wjpmr, 2023,9(9), 273-285

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

FORMULATION AND DEVELOPMENT OF NANOGEL CONTAINING GREEN TEA EXTRACT: AS TOPICAL DOSAGE FORM

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Article Received on 19/07/2023 **Article Revised on 09/08/2023** **Article Accepted on 29/08/2023**

ABSTRACT

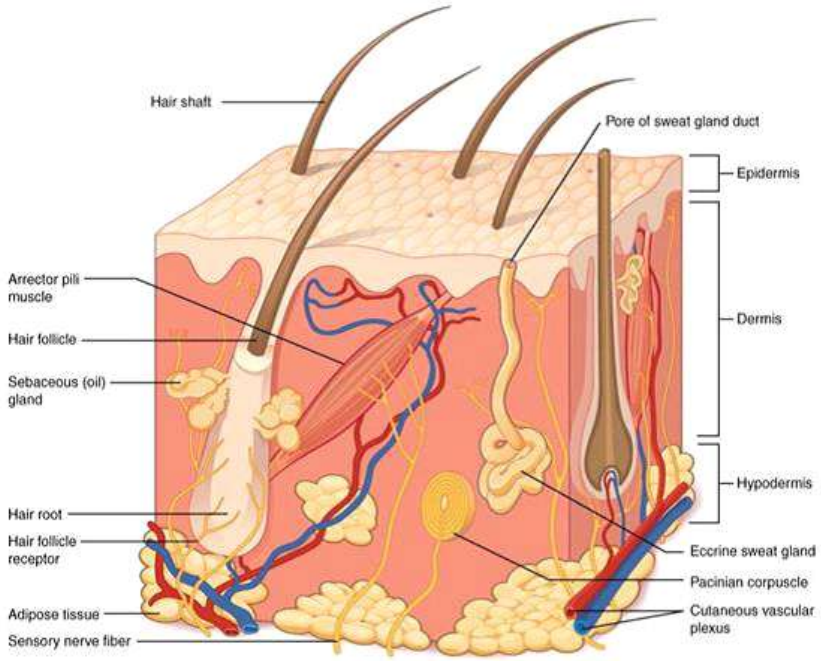
The present study aims at formulating Nano sized green tea extract by the method of nanoprecipitation. The prepared nanoparticle was evaluated for particle size, zeta potential, electron Scanning Microscopy (SEM), percentage of encapsulation efficiency (EE) and in vitro drug release. To formulate Nano gel, the selected formulation of Green Tea Extract Nanoparticles has been integrated into a gelling agent. The Box-Behnken method has prepared a total of 17 formulations used to test the effect of three selected independent variables such as Carbapol 934 (X1), triethanolamine (X2), and stirring speed (X3). The dependent variables analysed were the Nanogel pH (Y1), Nanogel viscosity (Y2), and gel diffusion in-vitro (Y3). Besides, pH, homogeneity, spreadability, viscosity, extrudability and drug content tests characterized the formulation. The nanoparticles were characterized through particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency and Percentage yield. The resulting shave found F5was best formulation among all batches and has transformed into topical Nanogel. In order to find the compositions of the optimised formulation, the statistical validity of the polynomials was established. Further response surface plots were drawn. Therefore, it can be inferred that the design shows the role of the derived polynomial equation and contour plots in predicting the values of dependent variables for preparation and optimization of Nanogel formulation. It was concluded that the GTE-loaded Nanogel was stable and could be used for the delivery of topical drugs with promising potential.

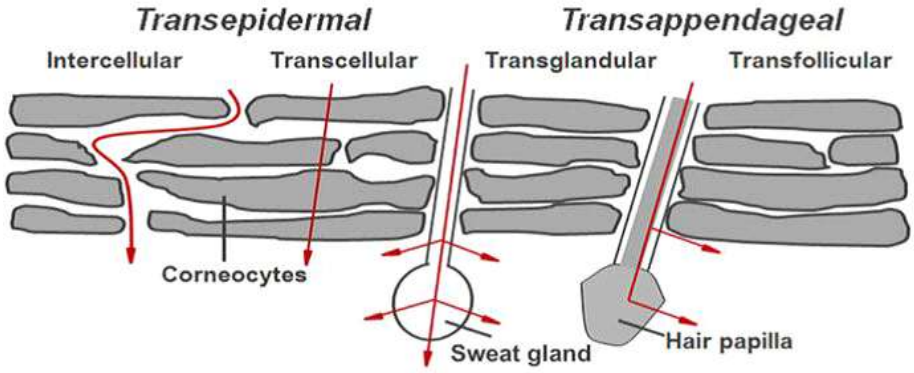
KEYWORDS: Green tea Extract, Chitosan, Sodium tripolyphosphate, Carbapol 934 and Box-Behnken design.

INTRODUCTION

Developing new formulations introduces unique challenges in selecting the best kind of formulation for a given active ingredient. The topical route has long been used to deliver drugs directly through the skin to the affected target site. Current approaches in design and optimization of topical formulations necessitate extensive decisions in choosing the right components for the formulation to achieve high safety, clinical efficacy, and patient compliance. It is a resource-intensive process on the part of the formulator which requires a certain degree of hit-and-trial. The solubility of the active ingredient in the vehicle and skin is an important parameter in the development and optimizing of vehicle components for the development of topical formulations. The Design-Expert Software Version 12.0.1 presents a new approach to design topical formulations aimed at selecting the components, which work synergistically to drive for active ingredient past the skin barrier while achieving sufficient solubility in the vehicle. Grounded to the theory of experiment design, the process requires the usage of several kinds of experimental structures, generating polynomial equations, and projecting the result across the scientific field to define the optimal

processing variables. This research project was aimed at developing a Nano gel of Green tea extract using Design expert software for the design and optimization of the Nano gel. In this study, seventeen Nano gel formulations of Green tea extract (1% w/w) in the form of green tea nanoparticles were developed and characterized. Furthermore, the Nano gels were evaluated for viscosity, pH of the formulation, spread ability, in vitro drug diffusion, and drug permeation. In summary, the results from this research establish a new and useful approach in designing the Nano gel formulation where the active ingredient shows the best result. Besides the use of Nano gel for the topical delivery of hydrophobic drugs was shown to be effective at maintaining a higher concentration of drugs through the skin barrier for an extended era of time.





