skin tone. The dermis, underneath the epidermis, contains intense connective tissue, hair follicles, and sweat organs. The more profound subcutaneous tissue (hypodermis) is made of fat and connective tissue. The skin's tone is made by uncommon cells called melanocytes, which produce the color melanin. Melanocytes are situated in the epidermis. Skin Conditions

Rash: Almost any adjustment of the skin's appearance can be known as a rash. Most rashes are from straightforward skin disturbance; others result from ailments.

Dermatitis: An overall term for irritation of the skin. Atopic dermatitis (a sort of skin inflammation) is the most well-known structure.

Dermatitis: Skin irritation (dermatitis) causing a bothersome rash. Frequently, it's anything but's an overactive insusceptible framework.

Psoriasis: An immune system condition that can cause an assortment of skin rashes. Silver, textured plaques on the skin are the most well-known structure.

Skin break out: The most well-known skin condition, skin inflammation influences more than 85% of individuals sooner or later throughout everyday life.

Cellulitis: Aggravation of the dermis and subcutaneous tissues, generally because of a disease. A red, warm, frequently agonizing skin rash for the most part results.

Skin canker (bubble or furuncle): A restricted skin disease makes an assortment of discharge under the skin. A few abscesses should be opened and depleted by a specialist to be restored.

Rosacea: A persistent skin condition causing a red rash on the face. Rosacea may look like skin break out, and is inadequately perceived.

Moles: An infection taints the skin and makes the skin develop exorbitantly, making a mole. Moles might be treated at home with synthetics, conduit tape, or freezing, or eliminated by a doctor.

Melanoma: The most perilous kind of skin malignant growth, melanoma results from sun harm and different causes. A skin biopsy can distinguish melanoma.

Basal cell carcinoma: The most well-known sort of skin malignant growth. Basal cell carcinoma is less perilous than melanoma since it develops and spreads all the more leisurely.

Seborrheic keratosis: A considerate, frequently irritated development that seems like a "stuck-on" mole. Seborrheic keratoses might be eliminated by a doctor, if irksome.

Actinic keratosis: A dried up or layered knock that structures on sun-uncovered skin. Actinic keratoses can in some cases progress to malignancy.

Squamous cell carcinoma: A typical type of skin disease, squamous cell carcinoma may start as a ulcer that will not mend, or a strange development. It normally creates in sun-uncovered regions.

1.1 NANOGEL OVERVIEW: RATIONALE FOR THEIR BIOMEDICAL USE

As of late, there has been colossal advancements in the feld of conveyance frameworks to give helpful specialists or regular based dynamic mixtures to its objective area for treatment of different aliments [33, 34]. Tere are a number of medication conveyance frameworks effectively utilized in the ongoing occasions, nonetheless there are as yet certain difficulties that should be addresses and a trend setting innovation should be created for effective conveyance

of medications to its objective locales. Consequently the nano based medication conveyance systerms are as of now been examined that will work with the progressed arrangement of medication conveyance.

Nanomedicine, is an emerging field carrying out the utilization of information and techniques of nanoscience in clinical science and sickness anticipation and remediation. It ensnares the utilization of nanodimensional materials including nanorobots, nanosensors for determination, conveyance, and tangible purposes, and impel materials in live cells . For model, a nanoparticle-based technique has been levelopend which consolidated both the treatment and imaging modalities of malignant growth analysis [20]. The very first generation of nanoparticle-based treatment included lipid frameworks like liposomes and micelles, which are currently FDA-endorsed [21]. Tese liposomes and micelles can contain inorganic nanoparticles like gold or attractive nanoparticles [22]. Tese properties let to an expansion in the utilization of inorganic nanoparticles with an accentuation on drug conveyance, imaging and therapeutic capacities. Also, nanostructures supposedly help in preventing drugs from being discolored in the gastrointestinal area and help the conveyance of sparingly water-dissolvable medications to their objective area. Nanodrugs show higher oral bioavailability since they display average take-up components of absorptive endocytosis.

Nanostructures stay in the blood circulatory framework for a drawn out period and empower the arrival of amalgamated sedates according to the specified portion. Thus, they cause less plasma fluctuations with decreased unfavorable effects [23]. Being nanosized, these constructions infiltrate in the tissue framework, work with simple take-up of the medication by cells, grant an efficient drug conveyance, and guarantee activity at the designated area. Te take-up of nanostructures by cells is a lot higher than that of enormous particles with size going somewhere in the range of 1 and 10 µm [17, 24]. Thus, they straightforwardly interact to treat the sick cells with further developed efficiency furthermore, diminished or immaterial side effects. At all phases of clinical practices, nanoparticles have been discovered to be valuable in securing data owing to their utilization in various novel examines to treat and dignose illnesses. Te primary benefits of these nanoparticles are related with their surface properties; as different proteins can be a fixed to the surface. For example, gold nanoparticles are utilized as biomarkers and tumor marks for different bimolecular location procedural measures.

