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Design of Experiment Approach for Formulation and Development of Nanosponges Loaded Gel of *Chenopodium Album* Leaves Extract

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KEYWORDS

Chenopodium album, Percentage yield, Nanosponge formulations, nanosponge-loaded gel, Design of Experiments (DoE), Hot melt method, Zeta potential, Particle size.

ABSTRACT:

The present study was carried out to design and develop a nanosponge-loaded gel containing Chenopodium album leaves extract using a Design of Experiments (DoE) approach. Initially, phytochemical screening of the extract confirmed the existence of alkaloids, flavonoids, saponins, and tannins. Nanosponge formulations were prepared using the hot melt method and optimized based on various parameters. The optimized nanosponge formulation exhibited a percentage yield in pet. ether 0.87% and ethanol 1.79%, particle size of 352.2 nm, zeta potential of 29.2 mV indicating good stability, and spherical morphology as confirmed by SEM analysis. The formulation showed smooth physical appearance. The optimized nanosponge formulation was further incorporated into a gel base to develop a nanosponge-loaded gel. A number of factors were assessed for the manufactured gel, showing desirable organoleptic properties, good homogenicity, pH of 6.9, viscosity of 5406±0.28 cps (measured by Brookfield viscometer), and spreadability of 12.36 g·cm/sec. In vitro antimicrobial activity conducted via the well diffusion method demonstrated significant zones of inhibition against Staphylococcus aureus and Escherichia coli, confirming the gel's antimicrobial potential. Stability studies performed as per ICH guidelines over a period of three months revealed no significant changes in physical appearance, pH, or viscosity, indicating that the formulation was stable under accelerated conditions. Overall, the study successfully formulated a stable and effective nanosponge-based gel with promising antimicrobial properties from Chenopodium album leaves extract.

1. INTRODUCTION

Novel drug delivery system: It is a novel approach to drug delivery system (the method or process of administrating a pharmaceutical compound to achieve a therapeutic effect in humans or animals) that includes various newer methods of drug delivery like; Oral controlled release, Large-molecule delivery, Taste masking, Transdermal and topical delivery, Oral fast-dispersing dosage forms, Technology for insoluble drugs, Colonspecific delivery, Intranasal delivery/Pulmonary delivery, Vaginal/rectal delivery, Site-specific drug delivery; Targeting is the ability to direct the drug-loaded system to the site of interest- Implants, Liposomes, Microspheres/Microcapsules, Nanotechnology, Cochleates, Transferosomes, Magnetic micro-carriers **(Rai *et al.*, 2016).**

Herbal Plants: Plants with medicinal potentials and their secondary metabolites have been identified and implicated in dishes from the earliest annals of human habitancy; herbal medicine in ancient systems as well as advanced medicine has created one of the most important science bases for security in various lands of the mankind. For many of years, herbal plants have been used for distinct goals. Herbal plants are generally defined as one year gramineous herbs with not any strict contexture **(Srivastava, 2018).**

Nanosponge: The nanosponges are a network of polyester or a three-dimensional scaffold (backbone) that can break down spontaneously. To create nanosponges, these polyesters are combined with a crosslinker in a solution. In this case, the polyester decomposes in the body in a modest manner because it is typically biodegradable. When the nanosponge scaffold disintegrates, the loaded drug molecules are released in an unfavorable way**(Moin*et al.*, 2020).**

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The purpose of this study is to the potential of nanosponge loaded gel formulation of *Chenopodium album* extract for enhancing the bioavailability. Moreover, the results demonstrated that the nanosponge based drug delivery approach could be a valuable tool to improve the therapeutic efficacy of phytochemicals by improving their absorption, and bioavailability via altering their physicochemical and release properties**(Kadian and Rao 2023).**

2. MATERIAL AND METHODS

2.1 Chemicals

Glacial Acetic Acid, Nitroprusside**,** Sodium Hydroxideand Ammonia were obtained from Merck, a reputable supplier of analytical reagents.Sulab provided the Carbopol 940 and Fizmerck provided the concentrated sulfuric acid.Lobachemie provided the Propylene glycol, Methyl paraben, Triethanolamine, while Clorofiltind supplied the concentrated hydrochloric acid, chloroform and 95% alcohol. Himedia supplied the magnesium, and Rankem provided the 1% copper sulfate solution. Narang chemical supplied the Carboxymethyl cellulose.

2.2 Plant collection

The medicinal plant *Chenopodium Album*(300 g) was collected. After cleaning, the plant components (leaves) were dried in the shade at normal temperature for three days.To prevent contamination and deterioration, dried plant components were kept in airtight glass containers in a dry, cool environment.