MATERIALS AND METHODS

S. No	Materials	Supplied by
1	Green tea extract	Suraksha pharmaceuticals, Hyderabad
2	Chitosan	BMR Chemicals, Hyderabad
3	Sodium tripolyphosphate	BMR Chemicals, Hyderabad
4	Carbopol	BMR Chemicals, Hyderabad
5	Propylene glycol	BMR Chemicals, Hyderabad
6	Triethanolamine	BMR Chemicals, Hyderabad
7	Acetic acid	BMR Chemicals, Hyderabad
8	Glycerin	BMR Chemicals, Hyderabad
9	Cellophane membrane	-

Equipments used

1.	**Homogenizer**	**Kinematica AG(Poly tron PT2100)**
2.	Rotary evaporator	Super fit
3.	Analytical balance	ShincoDeshi .,Ltd, Japan
4.	pH meter	Polmon, LP-139S
5.	Microscope	Olympus, CH20
6.	Particle size analyzer	MALVERN
7.	Zeta potential analyzer	MALVERN
8.	Magnetic stirrer	Rimek

Method of Preparation

Preparation of Green tea extract nanoparticles

Green tea extract nanoparticles were prepared by Nano precipitation technique. The correctly weighed drug quantity was dissolved with magnetic stirring in chitosan & acetic acid (1.6 ml each) and sodium tripolyphosphate (TPP) (5 percent) was added in dropwise at a uniform rate through a syringe. The five formulations of the nanoparticles cited in Table.7.4 have been prepared. It was further mixed for 2 hours, followed at 13000 rpm by centrifugation for 5 minutes. The supernatant was discarded, and the pH 6.8phosphate buffer nanoparticles were suspended again. At 15,000 rpm by centrifugation, the nanoparticle was extracted for 15 minutes and the obtained pellet was washed three times with filtered water. The prepared green tea extract nanoparticle was held for further use at room temperature.

The Formula of different formulations of GTE loaded chitosan nanoparticle

S.No.	Ingredients	F1	F2	F3	F4	F5
1	Green Tea Extract (%)	5	5	5	5	5
2	Chitosan	0.25	0.5	0.75	1	1.25
3	Acetic Acid (%V/V)	2	2	2	2	2
4	Tripolyphosphate	1	1	1	1	1

Preparation of Green tea extract (GTE) Loaded Nano gel

The GTE (Green tea extract) loaded Nano gel was prepared using the concentrations shown in Table 3 using the dispersion process. Dispersing Carbapol 934 for swelling in distilled water for 2 hours. Once the Carbapol was swelled, it was held for stirring on a magnetic stirrer, and the prescribed amount of GTE-loaded nanoparticles were applied to the dispersion to bring 1% of the substance into the gel, together with propylene glycol and glycerine were injected into the mixture and stirred at 500rpm using a magnetic stirrer. Stirring continued until the Carbapol 934 was completely dispersed to obtain the homogeneous gel. To shape an attractive gel formulation, the final pH was changed to 6.1–6.8 by adding triethanolamine.

Evaluation Parameters

Characterization of Nanoparticles

a) Particle Size and PDI

For particle size measurements, the dynamic laser scattering was used. Zetasizer (Nano ZS, Malvern Instruments, and Malvern, UK) has established the particle size and PDI of NPs. To assess the particle size, the suspension of NPs was mixed with distilled water. There were twelve measurements, and the average was determined.[37]

b) Entrapment Efficiency

The EE of GTE was calculated by an indirect method in the Chitosan nanoparticles. In short, for 10 minutes, the NPs suspension was centrifuged at 12000 rpm. The collected supernatant was mixed with methanol, and to calculate the amount of free GTE present in the supernatant, a 296 Nm UV-visible spectrophotometer was used. Using the formula below, EE was determined:

$$\%\ \boldsymbol{Entrapment\ Efficicency} = (\boldsymbol{Amonut\ of\ GTE\ added\ in\ formulation - Amount\ presemt\ in\ supernatant}) / \boldsymbol{Amount\ of\ GTE\ added\ in\ formulation} \times 100$$

c) The percentage yield of nanoparticle

The nanoparticle yield was determined by measuring the whole nanoparticle shaped against the combined weight of the copolymer and drug. With the following formula, the percentage yield was calculated

$$\%\ \boldsymbol{Yield} = \boldsymbol{Amount\ of\ Drug / Amount\ of\ drug + polymer} \times 100.$$

d) Morphological studies by scanning- electron microscopy

To measure the morphology of the nanoparticle, scanning electron microscopy is used. 100µl of the chitosan nanoparticle formulation was applied to a 10 mm glass slide in a preliminary phase and dried overnight in a vacuum desiccator at room temperature prior to the SEM study [38]. Using the gold sputter module in a higher vacuum evaporator, the nanoparticle study was fixed on sufficient support and coated with gold. The observation was carried out at 15kv under various magnifications.