2 MATERIALS –

Piperine is obtain as a gift sample from Herbal creation Pvt.Ltd. Delhi, India, Chitosan, carbopol 934&974, acetic acid, methanol, glycerin are from department of pharmaceutical science, Saurashtra university, Rajkot

3 METHOD-

3.1 ANALYTICAL METHOD

3.1.1 STANDARD CALIBRATION CURVE WITH BUFFER 7.4

Standard calibration curve of piperine was developed using phosphate buffer pH 7.4 and estimated by UV-Visible spectrophotometer at 342nm

3.1.2 GENERAL PROCEDURE FOR PREPARATION OF CALIBRATION CURVE BY UV.

A stock solution of (1mg/ml) of standard piperine was prepared, later required dilution wee made with phosphate buffer pH 7.4 to a series of 10ml volumetric flask standard solution was taken and volume was made up using phosphate buffer pH 7.4 absorbance of these solution was measured at respective wavelength of maximum absorbance using 1ml cuvette in UV Visible spectrophotometer. Absorbance value were plotted against respective concentration to obtain standard calibration curve

3.1.3 FTIR FOR IDENTIFICATION OF DRUG SAMPLE

The spectra produced a profile of the sample for many different compounds FTIR is effective analytical instrument for detecting functional group and characterization bond information

3.1.4 DSC STUDY FOR IDENTIFICATION OF DRUG SAMPLE

DSC is thermal analysis apparatus measuring how physical properties of sample with temperature against time in small pan 2-2.5mg of drug is kept and sample is run and graph is generated

3.1.5 SOLUBILITY STUDY OF DRUG

Solubility is focused on drug solvent system 1mg of piperine is dissolved in different solvent such as methanol, hot water, water, DMSO, buffer Ph 7.4 and then solubility has been measured

3.1.6 MELTING POINT

Melting point of piperine was measured by melting point apparatus in a capillary small amount of drug is filled and kept in melting point apparatus after certain temperature drug start melting and that temperature is recorded

3.2 PRELIMINARY SCREENING –

Preliminary screening was done on the bases of Selection of solvent, Selection of cross linking agent, Selection of homogenization speed, Selection of homogenization time, Selection of temperature

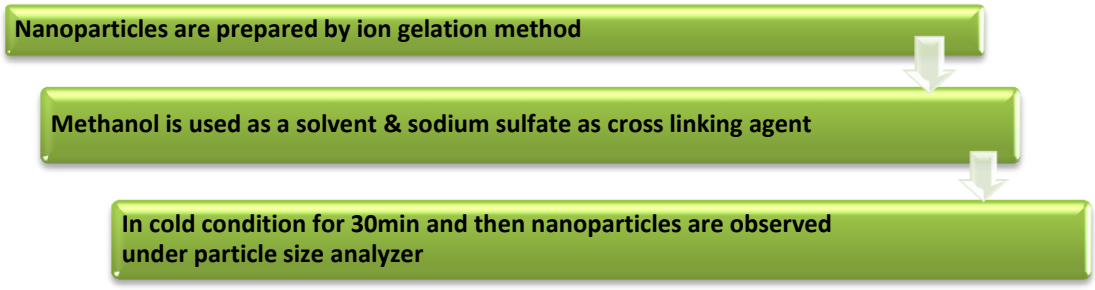

Figure 1. Preparation of nanoparticles

3.2.1 PREPARATION OF NANOPARTICLES-

Selected amount of drug is dissolved in methanol and added in 1% Chitosan solution with syringe drop wise in cold temperature after 30 min and observed particle size under particle size analyzer as given in fig.1.

3.2.2 PREPARATION OF GEL-

Selected amount of carbopol is dissolved into water and kept for 24 hr in room temperature after that carbopol is completely dissolved in water and adjusted pH with TEA and gel phase is ready

3.2.3 PREPARATION OF NANOGEL-

Take nanoparticles and gel phase in equal amount (1:1) and mix it well

4 EVALUATION OF NANOGEL-

4.1 Particle size determination

Particles sizes of the nanoparticles were measured in the Zetatract Instrument. Here, the turbid solution obtain from homogenizer was measured in nanometer

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4.2 Entrapment Efficiency

Entrapment efficiency was determined by centrifugation method. Aliquots (1ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (REMI CM-12 PLUS) at 5000 RPM for 30min at 4˚c. Clear supernatants were removed carefully to separate non-entrapped and absorbance were recorded at 342nm. Amount of piperine is supernatant and sediment gave a total amount of piperine in 1ml of dispersion

4.3 Screening electron microscopy (SEM)

For SEM study of nanoparticles the optimized batch was sent to Department of Biotechnology Junagadh Agriculture University Junagadh to check the size of nanoparticles

4.4 Physical examination

The prepared nanogel formulation was inspected visually for their appearance, color, smoothness, transparency.

4.5 Drug content

1gm of nanogel was dissolved in specific buffer and at specific volume. Then solution is filtered and analyze the drug contain with suitable method (UV method)

4.6 Spread ability testing

A small quantity of gel was applied on a glass slide and another slide was place on it manually 500gm of weight is kept and time (sec) is calculated when gel is removed from slide

$$S=M/T$$

S=spread ability

M=mass

T=time in second

4.7 Extrubability study

Selected quantity of gel was fill in collapsible tube and it was extruded from tube and time is calculated

4.8 Stability study

Stability testing is to identify how the quantity of formulation in different temperature and it is done under different temperature room temperature and temperature (0-2˚C) for 4 week and effect has been observed

4.9 Wash ability

A small quantity of gel will be applied on skin after washing with water checked for whether gel was completely wash or not any stickiness

4.10 Viscosity

Viscosity is use to identify internal flow resistance it is express by centipoises (cP) which is evaluated in millipascal second final nanogel is identify with Brookfield viscometer

4.11 In vitro drug permeation study

The in vitro diffusion was carried out with cellophane membrane which is placed between donor and receptor compartment and donor compartment is open from the top and we can add sample from there. The temperature was maintain at 37˚C and receptor compartment provided sampling point diffusion media use as phosphate buffer 7.4 pH