2.3 Extraction

Chenopodium Album powder was placed in Soxhlet apparatus thimble. Soxhlation was carried out employing petroleum ether as the nonpolar solvent at 60°C. After being dried, the dried plant material (marc) was removed again with methanol solvent. For each solvent, soxhlation was continued until no visible color change was noted in the siphon tube, and extraction was confirmed by the lack of any residual solvent when evaporated. The obtained extracts were evaporated at 40°C in a rotary vacuum evaporator (Buchi type). The dried extract was weighed, and each extract's % yield was computed using the formula that follows:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use**(Asati*et al.*, 2020).**

2.4 Phytochemical investigation

An investigation was carried out to determine the existence or disappearance of several phytoconstituents using thorough qualitative phytochemical analysis. Medical reactions to testing were based on colour intensity or precipitate formation. The following standard methods were followed

2.5 Quantitative Phytochemical Estimation

2.5.1 Total Phenolic Content (TPC): A few drops of a 5% ferric chloride solution were added to the Chenopodium Album extract in order to check for tannins and phenolic compounds. The development of a green or blue-black hue suggested the existence of phenolic and tanninic chemicals. The *Chenopodium Album* plant extract was treated with a 10% lead acetate solution. The presence of tannins was verified by the development of a white or yellow precipitate. To detect tannins, 2ml of 1% gelatin solution containing 10% sodium chloride was added to the *Chenopodium Album* extract. The development of a white precipitate indicated the presence of tannins **(Lone *et al.*, 2017).**

2.5.2 Total Flavonoid Content (TFC): To test for flavonoids in 1 mL of *Chenopodium Album* extract, a small amount of the extract was dissolved in ethanol, and a few magnesium turnings were introduced, and then droplets of strong hydrochloric acid. Flavonoids were indicated by the appearance of a pink, red, or orange tint**(Arora and Itankar 2018).**

2.6 Formulation of β-cyclodextrin Nanosponges

Beta-cyclodextrin nanosponges were prepared by ‘hot melt method’. Various ratios comprising Diphenyl carbonate (DPC cross-linker) and Beta- cyclodextrin (β-CD polymer) (Ratios of DPC and β-CD are given in table no.4) were chosen for the nanosponges preparation. For one to five hours, the finely homogenized anhydrous polymer (β-CD) and cross linker (DPC) were heated gradually to 90 to 100 oC while being stirred by a magnetic device. The substrate mixture (β-CD and DMC) was allowed to react for 1 to 5 h so as to ensure completion of cross linking reaction amongst them; creating nanosponges as a result. The one that obtained reaction mixture was subsequently cooled at ambient temperature. The solid thus obtained was washed

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repeatedly using double distilled water (to remove unreacted β-CD). Finally, the placebo nanosponges obtained were dried (at 40 ºC) and stored in desiccator, till further use**(Kumar *et al.*, 2018).**

2.7 Loading of extract in nanosponges

Extract was loaded in prepared nanosponges by using a freeze-drying technique. Placebo NS (1 gm) was distributed with a magnetic stirrer in 50 milliliters of double-distilled water. Chenopodium album extract (300 mg) was added to above dispersion. The obtained dispersion was then, sonicated (for 10 minutes), and subsequently kept for 1 to 3 hours (under stirring). To separate the un-entrapped extract, which was a residue underneath the colloidal supernatant, the resultant suspensions were centrifuged for ten minutes. The supernatant was then lyophilized at -81°C under a pressure of 0.0010 mbar. The NS powder loaded with dried extract was kept for later usage**(Dhakar*et al.*, 2019).**

2.7.1 Composition of Nanosponges formulation

Table 1: Composition of Nanosponges formulation

S. No	Beta-cyclodextrin-Polymer (mg) X1	Diphenyl carbonate-Cross linker (DPC) (mg) X2	Stirring time (hrs) X3	Extract (mg)	Temperature (ºC)
1	125	100	6	300	90 to 100
2	125	300	6	300	90 to 100
3	200	100	3.5	300	90 to 100
4	200	200	6	300	90 to 100
5	200	200	1	300	90 to 100
6	50	200	1	300	90 to 100
7	50	200	6	300	90 to 100
8	125	300	1	300	90 to 100
9	125	100	1	300	90 to 100
10	200	300	3.5	300	90 to 100
11	50	300	3.5	300	90 to 100
12	125	200	3.5	300	90 to 100
13	50	100	3.5		

2.8 Design of experiment

Design of the experiment to formulate Nano sponges was performed by Design Expert (Version 12.0.1.0) software. The quadratic response surfaces were represented by the second-order polynomial model.

2.8.1 Independent and Dependent variables

Table 2: Independent and Dependent variables

Independent variables	Dependent variables
(X1) Polymer (mg)	(Y1) Particle size (nm)
(X2) Cross linker (mg)	(Y2) Zeta potential (mV)
(X3) Stirring time (hrs)	

2.8.2 Values of variables

Table 3: Values of variables

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Polymer	mg	Numeric	50.00	200.00	-1 ↔ 50.00	+1 ↔ 200.00	125.00	61.24

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B	Crosslinker	mg	Numeric	100.00	300.00	-1 ↔ 100.00	+1 ↔ 300.00	200.00	81.65
C	Stirring time	hrs	Numeric	1.0000	6.00	-1 ↔ 1.00	+1 ↔ 6.00	3.50	2.04