Characterization of GTE Nanoparticles Loaded Nano gel

a) Physical examination

The prepared GTE loaded Nano gel formulation was inspected visually for its colour, appearance and consistency.

b) Measurement of viscosity

The viscosity of the GTE filled Nano gel was measured using the Brookfield DV-III Rheometer (Brookfield Engineering Laboratories Inc., Middleboro, MA). The CCT-14 spindle was attached to the Brookfield Rheometer. The spindle was previously packed into the sample holder with a dip in the topical Nanogel.[39] Then the holder of the sample was connected to the instrument and measured the viscosity of the topical nanogel.

c) Measurement of spreadability

Using two slides (5 cm2), the spreadability of the Nanogel was measured. Every batch of 0.5 g topical Nanogel was put between two slides and left for 1 min. The diameter of the topical nanogel spread circle was measured and compared with each other.

d) Homogeneity test

Using topical nanogel visual inspection, the study of nanogel homogeneity was studied. In order to check whether the prepared topical nanogel was homogeneous and whether aggregates were present or not, all formed nanogels were filled into vial containers of flint colour and subjected to visual inspection.

e) Drug content

Nano gel was weighted with GTE loading and dissolved into the 50 mL of methanol. To dissolve the GTE fully into the methanol, it was sonicated for 15 min. The solution was filtered through the Whitman filter paper and methanol was diluted with the resultant filtrate. Using UV visible spectroscopy and drug material, the aliquot subjected to 296 nm wavelengths scanning was measured.

f) Extrudability test

The formulations were finished in the collapsible tube after gels were poured in the pipe. The formulation extrudability was calculated in terms of the weight into grams needed for the 0.5 cm ribbon gel to be extruded in 10 seconds.

g) FTIR Studies

Compatibility study was studied by recording the sample using Perkin Elmer Fourier transformation Infrared spectroscope (FT-IR) combined to PC (with spectrum 2000 analysis software) in the range of 4000 cm-1 to 400 cm-1. Potassium Bromide Press Pellet was prepared by applying pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in light path and the spectra were analysed.

h) Differential scanning calorimetry

GTE and green tea extract loaded Nanogel (DSC, 821 e, Metter Toledo, DSC, 821e, Schwerzenbach, Switzerland) differential scanning calorimetry (DSC) thermos grams were recorded. Previously, the temperature range and the DSC cell constant were calibrated with indium. Over a temperature range of 25–2000 C with nitrogen purging (100 ml / min), a heating rate of 10 C / min was used. In an aluminium bowl, samples (2-8 mg) were measured and analysed using an empty bowl as a reference.[40]

i) In-Vitro Drug Diffusion Studies

Using a Franz diffusion cell apparatus, the in-vitro drug release of topical Nanogel from all batches was studied. Prior to its use for diffusion research, the dialysis membrane was soaked in the phosphate buffer of pH 7.4. A weighted amount (0.5 g) of Nanogel was placed in the donor compartment and then pH 7.4 phosphate buffer was filled into the receptor compartment. The dialysis membrane was positioned as a diffusion membrane between the donor and the receptor compartment and the use of the clamp remained tight. Using the Teflon coated stirring the bar, the receptor compartment media temperature was held at 37 ° C (± 0.5 ° C) below 100 rpm. At each prefixed time interval of 1, 2, 3, 4, 5 and 6h, 3ml aliquots were collected and sink condition was preserved over a period of full diffusion analysis. Using UV at wavelength 296 Nm, the collected aliquots were scanned and the percentage of cumulative drug release of Nanogel was calculated.

RESULTS AND DISCUSION

a. Physicochemical Characterization of Drug

S. No.	Description	Green tea extract
1.	Color	Dark Olive to Dark Brown
2.	Nature	Hygroscopic
3.	Form (Crystalline/Amorphous)	Amorphous powder
4.	Melting point	217oC
5.	Odor	Unpleasant

Solubility Profile

S. No.	Solvent	Solubility Observation
Green tea extract		
1.	Distilled water	+ ++
2.	Methanol	+ + +
3.	Acetone	+ +
4.	Ethanol	+ + +
5.	PBS (pH 7.4)	++

Keys:
++++ Freely soluble 1-10 parts of solvent
+++ Soluble 11-30 parts of solvent
++ Sparingly soluble 31-100 parts of the solvent
+ Slightly soluble 101-1000 parts of solvent
- Insoluble <1000 parts of solvent.

c. Determination of λ max
In order to determine the maximum absorption, the formulated stock solution was scanned Between 200 to 400 Nanometres. It was found to be 296 Nm.

d. The Calibration curve of Green tea extract
The standard curve of green tea extract was obtained and good correlation was obtained with
R2 value of 0.9992, the medium selected was pH 7.4 phosphate buffer.

Table: Standard graph values of Green tea extract at 296 Nm in pH 7.4 phosphate buffer.

Concentration	Absorbance
0	0
0.2	0.06
0.4	0.11
0.6	0.17
0.8	0.23
1.0	0.28

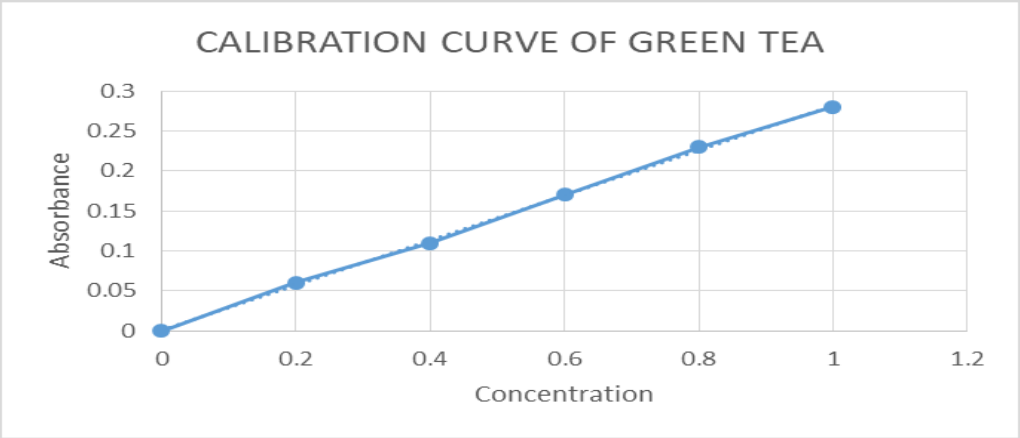


Figure: The Calibration curve of Green tea extract.

