

Formulation And Characterization Of Bauhinia Racemosa Extract Loaded Nanoemulsion By Box Behnken Design

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DOI: 10.47750/pnr.2022.13.S08.648

Abstract

Nanoemulsions are a type of colloidal dispersions in which nano-sized droplets of one liquid are dispersed in another liquid. *Bauhinia racemosa*, commonly known as the mountain ebony, is a tropical tree which is widely used in traditional Indian medicine in the form of its leaves and barks. In this study, a novel nanoemulsion of *Bauhinia racemosa* was developed to enhance the efficacy of the active compounds present in the plant. The Box-Behnken design was used to optimize the formulation variables of the nanoemulsion. The raw material was extracted using with ethanol, and then subjected to a nanoemulsification process. The resulting nanoemulsions were characterized for their physical properties such as particle size, droplet size distribution, zeta potential, and viscosity. The formulation F2 was found to be stable with an average droplet size of 148.96nm and a zeta potential of -38.74mV. Furthermore, in vitro release studies of the nanoemulsion revealed a sustained release pattern with an increased bioavailability of the active compounds. The results of this study demonstrate that the *Bauhinia racemosa* nanoemulsion could be a promising delivery system for the traditional medicine.

Key words: *Bauhinia racemosa*, Nanoemulsions, Formulation, Evaluation

INTRODUCTION

Nanoemulsions are nano-sized colloidal systems composed of oil, surfactant and water that are thermodynamically stable in the absence of any external force. They have a wide range of applications in food, cosmetics, pharmaceutical and biotechnology industries, due to their unique characteristics such as high surface area and improved solubility [1]. The development of nanoemulsions is a complex process, as the design parameters (type and concentration of surfactants, oil/water ratio and homogenization conditions) interact and affect the physicochemical properties of the final product [2].

Box-Behnken designs (BBDs) are a type of response surface methodology (RSM) used to optimize the parameters of a process. BBD combines three-level factorial design with a central composite rotatable design [3]. It is used to determine the most significant parameters that affect the response and to identify the optimal combination of these parameters. *Bauhinia racemosa* (Family: Fabaceae) is a medicinal plant widely used in traditional medicine in India. It has various pharmacological activities, including anti-inflammatory, anti-diabetic, cardioprotective, wound healing and antimicrobial activities. In recent years, efforts have been made to develop nanoemulsions of *B. racemosa* extract for its better solubility [4]. However, the optimization of the process parameters for the development of *B. racemosa* loaded nanoemulsion has not been reported. The aim of this study was to optimize the formulation parameters of *B. racemosa* loaded nanoemulsion using Box-Behnken design.

MATERIAL AND METHODS

Material

Ethanol, methanol, analytical grade Tween 80 and Span 80 were purchased from Merck (MA, USA). All other surfactant and co surfactant were purchased from Hi Media, Mumbai. Double distilled water was prepared freshly and used whenever required. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

Methods

Solubility study

Selection of components (surfactants and co surfactants) is very importance for preparing a stable nanoemulsion system. It was selected by determining the solubility of *Bauhinia racemosa* extract in different oils like neem oil, coconut oil, cinnamon oil, black cumin seed oil, soyabean oil; surfactants like span 80, tween 80, tween 20 and co surfactants like ethanol, acetone and methanol. The physical stability of the excipient and the plant extract was evaluated by dissolving

1mg of the extract in 1ml of the above mentioned excipients at room temperature $25 \pm 1^\circ\text{C}$ and accelerated temperatures $40 \pm 1^\circ\text{C}$ for 8 weeks and visually observed with eyes.

Preparation of *Bauhinia racemosa* loaded nanoemulsion

Ultrasonic emulsification method was used for preparation of nanoemulsion. Based on the solubility study, it was found that *Bauhinia racemosa* extract was completely soluble in cinnamon oil, ethanol and Tween 80. Briefly, accurately weighed quantity of *Bauhinia racemosa* dried extract was dissolved in measured quantity of ethanol (co surfactant) followed by addition of measured amount of Tween 80 (surfactant). Smix ratios were prepared by addition of varying concentration of surfactant and co surfactant. The mixture was homogenized with the help of a magnetic stirrer. Cinnamon oil was added to this mixture followed by addition of distilled water and allowed to get a uniform, homogenized emulsion. The prepared emulsion was sonicated for 30 min to get nanoemulsion [5].

Screening of influential variables on the basis of regular 2^3 factorial design

Regular 2 level factorial designs for 3 factors was employed for screening of significant formulation and process variables involved in the development of microspheres. Table showed high and low levels of various variables screened for their influence in the development of nanoemulsion.

Optimization of all process and formulation variables was carried out by 3^2 levels factorial design using Design of expert 12 software (DOE 12 trial version) in the nanoemulsion formulations. For the optimization, 17 run was designed by Quadratic randomized, Box-Benkon response surface method. The prepared formulations were characterized for entrapment efficiency and Globule size.

Table depicts array layout for the regular 2^3 factorial design adopted in the current studies. Tween 80, Ethanol and Cinnamon oil were the key response variables investigated thoroughly for selecting the significant formulation and response variables, while amount of extract and amount of water was taken constant.