4.12 In vitro drug release study

The in vitro dissolution was carried out with dialysis membrane which is fill with suspensions and gel and tie with paddle. The temperature was maintain at 37˚C and phosphate buffer 7.4 pH specific time sampling is done and analyzed under uv spectrophotometer and buffer will be replaced with fresh buffer

4.13 Ex-vivo drug release study

The Ex vivo diffusion was carried out with goat mucosa which is placed between donor and receptor compartment and donor compartment is open from the top and we can add sample from there. The temperature was maintain at 37˚C and receptor compartment provided sampling point diffusion media use as phosphate buffer 7.4 pH

4.14 Skin irritation test

Skin irritation testing was performed on rabbit and it has been approved by animal ethic committee. The protocol number was **IAEC/DPS/SU/2105.** Here for the study 5 cm of skin fur was removed at 3 different sides and formulation was applied at left side, in center standard gel is applied and at right side drug suspension is applied and it was observed after 24hr and after 8days for any kind of redness, itchiness or swelling in the area

5 RESULT AND DISCUSSIONS

5.1 Standard calibration of estimation of piperine in phosphate buffer pH 7.4

Standard calibration curve of piperine was developed using phosphate buffer pH7.4 and estimated by UV-Visible spectrophotometer at 342nm shown in figure 2.

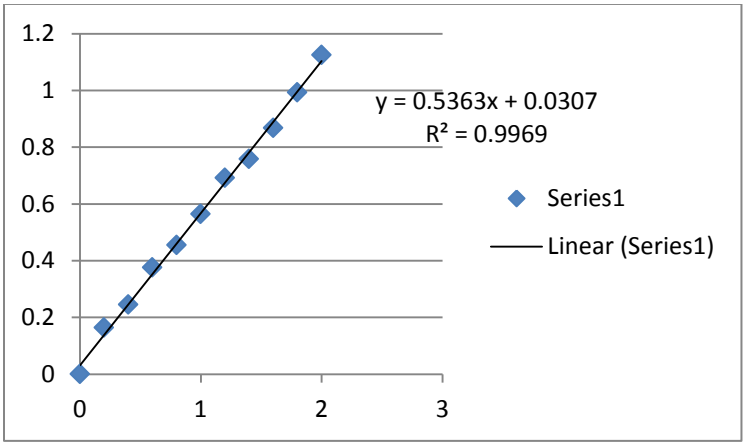

Figure 2. Calibration curve of piperine

A stock solution of (1mg/ml) of standard piperine was prepared, later required dilution were made with a phosphate buffer 7.4pH. To a series of 10ml volumetric flask aliquate standard solution were there and volume was made up using phosphate buffer pH 7.4 absorbance of this solution was measured by respected wavelength of maximum absorbance using 1ml cuvette in UV Visible spectrometer. Absorbance value is plotted against respective concentration to obtain standard curve reading is shown in table 1 and calibration curve graph in fig.2

5.2 DRUG IDENTIFICATION STUDY

5.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

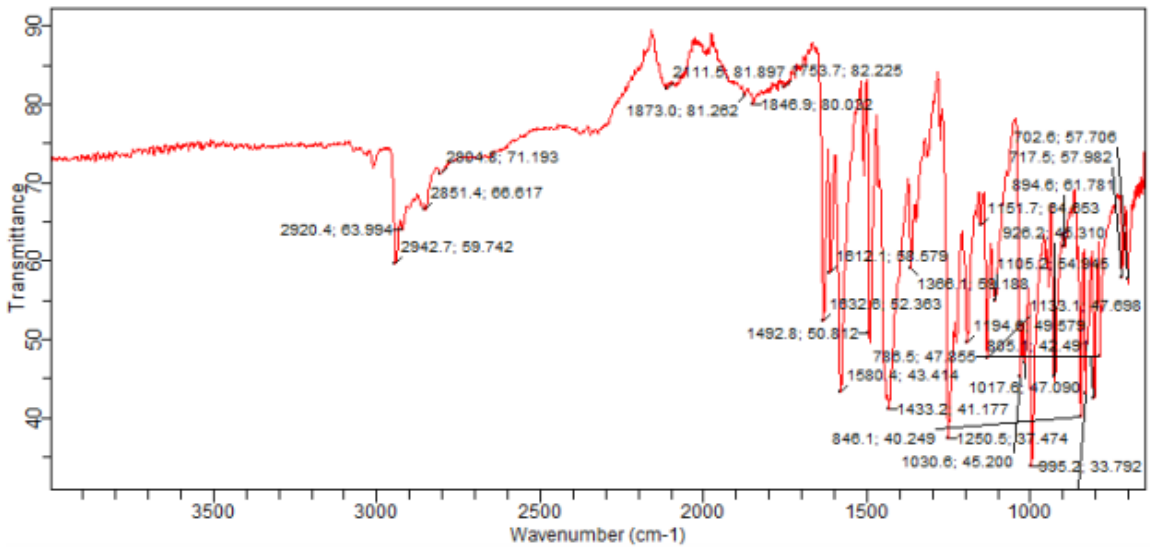

Figure 3 FTIR of drug

The spectra produced a profile of the sample for many different compounds FTIR is effective analytical instrument for detecting functional group and characterization bond information & interpretation is shown in figure 3.