Characterization of Nanoparticles
Particle Size and PDI
The average size and zeta potential study of GTE loaded chitosan nanoparticles was carried out by using zeta sizer. The maximum zeta potential of chitosan nanoparticle was found to be 40.2mV. the zeta potential was found to increase with the particles surface charge also will Increase. The results have also shown that the zeta potential was found to get an increase with the increase in particle surface charge. The average particle size and Zeta Potential of Nanoparticles were recorded in Table 7.5. The average particle size of nanoparticles was Found to be 159.9 to 354.5 Nm. The dynamic light scattering has been used to evaluate the particle size and polydispersity index of GTE loaded chitosan nanoparticles, as shown in Fig. 1-5.

Table The Mean size of particles and zeta potential value of different formulations.

S.No.	Formulation Code	Particle size(nm)	PDI	Zeta potential(mv)
1	F1	345.5	1.00	+40.2
2	F2	241.0	0.288	+38.6
3	F3	217.3	0.035	+35.8
4	F4	196.7	0.521	+34.7
5	F5	159.9	0.308	+31.4

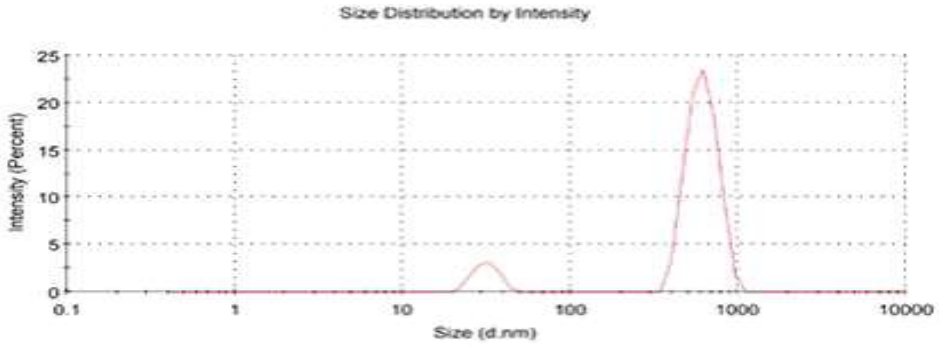


Fig. 1: Size distribution of F1.

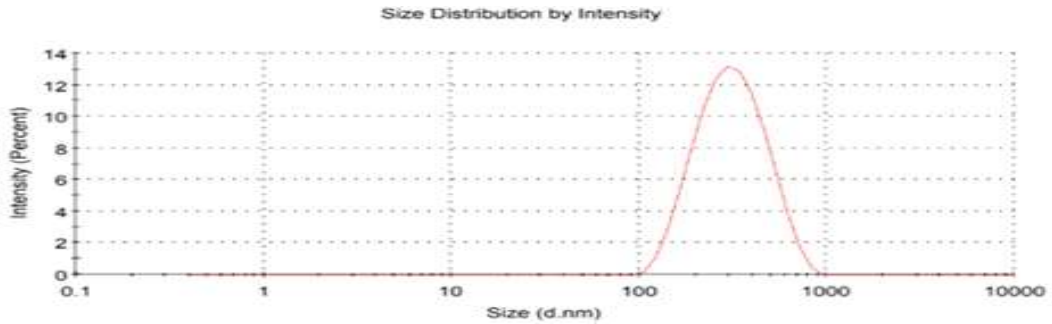


Fig. 2: Size distribution of F2.

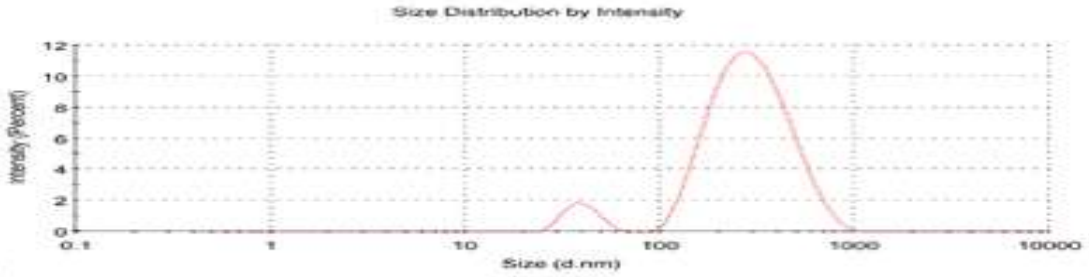


Fig. 3: Size distribution of F3.

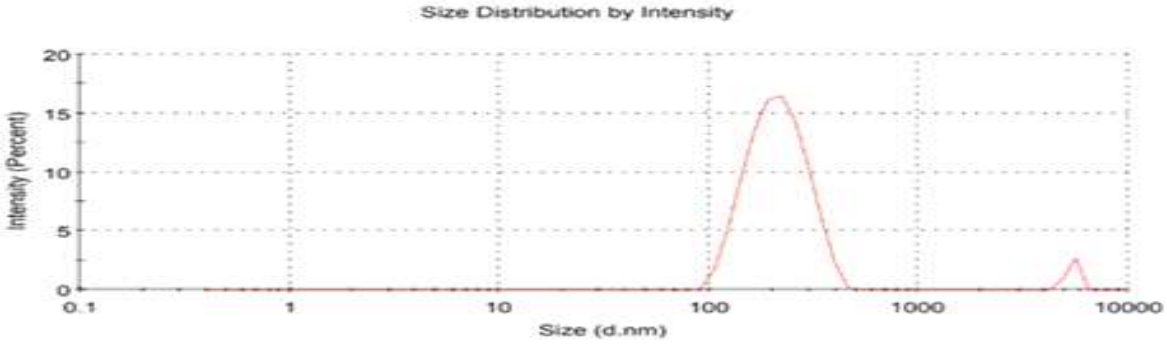


Fig. 4: Size distribution of F4.