2.9 Characterization of Nanosponges

2.9.1 Particle size

A Malvern Zeta sizer (Malvern Instruments) was used to measure the nanosponge's size. At 25°C, the dispersions were mixed with Millipore filtered water to achieve the desired scattering intensity prior to being put in a disposable sized cuvette **(Khosla *et al.*, 2024).**

2.9.3 Zeta potential

Using a Malvern zeta sizer, zeta potential calculates the surface charge of prepared nanosponges. The difference in potential between two fluid layers (the immobile layer and the dispersion medium) that are locked up with scattered particles is known as the zeta potential. The main indicator of the colloidal dispersion's stability is the zeta potential. The stability of a colloidal dispersion increases with its zeta potential value. Samples to be analyzed were suitably dispersed in double distilled water a clear disposable zeta cell**(Samari-Kermaniiet al., 2021).**

2.9.4 Scanning Electron Microscopic (SEM)

SEM images of the optimized Nanosponges formulation were taken by scanning electron microscope. A concentrated aqueous sample was spread over a slide and dried under vacuum. Surface topography was captured by the machine operated at 15 kV acceleration voltages. The sample was shadowed in a cathode evaporator with a gold layer of 20 nm thick.

2.10 Formulation of Nanosponges loaded Gel

Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. To create a stiff gel, 50 milliliters of warm water (B) was combined with carboxymethyl cellulose and methyl paraben in a different container and constantly agitated. Stirring continuously, mixtures A and B were combined. The pH was then balanced by adding triethanolamine dropwise, and the dispersion was then mixed with optimized-formulation nanosponges to create gel. Propylene glycol, a permeability enhancer, was introduced at this point. Agitating the final dispersion produced a lump-free, smooth gel (**Nagaich and Gulati 2016).**

2.10.1 Composition of gel formulation

Table 4: Composition of gel formulation

S. No	Excipients	Quantity (gm)
1.	Carbopol 934	1.00 gm
2.	Carboxymethyl cellulose	1.00 gm
3.	Propylene glycol	0.5 ml
4.	Methyl paraben	0.2 ml
5.	Nanosponges	1.0 gm
6.	Tri-ethanolamine	q.s
7.	Water	100 ml

2.11 Characterization of Nanosponges loaded Gel

2.11.1 Physical appearance: The prepared gel formulation's appearance, color, and odor were assessed

2.11.2 Determination of pH: The formulation's pH was measured with a digital pH meter (EI).

2.11.3 Viscosity: Using a Brookfield viscometer with spindle number 61 at 100 rpm and 250C, the viscosity of the gel compositions was assessed

2.11.4 Spreadability:A topical gel that is placed or rubbed onto the skin's surface should have a high enough spreading coefficient. The formula used to determine spreadability was as follows:

S= M*L/T

2.12 Anti-microbial activity

2.12.1 Preparation of Nutrient Agar Media:2.8 g of Nutrient Media was dissolved in one hundred milliliters of purified water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121ºC at 15 lbs pressure for 15 min. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

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2.12.2 Preparation of nanosponges gel dilutions: Weigh out 2mg, 4mg, and 8mg of the nanosponges gel separately. Transfer each weighed amount into three separate clean and dry test tubes. To each test tube, add 1mL of double-distilled water. Mix thoroughly until the nanosponges gel was completely dispersed or dissolved in the water. If required, gently agitate the solution with stirring or vortexing to guarantee that the gel of the nanosponges is evenly distributed. Label each test tube appropriately based on the respective concentration for subsequent use.

2.12.3 Culture inoculation and well preparation: : Using a sterile cork-borer, create four wells in both agar plates. Lawn culture of bacteria *E. coli* and *S. aureus* were spread on the Nutrient Agar Media using spreader. Then, add the extract- loaded gel in a different concentration, into the wells. The incubation time for the plate is 37 °C temperature for 24 hours. After incubation, the zone of inhibition around each well is measured. A larger zone indicates stronger antimicrobial activity, while the absence of a zone suggests no antimicrobial effect.

2.13Stability studies: The gel formulation filled with nano sponges was packed, put in the stability test chamber, and then tested for stability at accelerated temperatures (250C±20C and 60±5% RH) and 400C±20C and 70±5% RH) for three months. The wording was checked for evaluation parameter viscosity, and pH studies at the interval of 30, 45, 60, 90 days (3 month) months. The mixture was examined for stability under accelerated storage conditions for three months in accordance with the International Conference on Harmonization (ICH) criteria. Formulation was investigated for changes in evaluation parameter studies of pH and viscosity. Every result was contrasted against final formulation of 0 days as the reference**(Mahmood *et al.*, 2023).**

3. RESULT AND DISCUSSION

3.1 Percentage Yield

Table 5: Percentage Yield of crude extracts of *Chenopodium Album*extract

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Chenopodium Album*	Pet ether	300	3.42	0.87%
2		Ethanol	278	5.75	1.79%