1050-1150	-C-H -Bending
1200-1250	=C-O-C Asymmetrical stretching
1300-1580	C=C Aromatic stretching (benzene ring)
~1450	C-H2 Bending
~1634	C=O Keton
1700	-CO-N Stretching
3200-3500	N-H
3300-3600	O-H Alcohol strong bond

5.2.2 Differential Screening Calorimetric (DSC)

DSC was done of drug piperine and Chitosan polymer is shown in (figure 4)

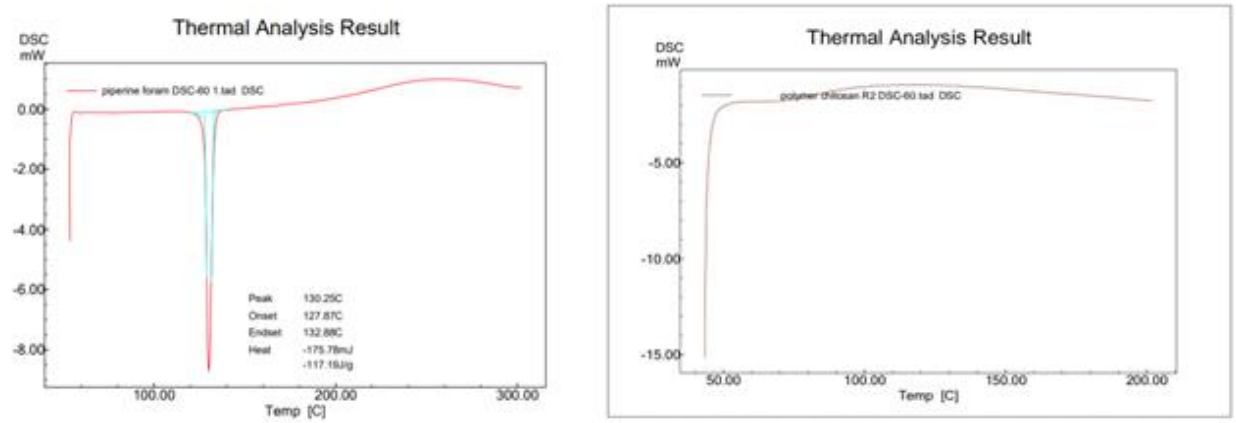

Figure 4. DSC of drug piperine & DSC of polymer chitosan

5.2.3 Solubility of drug

compound	Solubility
Methanol	Freely soluble
Buffer pH7.4	Partially soluble
DMSO	Partially soluble
Water	Not soluble

Table 2 solubility of drug

5.2.4 Melting point

It is done by melting point apparatus after drug start melting that temperature is recorded and shown in table 3

DRUG	STANDARD VALUE	OBSERVED VALUE
Piperine	125 °C-126 °C	126 °C-127 °C

Table 3 melting point of drug

5.3 PRELIMINARY STUDY

Selection of solvent ,cross linking agent, polymer concentration, homogenization speed, homogenization time & temperature was selected and batch were calculated result are shown in Table 4,Table 5,Table 6,Table 7, Table 8, Table 9

5.3.1 Selection of solvent

parameters	Methanol	Dimethyle sulfoxide (DMSO)
P.S (nm)	175	359
EE (%)	78	65
PDI	0.790	1.39

Table 4 solvent selection

5.3.2 Selection of cross linking agent

parameters	Sodium tripoly Phosphate (STPP)	Sodium sulfate (SS)
P.S (nm)	549	164.4
EE (%)	100	91.5
PDI	0.213	0.381

Table 5 selection of cross linking agent

5.3.3 Selection of polymer concentration (chitosan)

parameters	0.1gm	0.2gm	0.5gm
P.S (nm)	6000	233	1913
EE (%)	73	75	43.2
PDI	1.48	0.86	Sticky

Table 6 selection of polymer concentration

5.3.4 Selection of homogenization speed

parameters	800 RPM	1000RPM	1200RPM
P.S (nm)	359	414	6000
EE (%)	65	44.3	100
PDI	1.27	0.413	Sticky

Table 7 Selection of homogenization speed

5.3.5 Selection of homogenization time

parameters	15min	30min	45min
P.S (nm)	1930	532	1708
EE (%)	30	100	44
PDI	2.0	Not appear	1.41

Table 8 selection of homogenization time

5.3.6 Selection of temperature

parameters	Room temperature	Cool temperature (2-4˚C)
P.S (nm)	256	175
EE (%)	78	92
PDI	2.9	1.1

Table 9 selection of temperature

SCREENING STUDY OF NANOPARTICLES

5.4 Box behnken design (BBD)

Screening design is the most powerful DOE technique that determines the most critical factors in pharmaceutical development. Most common screening design is box behnken design (BBD) it screen factors and identify critical one in a minimal number of runs with good degree of accuracy. Generally, number of run needed to investigate the main effect is equal batch was prepared and shown in figure 6. Value is applied in a table 12 and table 13.