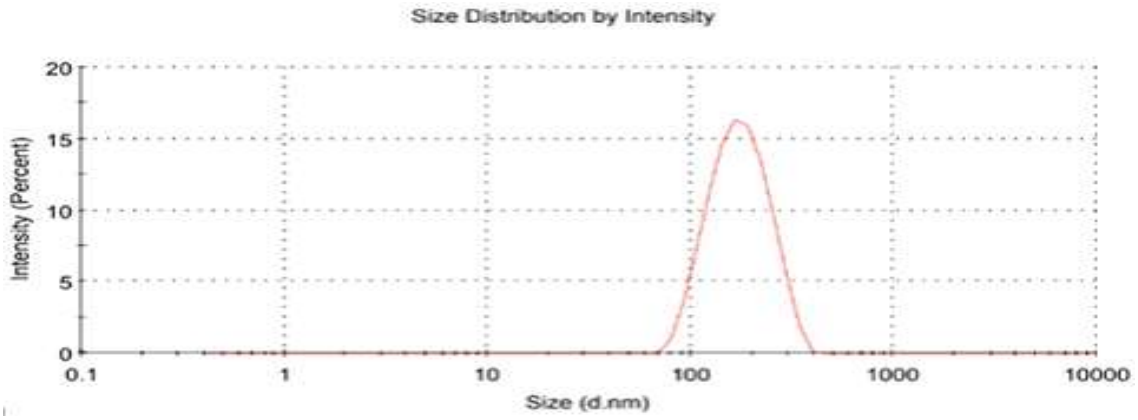


Fig. 5: Size distribution of F5.

Morphological studies by Scanning Electron Microscopy

Using scanning electron microscopy, the surface morphology of the GTE equipped chitosan Nanoparticle was investigated. The research gave a better understanding of the morphological features of the nanoparticle. The research offered a greater understanding of the nanoparticle's morphological characteristics. There have been a huge number of nanoparticles with an approximately spherical form, so they're going to split. At higher cross-linking time, The SEM image of dried chitosan nanoparticles, little forwarded but small spherical nanoparticles were obtained in Formulation F5, and all the formulations images have been shown in Fig no 1-5.

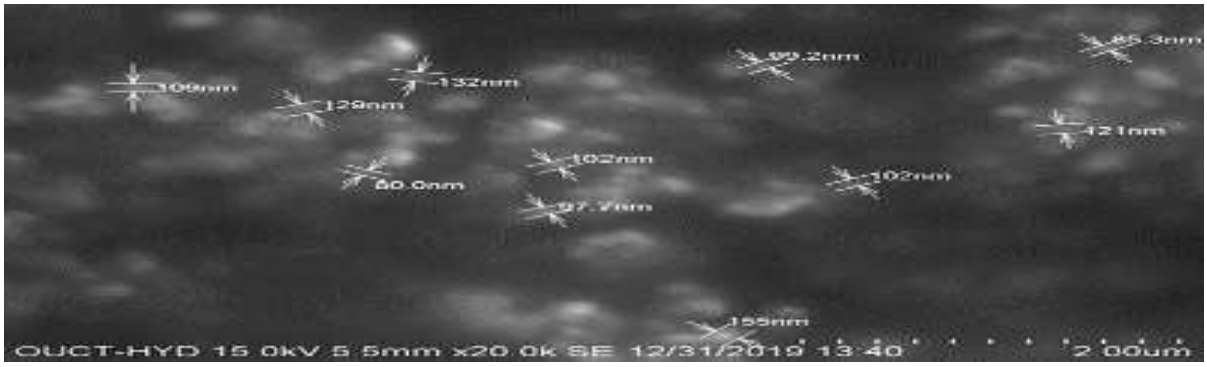


Fig 1: SEM image of Optimized formulation F1.

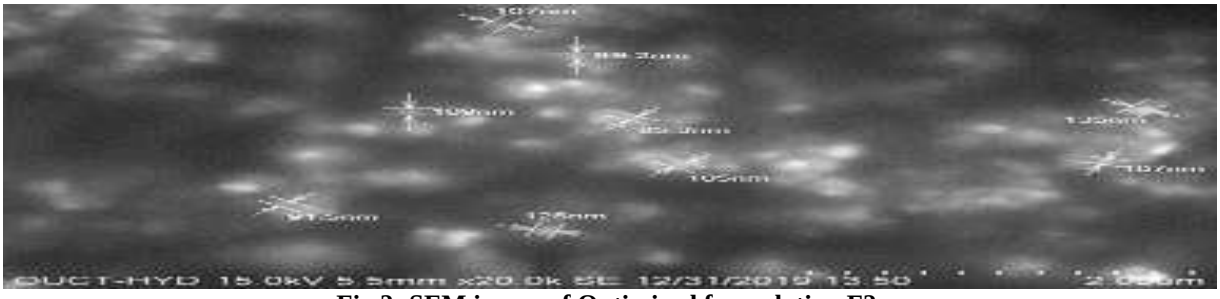


Fig 2: SEM image of Optimized formulation F2.

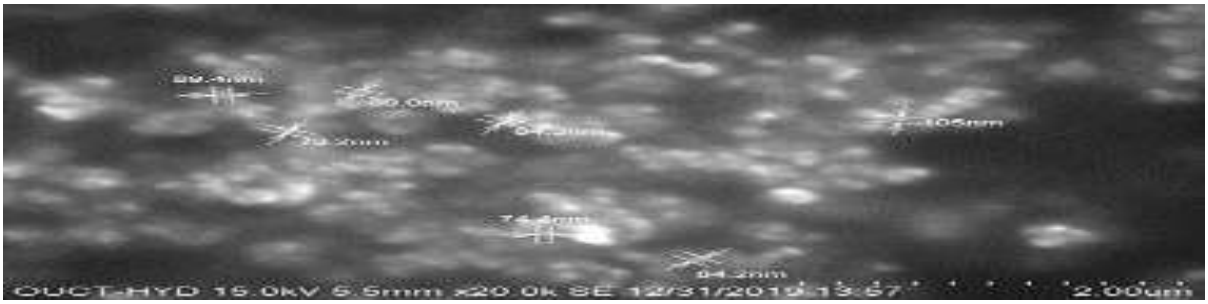


Fig 3: SEM image of Optimized formulation F3.