3.2 Preliminary Phytochemical study

Table 6: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Ethanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	Glycoside		
2.1	Borntrager test	Present	Present
2.2	Killer-Killiani test	Present	Present
3.	Carbohydrates		
3.1	Molish's test	Present	Present
3.2	Fehling's test	Present	Present
3.3	Benedict's test	Present	Present
3.4	Barfoed's test	Absent	Present
3.5	Iodine Test	Absent	Present

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4.	Flavonoids		
4.1	Shinoda Test	Absent	Present
5.	Tannin and Phenolic Compounds		
5.1	Ferric Chloride test	Present	Present
5.2	Lead Acetate Test	Absent	Present
5.3	Gelatin Test	Absent	Present
6.	Saponin		
6.1	Foam test	Present	Present
6.2	Froth Test	Present	Present
7.	Test for Triterpenoids and Steroids		
7.1	Salkowski's test	Present	**Absent**
7.2	Libbermann-Burchard's test	Present	**Absent**

3.3.1 Build Information

Table 7: Build information of DOE software

File Version	**12.0.1.0**		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	13
Design Model	Linear and 2FI	**Blocks**	No Blocks
Build Time (m)	46.00		

3.3.2 Independent variables

Table 8: Independent variables

S. No.	Coding	Variables
1.	X1	A: Beta- cyclodextrin- Polymer (mg)
2.	X2	B:Diphenyl carbonate-Cross linker (DPC) (mg)
3.	X3	C:Stirring time (hrs)

3.3.2 Dependent variables

Table 9: Dependent variables

S. No	Coding	Variables
1.	Y1	Particle size (nm)
2.	Y2	Zeta Potential (mV)

3.3.3 Formulation trials as per Box–Behnken design

Table 10: Formulation trials

S. No	Factor 1 Beta-cyclodextrin-Polymer (mg) X1	Factor 2 Diphenyl carbonate-Cross linker (DPC) (mg) X2	Factor 3 Stirring time (hrs) X3	Extract (mg)	Temperature (°C)	Response 1 Particle size (nm)	Response 2 Zeta Potential (mV)
1	125	100	6	300	90 to 100	256.3	28.6
2	125	300	6	300	90 to 100	190.5	31.9
3	200	100	3.5	300	90 to 100	486.9	24.6
4	200	200	6	300	90 to 100	322.8	30.1

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5	200	200	1	300	90 to 100	762.5	29.8
6	50	200	1	300	90 to 100	701.6	27.4
7	50	200	6	300	90 to 100	253.6	32.6
8	125	300	1	300	90 to 100	844.6	25.7
9	125	100	1	300	90 to 100	689.1	29.8
10	200	300	3.5	300	90 to 100	552.3	31.8
11	50	300	3.5	300	90 to 100	410.5	28.2
12	125	200	3.5	300	90 to 100	364.1	27.5
13	50	100	3.5		90 to 100	400.7	30.8

3.3.4 Limits of Variables (Constraints)

Table 11: Variables operating range for Nano sponge's formulation

Name	Goal	Lower Limit	Upper Limit	Importance
A:Polymer	is in range	50	200	3
B:Crosslinker	is in range	100	300	3
C:Stirring time	is in range	1	6	3
Particle size	none	190.5	844.6	3
Zeta potential	none	24.6	32.6	3

3.3.5 Fit Summary

Table 12: Response 1: Particle size

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9061**	**0.9698**	**Suggested**
2FI	0.4446	0.9069	0.8481	
Quadratic	0.1886	0.9546		
Cubic				Aliased

3.4 Effect of formulation variables on Particle size (ANOVA for linear model)

3.4.1 Response 1: Particle size

Table 13: Response 1: Particle size (ANOVA for Linear model)

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	5.068E+05	1.689E+05	39.59	< 0.0001	significant
A-Polymer	16029.45	16029.45	3.76	0.0846	
B-Crosslinker	3399.00	3399.00	0.7965	0.3954	
C-Stirring time	4.874E+05	4.874E+05	114.21	< 0.0001	
Residual	38405.31	4267.26			
Cor Total	5.452E+05				

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Coefficients in Terms of Coded Factors