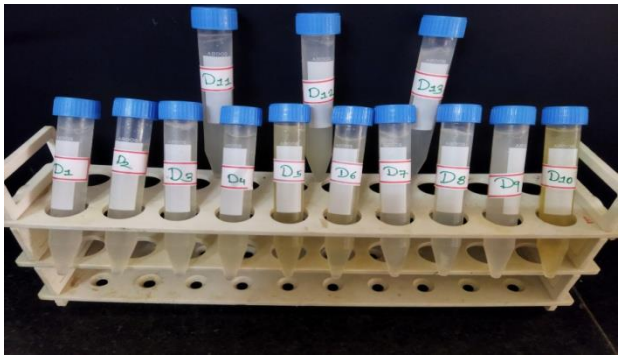

Figure 6 prepared batch

Independent variable of box behnken design screening design

Code	Independent variable	levels		
		-1	0	+1
X1	Stirring speed	800 RPM	1000 RPM	1200 RPM
X2	Sodiumsulfate concentration	5 ML	10 ML	15 ML
X3	Stirring time	10 MIN	20 MIN	30 MIN

Table 10 independent variables

Dependent variables of box behnken design screening design

Dependent variables	code
Particle size (nm)	Y1
Entrapment efficiency (%)	Y2

Table 11 dependent variable

Dependent variable and independent variables were separated and shown in table 10 and table 11

Box behnken design

RUN	STIRRING SPEED (RPM)	SODIUM SULFATE CONC. (ML)	STIRRING TIME (MIN)	Particle Size (nm)	Entrapment Efficiency (%)
D1	800	10	30	350	86
D2	800	5	20	178	76
D3	1200	10	10	282	93
D4	1000	5	30	340	90
D5	1000	10	20	148	82
D6	1200	15	20	236	72
D7	1000	15	10	270	88
D8	1200	10	30	146	84
D9	800	10	10	218	73

D10	1000	15	30	250	87
D11	1200	5	20	231	83
D12	800	15	20	280	95
D13	1000	5	10	209	92

Table 12 Box behnken design

Regression analysis	
R^2	0.9838
Adjusted R^2	0.9351

Table 13 R value

5.4.1 Counter Plot

The relationship between and the independent variables are further elucidated by consideration counter plot and response on surface area analysis. These types of plot are useful in study of the effect of two factors on the response at one time. When these plots are carefully observed, the qualitative effect on each variable on each response parameters could be visualized The analysis of counter plot of Homogenization speed and concentration of sodium sulfate on particle size. As see here, increased stirring time Particle size decreases and concentration of sodium sulfate increase particle size decrease by this picture we can say that concentration of sodium sulfate and stirring speed is having effect on particle size. It is show in figure 7.The analysis of counter plot of sodium sulfate concentration and homogenization speed is effect on entrapment efficiency as see here concentration of sodium sulfate increase entrapment efficiency of drug is increasing and same way increasing in stirring speed entrapment efficiency is decreasing.

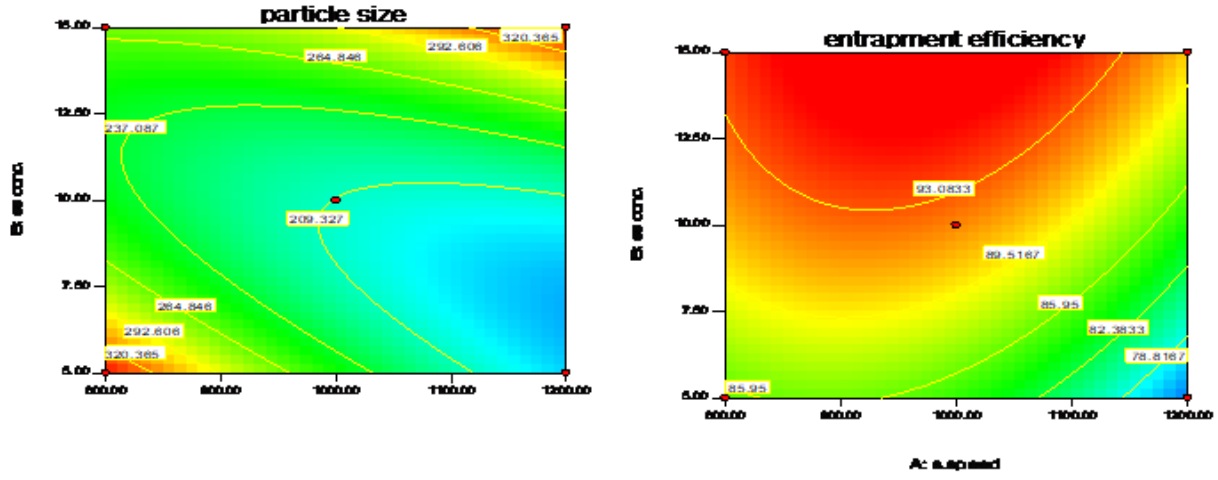

Figure 7 counter plot of particle size and entrapment efficiency

5.4.3 3D Plot for particle size

The analysis of 3D plot for box behnken design, shows effect on amount of sodium sulfate concentration and stirring speed as we can say that concentration of sodium sulfate and stirring speed is having effect on particle size. Which is shown in figure 8. The analysis of 3D plot for box behnken design shows effect on concentration of sodium sulfate

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increase entrapment efficiency of drug is increasing and same way increasing in stirring speed entrapment efficiency is decreasing