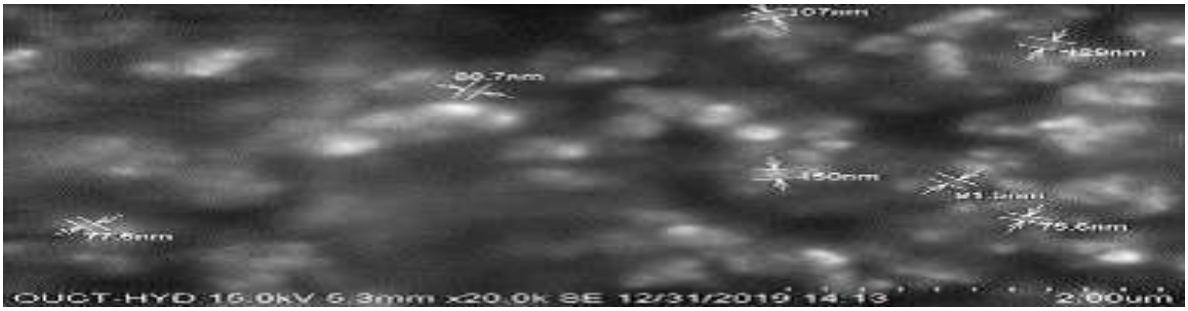


Fig 4: SEM image of Optimized formulation F4.

Fig 5: The SEM image of Optimized formulation F5.

Characterization of GTE Nanogel

Physical examination

All batches of Nanogel were characterized for their physical properties such as colour, Texture and consistency. The prepared Nanogel were yellow in colour with a pleasant, Smooth homogeneous appearance and texture. The homogeneity values of all the 17 Formulations were recorded in given Table.

S.No.	Formulation Code	Homogeneity
1	NG1	+
2	NG2	+
3	NG3	+
4	NG4	+
5	NG5	+
6	NG6	+
7	NG7	+
8	NG8	+
9	NG9	-
10	NG10	+
11	NG11	-
12	NG12	+
13	NG13	+
14	NG14	+
15	NG15	+
16	NG16	+
17	NG17	+
18	NG18	+

Note: +++Excellent, ++Good, +Not satisfactory

Differential Scanning Electron Microscopy

Based on the presence, change or absence of endothermal or exothermic peaks, DSC has Proved to be an essential tool for quickly obtaining information about possible interactions Between the active and the excipients. DSC study was conducted using DSC 60 instruments To determine the compatibility study of the drug excipients. During the study, a sharp endothermic peak for Green tea extract was obtained at 201.65°C corresponding to the melting point. However, in the formulation, there was a slight change in peak temperature and peak shape as seen in Fig.1 and Fig2, which reveals there was no interaction Between drug and excipients.

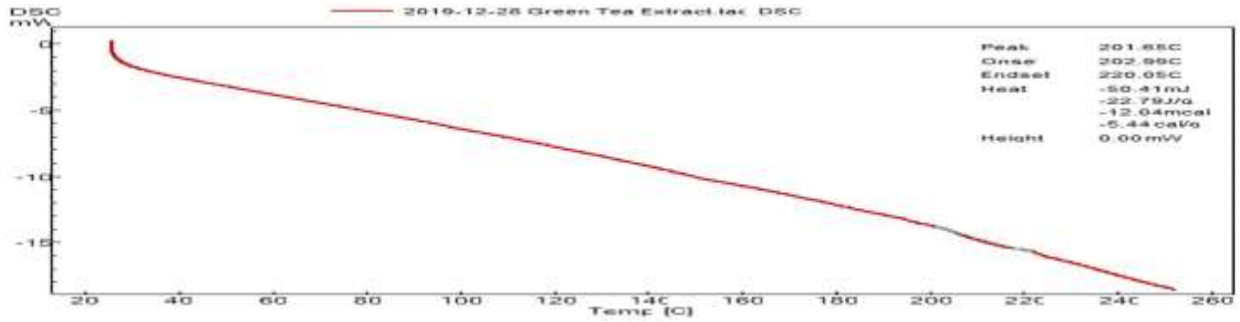


Fig 1: Differential Scanning Calorimetry of Pure Green Tea Extract.

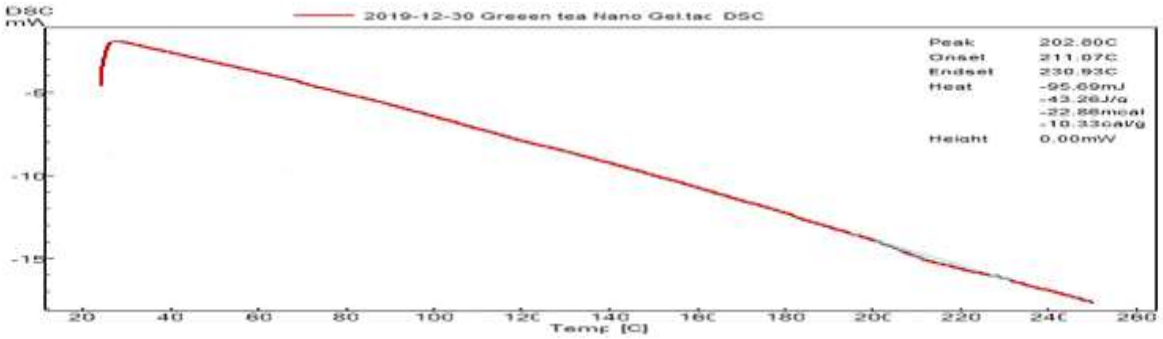


Fig 2: Differential Scanning Calorimetry of Green Tea Extract Nanoparticles Loaded Nanogel.