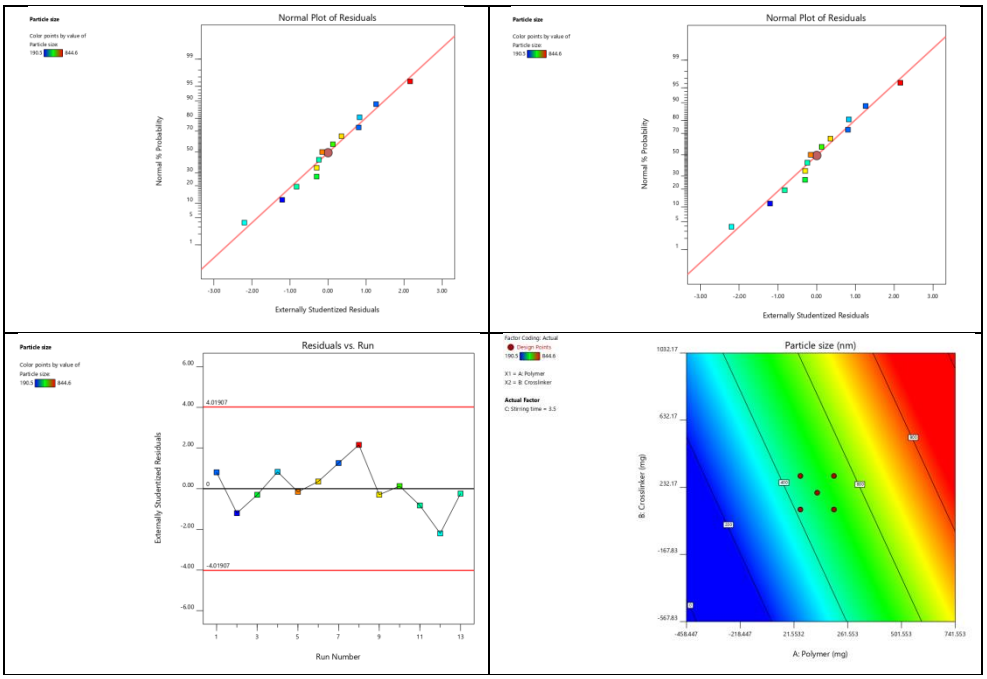

Figure 1: Three-dimensional response surface plots (normal plot vs residual, residual vs predicted and residual vs. run) and contour plots revealing relative effects of independent variables (*A*: polymer concentration; *B*: cross-linker concentration and *C*: stirring time) on dependent variable – particle size of extract loaded nanosponges formulation.

3.4.2 Predicted value and actual value of all formulations

Table 14: Predicted value and actual value of all formulations

S. No	Formulation code	Actual Value of particle	Predicted Value of particle size
1	NDDS 1	256.30	212.22
2	NDDS 2	190.50	253.44
3	NDDS 3	486.90	503.80
4	NDDS 4	322.80	277.59
5	NDDS 5	762.50	771.24
6	NDDS 6	701.60	681.72
7	NDDS 7	253.60	188.07
8	NDDS 8	844.60	747.09
9	NDDS 9	689.10	705.87
10	NDDS 10	552.30	545.03
11	NDDS 11	410.50	455.50
12	NDDS 12	364.10	479.65
13	NDDS13	400.70	414.28

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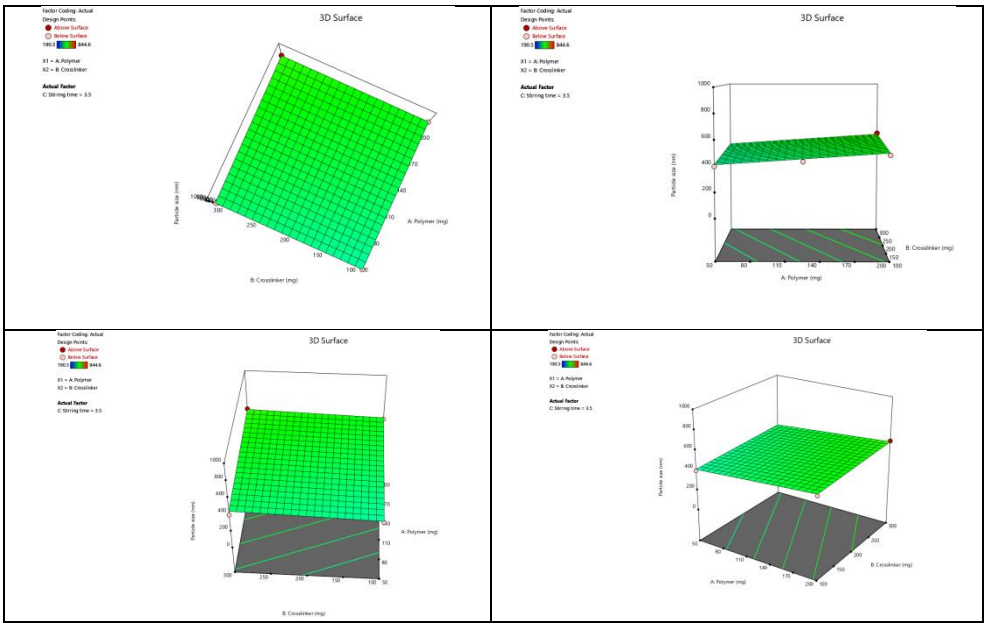

Figure 2: Response surface plot showing combined effect of polymer (beta-cyclodextrin) and cross linker (DPC) on particle size of nanosponges formulations

3.4.3 Effect of formulation variables on zeta potential of nanosponges formulation

Table 15: Response 2: Zeta potential (Fit Summary)

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	0.4714	-0.0215	-0.6518	
2FI	**0.0142**	**0.7066**	**0.3483**	**Suggested**
Quadratic	0.4120	0.7473		
Cubic				Aliased