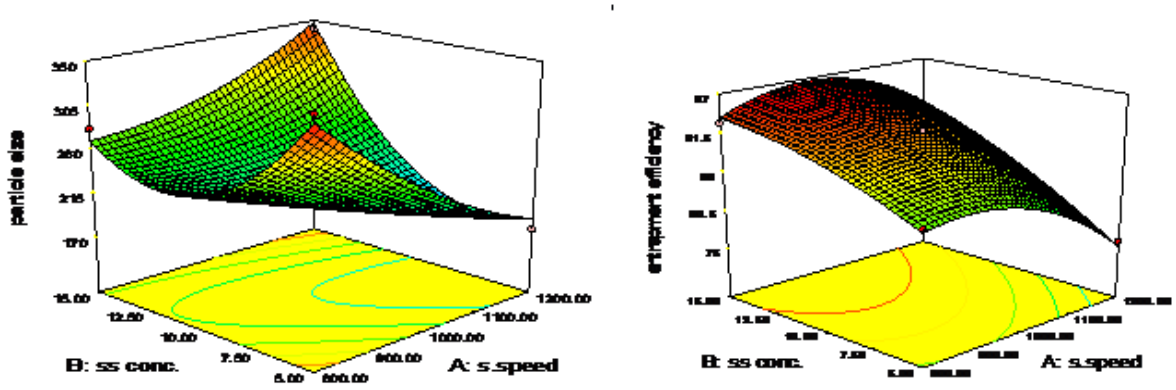

Figure 8. 3D plot of particle size and entrapment efficiency

5.4.5 Check point batch analysis

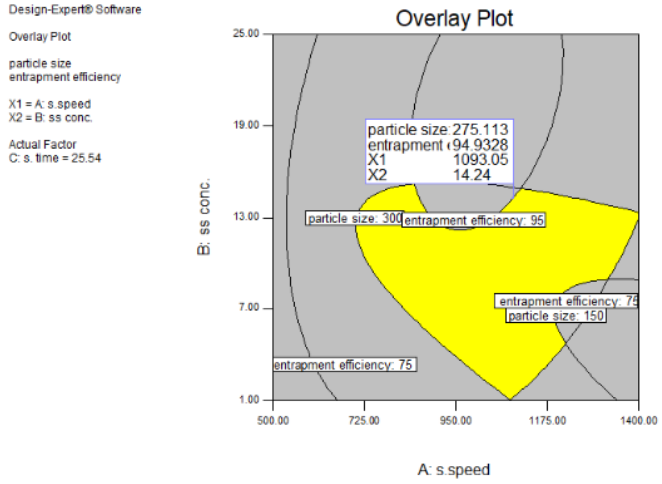

Figure 11 check point batch analysis

Overlay plots of the response could be used to determine desire concentration of the amount sodium sulfate concentration and homogenization speed. In the above picture show the desired range of response. By choosing any concentration and speed in this region desired region could be achieve. From the overlay plot, check point was selected in order to obtain desired value of factor. On the basis of desired criteria of particle size following batch was formulated to assess the reliability of the evolved equation. The experimental value and predicted value of each response is shown in this figure 11. The percentage relative error of each response was calculated using the following equation shown in table no 14.

%Relative error= predicted value- experimental value *100

Predicted value

Formula for check point batch

Ingredients	Levels
Particle size	275.113
Entrapment efficiency	94.93

Table 14 final batch

5.4.6 PARTICLE SIZE

Particle sizes of nanoparticles were measured in the zetatract instrument. To identify the particle size range of nanoparticles

5.4.6.1 Particles size for box behnken design (BBD)

Screening design are the most powerful DOE technique that determine the most critical factors to reduced it according to particle size as a response in the pharmaceutical development that screen large number of factor and identify critical one in a minimal number of run with more accuracy

Batch no.	Stirring speed (RPM)	Sod.sul. conc. (ml)	Stir .time (min)	Particle size (nm)
D1	800	5	20	350
D2	1200	5	20	178
D3	800	15	20	282
D4	1200	15	20	340
D5	800	10	10	148
D6	1200	10	10	236
D7	800	10	30	270
D8	1200	10	30	146
D9	1000	5	10	218
D10	1000	15	10	250
D11	1000	5	30	231
D12	1000	15	30	280
D13	1000	10	20	209

Table no 15 particle size of batch

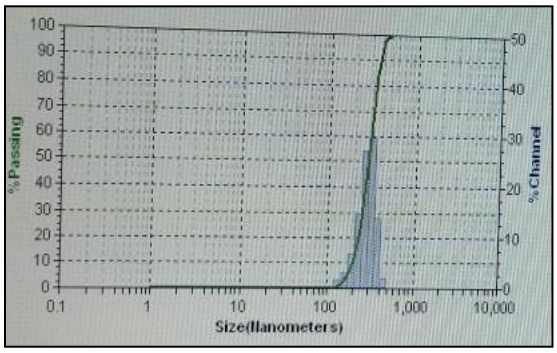

Figure no 12. Optimized batch

The particle size distribution was analyzed for box behnken design for D1 is optimized formulation of nanoparticles obtained from particle size analyzer and result shown in figure no 12.

5.4.7 Entrapment efficiency for box behnken design (BBD)

Batch no.	Stirring speed	Sod.sul. conc.	Stir .time	Entrapment Efficiency (%)
D1	800	5	20	86
D2	1200	5	20	76
D3	800	15	20	93
D4	1200	15	20	90
D5	800	10	10	82
D6	1200	10	10	72
D7	800	10	30	88
D8	1200	10	30	84
D9	1000	5	10	73
D10	1000	15	10	87
D11	1000	5	30	83
D12	1000	15	30	95
D13	1000	10	20	92

Table 16. entrapment efficiency

Screening design are the most powerful DOE technique that determine the most critical factors to reduced it according to entrapment efficiency as a response in the pharmaceutical development that screen large number of factor and identify critical one in a minimal number of run with more accuracy result is show in table no16.

5.4.8 Check Point Batch

Particle sizes of nanoparticles were measured in the zetatract instrument.

5.5 SEM Study

For SEM study of nanoparticles, the optimized sample was sent to department of biotechnology junagadh Agriculture University Junagadh to check the lamellarity and size of nanoparticles.