Fourier Transform Infrared Spectroscopy (FTIR)

Pure Green tea extract, Excipients and Optimized formulation were subjected to FT-IR Studies. The obtained spectra are shown in Fig no. 7.14. Characteristics peaks of pure Green tea extract were compared with the peaks of optimized formulation. The characteristic bands of pure green tea extract were identifiable and no major shifts were observed in optimized formulation which indicates that the drug is intact in the formulation has not reacted with the excipients.

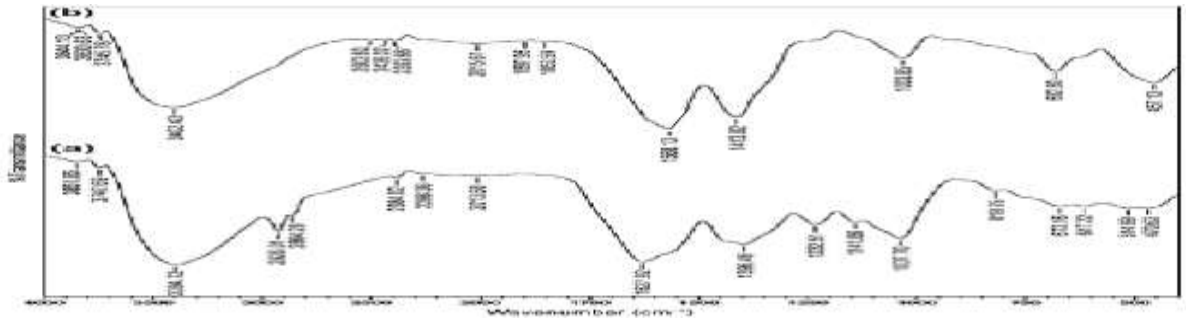


Fig. 1: FTIR of a) Green tea extract b) Optimized formulation.

***In vitro* drug Diffusion Studies**

By using Franz diffusion cell apparatus, drug release of topical Nanogel formulation was observed. The collected aliquots were scanned using UV-Visible spectrophotometer, and % cumulative drug release was calculated. % cumulative drug diffusion was shown in the Table.1. And % cumulative drug diffusion of each formulation are mentioned in Table.1 & Table 1. The % cumulative drug diffusion graphs are shown in Fig no. 1.

Table 1: % cumulative drug diffusion of Formulations NG1-NG17

S.No.	Formulation Code	% cumulative drug diffusion
1	NG1	92.3
2	NG2	110.5
3	NG3	85.8
4	NG4	92.3
5	NG5	59.5
6	NG6	68..2
7	NG7	48.7

8	NG8	92.3
9	NG9	92.3
10	NG10	90.2
11	NG11	52.7
12	NG12	92.3
13	NG13	40.8
14	NG14	41.9
15	NG15	92.3
16	NG16	99.9
17	NG17	41.5

% Cumulative Drug Diffusion					
Timei(Hrs)	**NG13**	**NG14**	**NG15**	**NG16**	**NG17**
0	**0**	**0**	**0**	**0**	**0**
0.5	**9.33**	**10.05**	**11.2**	**11.2**	**9.91**
1	**12.9**	**11.9**	**18.24**	**19.4**	**13.4**
2	**20.9**	**19.6**	**28.97**	**27.32**	**21.6**
3	**26.4**	**25.4**	**34.6**	**36.1**	**28.9**
4	**31.9**	**28.4**	**43.25**	**44.8**	**31.6**
6	**33.9**	**32.6**	**54.6**	**55.2**	**37.2**
8	**36.8**	**36.9**	**76.98**	**77.6**	**39.9**
10	**40**	**40.8**	**87.32**	**88.1**	**40.19**
12	**40.8**	**41.9**	**92.3**	**99.9**	**41.5**

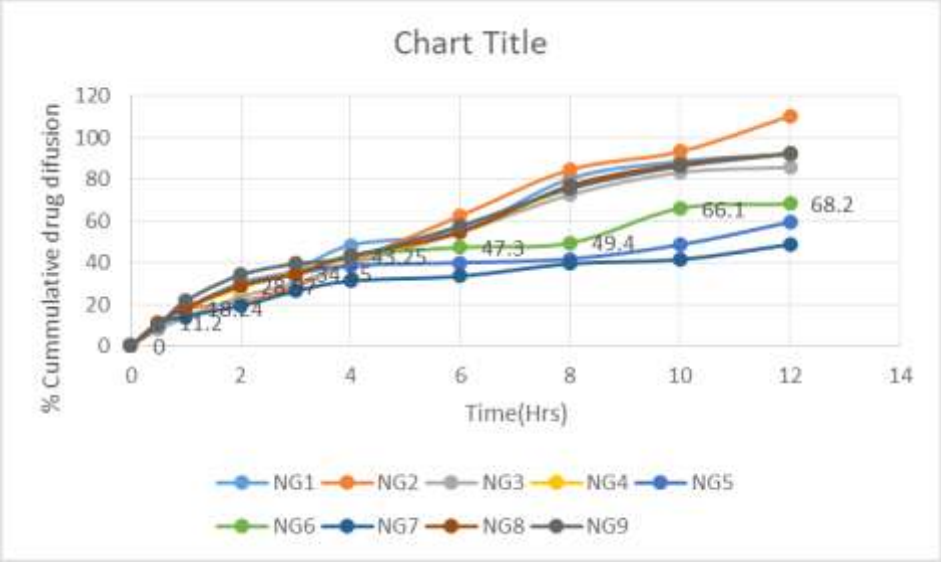


Fig: Cumulative %drug diffusion from formulations NG1-NG09.