7.5 ANOVA for 2FI model of zeta potential

7.5.1 Response 2: Zeta potential (ANOVA 2FI model)

Table 16: Response 2: Zeta potential (ANOVA 2FI model)

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	60.20	10.03	5.82	0.0250	significant
A-Polymer	0.9113	0.9113	0.5282	0.4947	
B-Crosslinker	1.80	1.80	1.05	0.3458	
C-Stirring time	13.78	13.78	7.99	0.0301	
AB	24.01	24.01	13.92	0.0097	
AC	6.00	6.00	3.48	0.1114	
BC	13.69	13.69	7.94	0.0305	
Residual	10.35	1.73			

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Cor Total	70.55				

Table 17: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	29.14	0.3643	28.25	30.03	
A-Polymer	-0.3375	0.4644	-1.47	0.7988	1.0000
B-Crosslinker	0.4750	0.4644	-0.6613	1.61	1.0000
C-Stirring time	1.31	0.4644	0.1762	2.45	1.0000
AB	2.45	0.6567	0.8431	4.06	1.0000
AC	-1.23	0.6567	-2.83	0.3819	1.0000
BC	1.85	0.6567	0.2431	3.46	1.0000

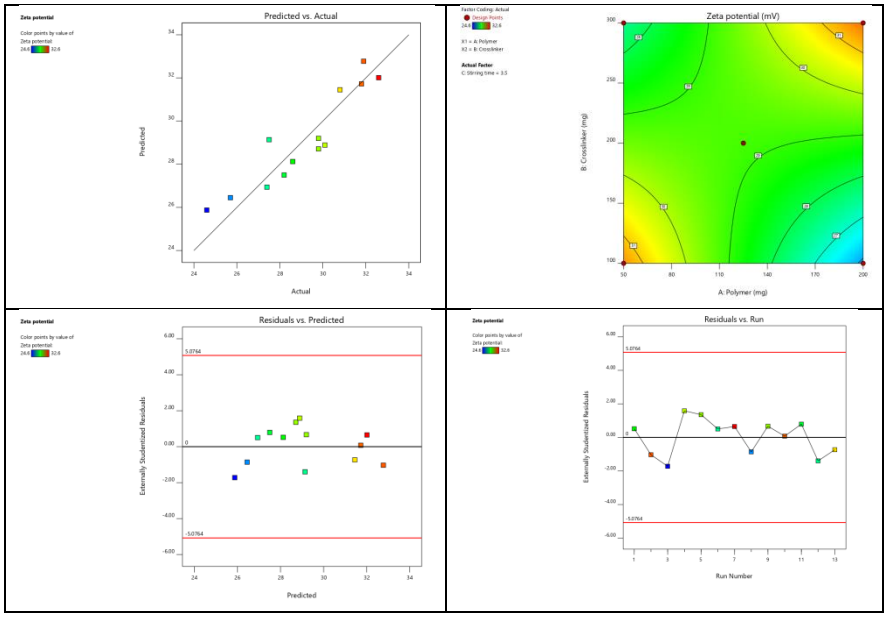

Figure3: Three-dimensional response surface plots (Predicted vs Actual, residual vs predicted and residual vs. run) and contour plots revealing relative effects of independent variables (*A*: polymer concentration; *B*: cross-linker concentration and *C*: stirring time) on dependent variable – zeta potential of extract loaded nano formulation.

3.5.2 Predicted and actual value of Zeta potential

Table 18: Predicted and actual value of Zeta potential

S. No	Formulation code	Actual Value	Predicted Value
1	NDDS 1	28.60	28.13
2	NDDS 2	31.90	32.78
3	NDDS 3	24.60	25.88
4	NDDS 4	30.10	28.89
5	NDDS 5	29.80	28.71
6	NDDS 6	27.40	26.94
7	NDDS 7	32.60	32.01
8	NDDS 8	25.70	26.45

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9	NDDS 9	29.80	29.20
10	NDDS 10	31.80	31.73
11	NDDS 11	28.20	27.50
12	NDDS 12	27.50	29.14
13	NDDS13	30.80	31.45