5.6 In vitro permission study

Time(min)	Percm² nanosuspension	Per cm² nanogel
30	0.0033	0.0198
60	0.0221	0.0535
90	0.0325	0.0698
120	0.0562	0.0965
150	0.0895	0.1536
180	0.1535	0.1998
210	0.1857	0.6297
240	0.2334	1.0726
270	0.3865	1.1544
300	0.4568	2.1948
330	0.5984	3.2687

Table no 17 In vitro permission study

The drug permeation profile by cellulose membrane demonstrated significant difference in the percentage of drug release according to pH media however formulation contain nanogel it give high drug release as compare to nanosuspension.shown in table no 17.

5.7 In vitro drug release study

Time(min)	% Drug release Nanosuspension	% Drug release Nanogel
30	9.099	27.9
60	11.295	34.398

90	15.093	39.897
120	21.798	50.796
150	28.8	51.894
180	30.6	52.2
210	38.295	52.29
240	46.899	58.594
270	57.195	69.098
300	66.897	85.599
330	65.297	87.993

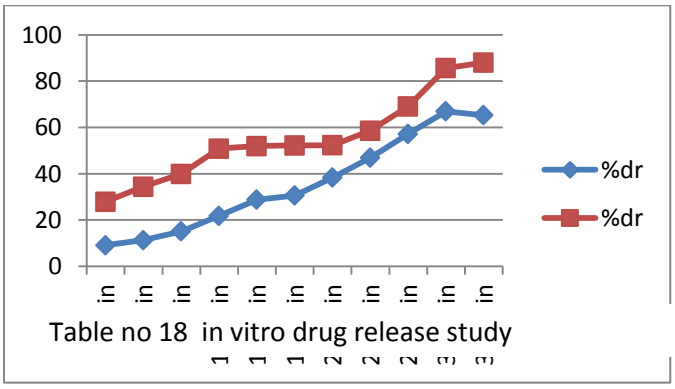

Figure 13 graph of drug release

The drug release profile demonstrated significant difference in the percentage of drug release according to pH media however formulation contain nanogel it give high drug release as compare to nanosuspension shown in table no 18 and graph of drug release is shown in figure no 13.

5.8 Ex-vivo drug release study

Time(min)	**Per cm² nanogel**	**Per cm² nanosuspension**
30	0.0128	0.0063
60	0.0326	0.0179
90	0.0568	0.0387
120	0.0865	0.0652
150	0.1216	0.0978

180	0.1596	0.1325
210	0.5897	0.1755
240	1.0726	0.2238
270	1.1247	0.2712
300	1.1842	0.3373
330	1.2539	0.4036

Table no 19 ex vivo drug release study

The drug permeation profile by goat skin and demonstrated significant difference in the percentage of drug release according to pH media however formulation contain nanogel it give high drug release as compare to nanosuspension.it is shown in table 19.

5.9 Infrared (IR) Spectroscopic analysis

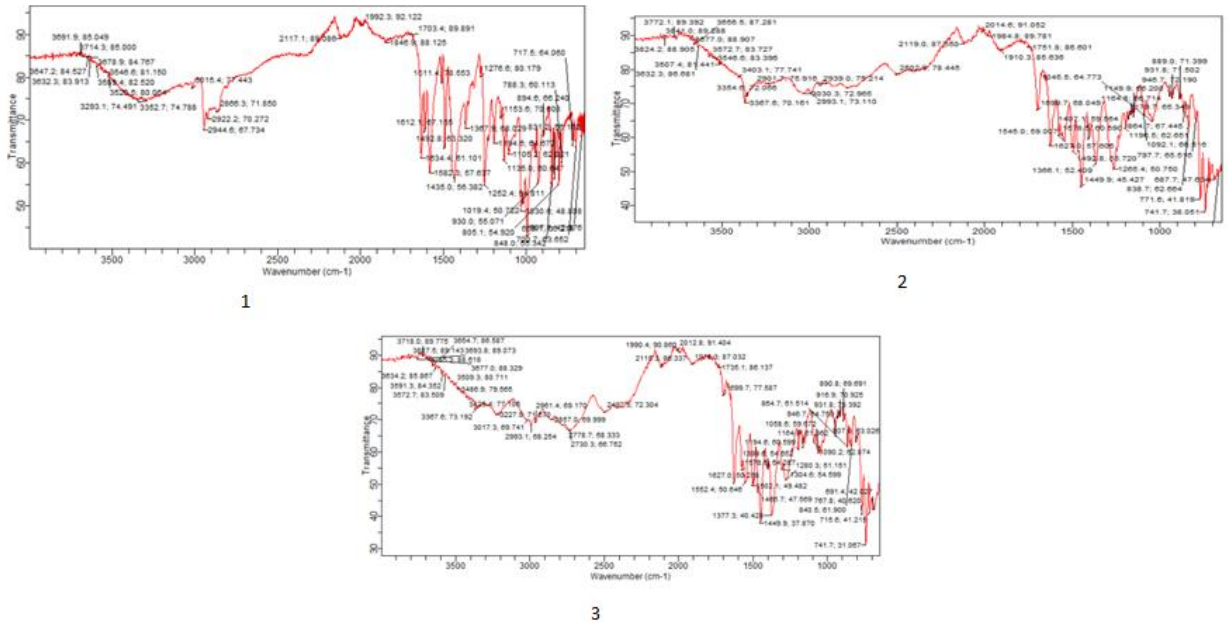

Figure 10. (1) drug polymer ,(2) nanoparticles of drug polymer,
(3) final formulation of nanogel

5.10 Incorporation of nanoparticles into gel

After the formulation of nanoparticles are prepared they are incorporated into a carbopol gel in equal amount of ration which is (1:1)(carbopol gel :nanoparticles)

5.11 Physical Examination

5.11.1 Drug content

5.11.2 Spreadbility test

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Sufficient amount of Nanogel was placed on glass slide and above that 1 glass slide is placed and 500mg weight on glass slide and spreadbility was calculated. On table 20 and figure no 18.