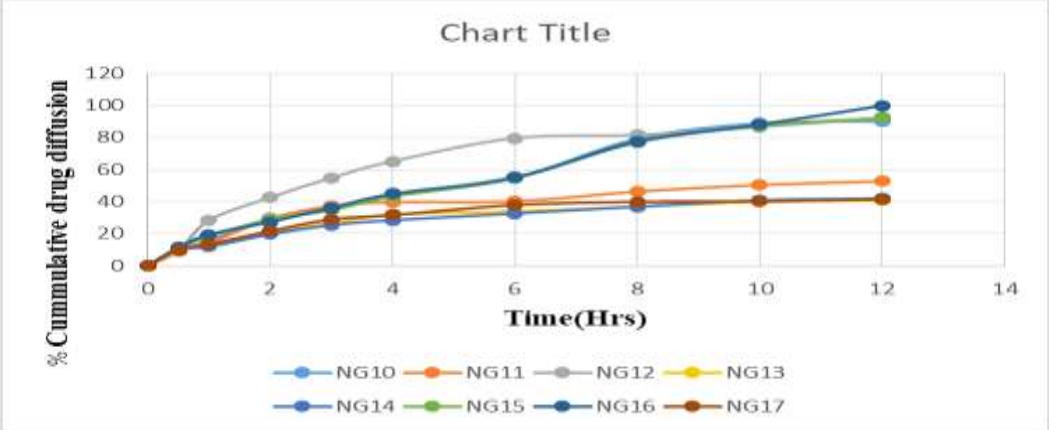
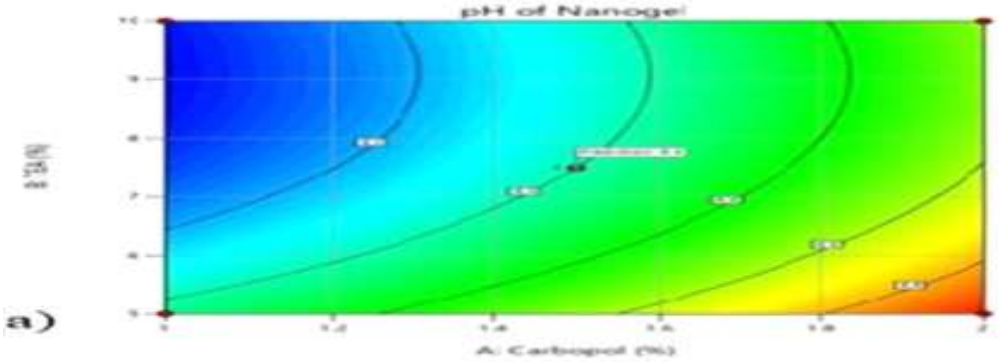


Figure: Cumulative %drug diffusion from formulations NG10-NG17.

The effect on pH of Nanogel formulation.

The following polynomial equation (1) was proposed by the model for pH of gel (Y1) Formulation.

Y1 = 6.30 + 0.1813A - 0.1025B +0.0038C +0.0025AB - 0.0010AC +0.0025BC +0.0225A2 +0.080B2 +0.0175C2 ------- (1). Where Y1 is the pH of the Nanogel formulations.



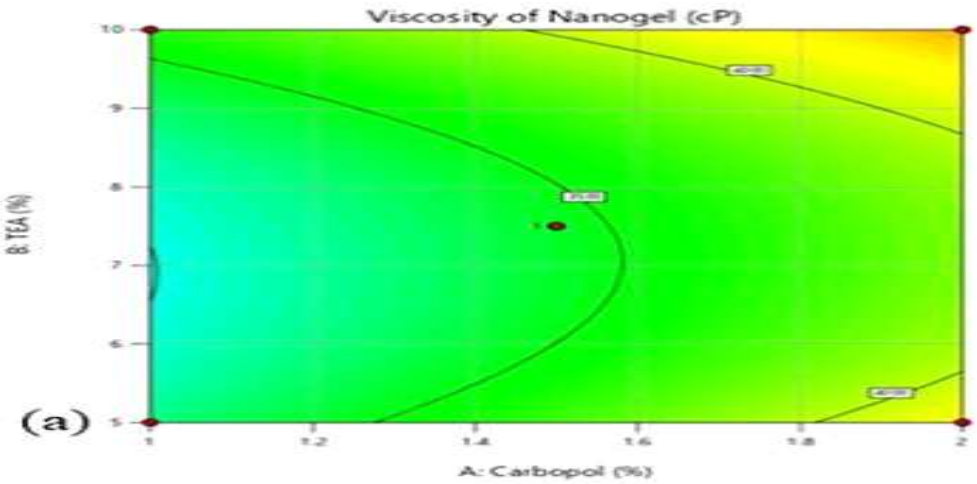
The effect on the viscosity of Nanogel formulation

The following polynomial equation (2) was proposed by the model for viscosity of gel (Y2)

Formulation

Y2 = +3445.70 + 418.42A +160.06B + 477.84C – 44.65AB +344.45AC -102.47BC -10.71A2 +424.06B2 – 485.94C2 ---------- (2)

The following Figure represents the contour plots and its 3D response plots which show the effect of different independent factors on viscosity.



SUMMARY AND CONCLUSION

The present work involves the formulation development, optimization and evaluation of Green tea extract nanoparticles loaded Nano gel. The Green tea extract Nanoparticles were prepared by Nano precipitation method and green tea extract loaded Nanogel were prepared by dispersion method and optimized by box-behnken method. Various concentration of chitosan was used to prepare Green tea extract nanoparticles. Total 17 formulations were formulated and evaluated for different evaluation parameters like drug loading, % entrapment efficiency, Percent Yield and particle size. The green tea extract and chitosan were found to be compatible in FTIR study and DSC studies. SEM study showed that prepared nanoparticles were spherical in shape with a smooth surface with spherical shape. Particle size of prepared nanoparticles was found to be in the range between 159 nm and 354.5nm.

In-vitro drug release of the optimized formulation shows 99.87% release at the end of 12 hours. Using Box-Behnken method of three factors and three rates helped to assess the Relationship between factors and response significantly. The final formula prepared with the predicted one based on expected values and the observed value pretty similar. It can therefore be inferred that prepared Green Tea Extract loaded with Nano gel was a suitable candidate for a topical drug delivery method.

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