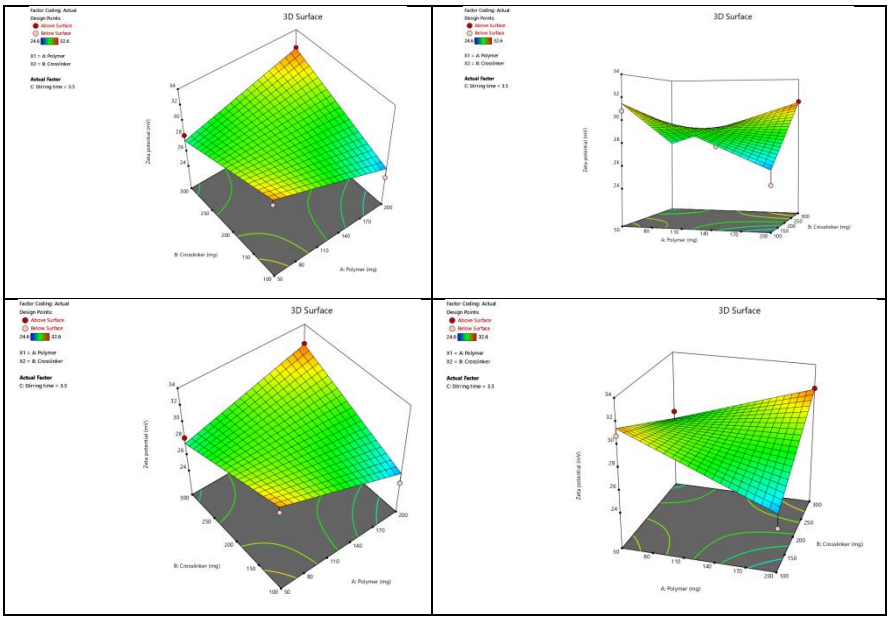

Figure 4: Response surface plot showing combined effect of polymer and cross linker on zeta potential of Nano sponge's formulation

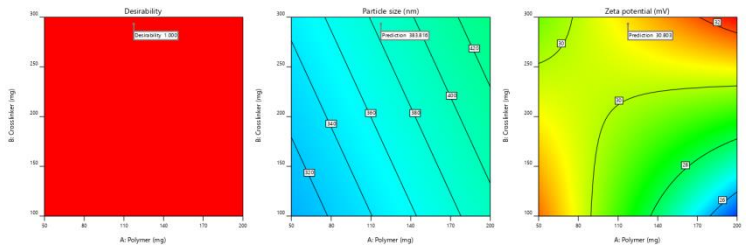

Figure 5: Response surface plot showing prediction data for optimization

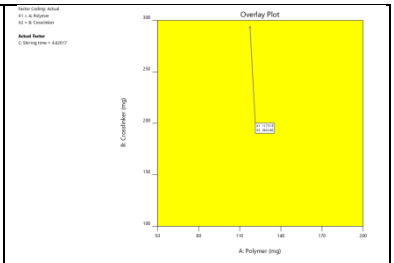

Figure 6: Overlay plot of optimization formulation

3.5.3 Optimized formula of nanosponges formulation (Point Prediction)

Table 19: Optimized formula of nanosponges formulation

S. No	Polymer (mg)	Cross linker (mg)	Stirring time (hrs.)	Particle size (nm)	Zeta potential (mV)	Desirability	
1	177.126	130.932	3.976	449.562	27.244	1.000	
2	**117.515**	**293.262**	**4.620**	**383.816**	**30.803**	**1.000**	**Selected**
3	158.120	159.593	3.731	468.318	28.363	1.000	

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3.6 Characterization of optimized formulation

3.6.1 Particle Size

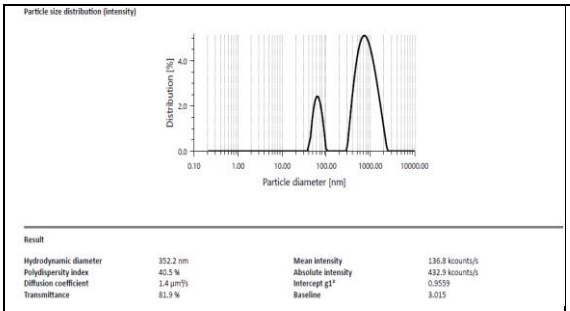


Figure 7: Particle size

Table 20: Particle size

S. No	Formulation	Particle size (Predicted value)	Particle size (Actual value)
1.	Nano sponges	383.816	352.2 nm

3.6.3 Zeta potential

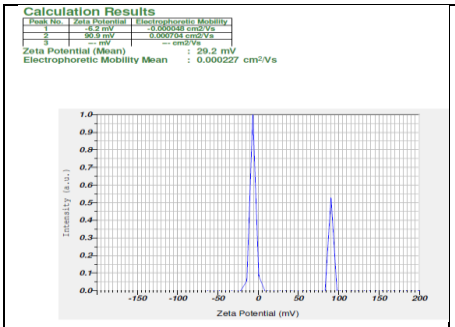
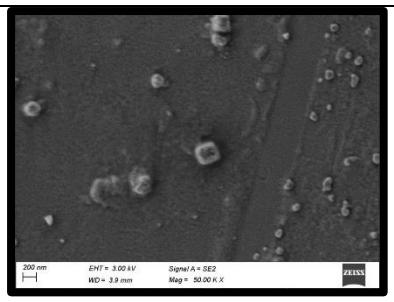


Figure 8: Zeta potential

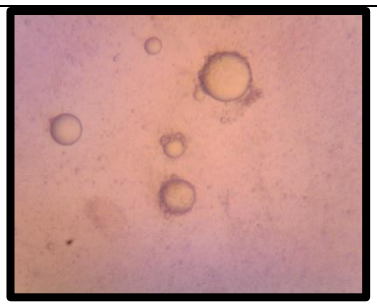
Table 21: Zeta potential

S. No	Formulation	Zeta potential (Predicted result)	Zeta potential (Actual result)
1.	Nanosponges	30.8mV	29.2 mV

3.6.4 Scanning electron microscope (SEM)



SEM Image of Nano sponge formulation



Simple Microscopy image of nanosponges formulation (100x)

Figure 9: Microscopic analysis of the nanosponges in 50 kx and 100×.