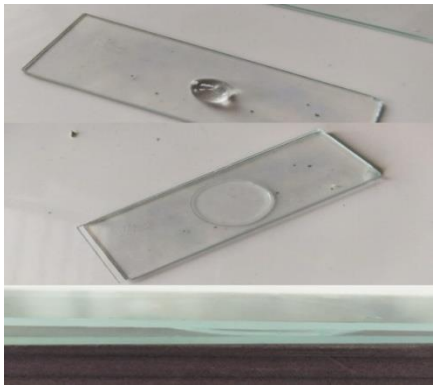

Figure 18 spradbility

1	۷	۳	Average
8sec	7sec	8sec	7.6sec
0.125	0.145	0.125	0.136

Table no 20 spradibility testing

5.11.3 Ex

Sufficient amount of Nanogel was kept in collapsible tube and time was calculated when gel is coming out from tube. Shown in figure 21

Figure 21 extrudibility testing

5.11.4 Stability test

Carbopol gel wash kept in room temperature and cold condition (2-4˚C) and wash observed after 8 week no contamination or no fungal growth is observed in figure 22.

Figure 22 stability study

5.11.5 Wash ability

Gel is easily washable and easily removed it is transparent, non sticky in nature.

5.11.6 Viscosity

Viscosity of final gel wash observed by Brook field viscometer at 100RPM by Spindal number64 triplicate trial was taken. figure 23. And table no 21.

Figure 23.viscosity

Final formulation of	**Viscosity in cp**	**Average**
Nanogel	6035 cp	
	5843 cp	5889 cp
	5789 cp	

Table no 21 average of viscosity

6 Summery And Conclusion

In present investigation it was expected to advancement of Nanogel for skin malignant growth is that so the practicality of nanoparticles for skin drug conveyance framework in the skin course drug is straightforwardly applied to the site of activity and there is no stomach result is oral conveyance through skin course is a best for treatment of skin cancer

Is first primer investigation was accomplished for determination of dissolvable crosslinking specialist, Homogenization speed, Homogenization time and temperature this factor has been resolved dependent on planning nanoparticles thinking about this factor and assessed the nanoparticles by Entrapment proficiency, molecule size, zeta potential, poly dispersity list, aggregate rate drug discharge. To decide as far as possible and lower cutoff and focus point. This decided alongside reliant and autonomous variable. After that we go for the to enter this factor to apply the plan hear what the financier configuration was applied pareto graph, counter plot was drawn, relapse investigation was applied to decide the significant factor in box behnken plan…

The mean molecule size was between 50 to 400 nm the examination of Counter plot and 3D plot for the crate behnken plan so the impact of sodium sulfate fixation, homogenization time and homogenization speed a molecule size here we can see that expanding the hour of homogenization the molecule size is diminishing and the speed of blending molecule size may diminish .Examination of Counter plot and 3D plot for the crate behnken configuration measure of sodium sulfate will give significant factor in Entrapment productivity as we increment the sodium sulfate fixation the more uninhibitedly and round Nano particles are shaped and Entrapment effectiveness of medication is expanded

The examination of Counter plot and 3D plot of otha box behnken plan so the impact of CPR% is the measure of sodium sulfate is expanded CPR% increment homogenization time will give legitimate medication discharge

The % relative mistake for the designated spot page of molecule size was 5.6 to the designated spot page for Entrapment proficiency was 4.86 which is under 8% so measurably worthy it very well may be reasoned that the exploratory worth and anticipated worth show great concurrence with one another

SEM investigation of the nanoparticles was played out the upgrade test was sended to check the size of nanoparticles. In vitro penetration investigation of a medication discharge saturation study was performed by utilizing USP disintegration rate test contraption paddle type it was discovered 87% IR Spectroscopy investigation was performed of an unadulterated medication and medication polymer likewise after the transdermal nano gel was set up by particle gelation strategy it was assessed by actual assessment like appearance, colou,r clearness, perfection, wash capacity, Spreadablity, Extrudablity, consistency, ex Vivo considers, skin aggravation study, drug content my old this test to the definition is past

Definitions show adequate fomoco method properties from the outcome acquired unmistakably there was appropriate conveyance of piperine in the nano gel detailing consequently it was reasoned that the truck was consistently disseminated in the plan with worthy deviations. The essential skin disturbance test () was performed on a sound bunny no skin bothering due to there was no erythema and edema were found on hare there is no indication of instruction has been seen just be two for which we can reason that the definition is alright for the utilization in human skin the consequence of the current examination demonstrate that the transdermal nanogel of piperine was test bye a few boundaries in this investigation has shown the promising outcome subsequently there is practicality of conveying Nanogel through transdermal course.

The advancement of transdermal Nano gel prepuce malignant growth plan Mein end up being a promising profession for piperine and other medication because of their straightforward creation and shortsighted scale up.

7 Declarations

Conflict of interest- there is no conflict of interest

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