3.7 Characterization of Nano sponges loaded gel

3.7.1 Physical appearance

Table 22: Physical appearance

S. No	Parameter	Result
1.	Colour	Yellowish
2.	Odour	Odourless
3.	Appearance	Slightly turbid
4.	Homogeneity	Homogeneous

Table 23: Viscosity

S. No	Formulation	Viscosity (cps)
1.	Nano gel	5406±0.28

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Table 24: pH

S. No.	Formulation	pH
1.	Gel	6.9

Table 25:Spreadability

S. No	Formulation	Spreadability (g.cm/s)
1.	Gel	12.36

3.8 Antimicrobial activity of nanosponges formulation

3.8.1 Antimicrobial activity of Formulation against *E.coli* and *S.aureus*

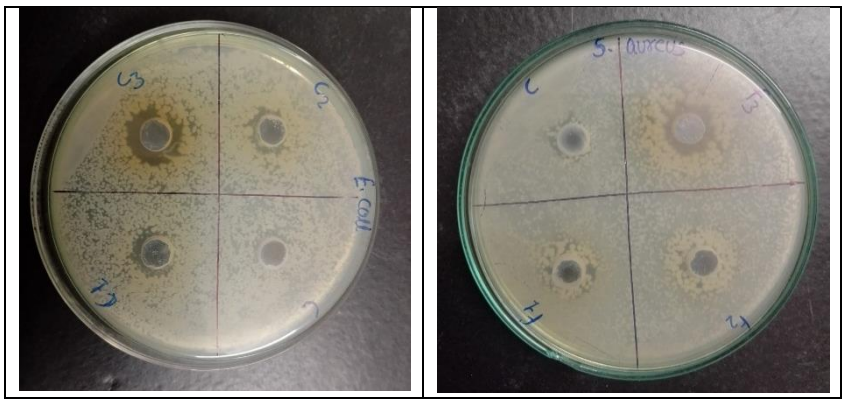

Figure10: Antimicrobial activity against *E.coli* and *S.aureus*

Table 26: Antimicrobial activity of Formulation against *E.coli* and *S.aureus*

S. No.	Sample Name (mg/ml) *E.coli*	Zone of Inhibition (mm) of *E.coli*	Sample Name (mg/ml) *S.aureus*	Zone of Inhibition (mm) of*S.aureus*
1.	C (Control)	0mm	F	0mm
2.	C1 (Extract mg/ml)	4 mm	F1 (Extract mg/ml)	3 mm
3.	C2 (Nanosponges mg/ml)	5 mm	F2 (Nanosponges mg/ml)	6 mm
4.	C3 (Nano gel mg/ml)	10 mm	F3(Nano gel mg/ml)	9 mm

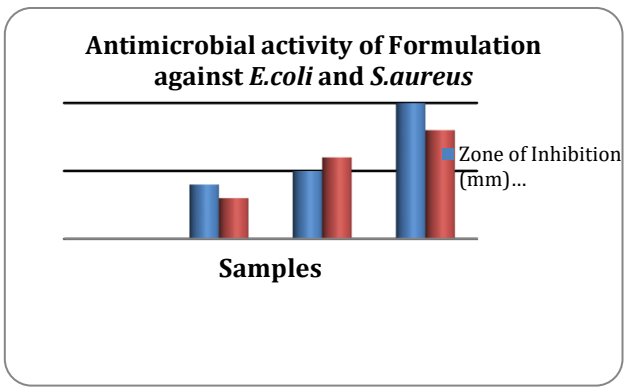

Figure11: Graphical representation of antimicrobial activity

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3.8.2 Stability study

Table 27: Stability Study of optimized formulation (Nanosponges gel)

S. No	Time (Days)	30^0C±2 ^{0}C and 60 ± 5% RH			40^0C±2 ^{0}C and 70 ±5% RH		
		Appearances	Viscosity	pH	Appearances	Viscosity	pH
1.	0	Semi solid	5406	6.9	Semi solid	5406	6.9
2.	30	Semi solid	5411	6.9	Semi solid	5401	6.8
3.	45	Semi solid	5507	6.8	Semi solid	5397	6.6
3.	60	Semi solid	5481	6.9	Semi solid	5399	6.7
4.	90	Semi solid	5419	6.7	Semi solid	5404	6.7

4. CONCLUSION

This work shows for the first time how Chenopodium album leaf extract nanosponges gel formulation can improve the solubility, stability, and bioavailability of herbal-based active ingredients. Also, the outcomes showed that the gel-based drug delivery method based on nanosponges can be a useful instrument to increase therapeutic efficacy.

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