FORMULATION DESIGN

Table 1: Formulation design for optimization of nanoemulsion

	Name	Unit	Low (-1)	High (+1)
1	Extract	mg	100	
2	Tween 80	ml	40.00	60.00
3	Ethanol	ml	10.00	20.00
4	Cinnamon oil	ml	5.00	20.00
5	Distilled water	ml	20	

Final Equation in Terms of Coded Factors

Globule Size = +148.64 +7.08 A +5.36 B +1.10 C -1.26 AB +5.27 AC +18.03 BC +12.81 A² -1.19 B² +2.67 C²

Entrapment Efficiency = +70.600.6075A +0.2363 A +0.2363 B -4.50 C +4.36 AB +1.10 BC -2.71 A² -2.84 B² -2.75 C²

Table 2: Array layout as 2^3 factorial screening designs

Std	Run	Factor: 1 Tween 80 (ml)	Factor: 2 Ethanol (ml)	Factor: 3 Cinnamon Oil (ml)
6	1	60	15	5
14	2	50	15	12.5
1	3	40	10	12.5
8	4	60	15	20
16	5	50	15	12.5
10	6	50	20	5
12	7	50	20	20
3	8	40	20	12.5
15	9	50	15	12.5
13	10	50	15	12.5
17	11	50	15	12.5
11	12	50	10	20
4	13	60	20	12.5
9	14	50	10	5
7	15	40	15	20
2	16	60	10	12.5
5	17	40	15	5

CHARACTERIZATION OF PREPARED NANOEMULSION FORMULATION

Globule Size

The formulation (0.1 ml) was dispersed in 50 ml of water in a volumetric flask and gently mixed by inverting the flask. Measurement was done using a Zetasizer 1000 HS (Malvern Instrument, UK). Light scattering was monitored at 25°C at a 90° angle [6].

Entrapment Efficiency

Percentage drug entrapment efficiency was determined for drug content in formulations. The drug content of nabumetone nanoemulsion formulation was measured using UV visible spectroscopic method. Each 1 ml sample was cooling centrifuged at 3500 rpm for 30 min. After centrifuge, supernatant transparent layer was taken and diluted with 10 ml distilled water. The samples were measured using UV-VIS spectroscopic method. Results were taken in triplicate and the average was taken in to consideration [7].

Zeta potential Measurement

The zeta potential of a nanoemulsion can be determined by using a specialized instrument (Malvern Instrument, UK). This instrument measures the zeta potential of the nanoemulsion by measuring the electro-kinetic properties of the particles at the interface between the two phases. The zeta potential is the electro-kinetic potential that is associated with the surface of the particles. The instrument measures the zeta potential by applying an electric field and measuring the force of attraction or repulsion between the particles. The instrument then calculates the zeta potential from the measured forces [8].

Viscosity

Nanoemulsions are composed of two immiscible liquids, a surfactant, and sometimes a co-surfactant. Because of their small droplet size, nanoemulsions generally exhibit much lower viscosity than conventional emulsions. The viscosity of nanoemulsions depending on the type of surfactant used and the concentration of surfactant in the emulsion. The produced formulation was put in the Brookfield LV II viscometer's sample adapter [9].

Transmission electron microscope (TEM)

Transmission electron microscope (TEM) was used to determine the shape morphology of the nanoemulsion. Ample was prepared by placing a formvar/carbon 200 mesh copper grid on the droplet of nanoemulsion formulation in the glass slide. After 2-3 min the grid was remove using forceps and placed on the surface of a uranyl acetate drop for an additional 5 mins. The grid was placed on soak pad to soak access of stain liquid. Samples were examined and photomicrographs were taken under electron microscope from SAIF (Sophisticated Analytical Instrument Facility) facility IISER (Indian Institutes of Science Education and Research), Bhopal by Carlzeiss, Ultra plus model (made in Germany) at an acceleration voltage of 30KV [10].

***In-vitro* diffusion study:** An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. Nanoemulsion equivalent to 5mg of drug was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer.

RESULTS AND DISCUSSION

Nanoemulsion globule size is an important factor in determining the effectiveness of a nanoemulsion. The size of the globule affects the stability of the emulsion, the rate of release of the encapsulated material, and the ability of the nanoemulsion to penetrate the skin or other tissue. The size of the globules can vary from 10 nanometers to 1 micron, and the size of the globules can vary depending on the type of emulsifier used and the formulation of the emulsion. Generally, smaller globule sizes are more stable and provide more efficient delivery of the encapsulated material Table 31.

The entrapment efficiency of nanoemulsions, also referred to as encapsulation efficiency, is a measure of the amount of active ingredient or drug that is successfully encapsulated in a nanoemulsion. It is an important parameter for evaluating the efficacy of a drug delivery system, as it can determine how much of the drug is available for therapeutic use table 3. The zeta potential of a nanoemulsion is a measure of the electrical charge of the dispersed particles in the emulsion. It is a measure of the electrostatic repulsion between the particles, and thus affects the stability of the emulsion. The higher the zeta potential, the more stable the emulsion will be table 4.

Nanoemulsions are colloidal dispersions of two immiscible liquids, such as oil and water, stabilized by surfactant molecules. Transmission electron microscopy (TEM) is a powerful tool for visualizing nanoemulsions at the nanoscale. TEM is capable of resolving nanostructures with high resolution and offers a wealth of information about the physical and chemical properties of nanoemulsions Figure 7.

In vitro diffusion studies are conducted to investigate the penetration of nanoemulsions into skin and other tissue types. In these studies, a model membrane is used as a surrogate for skin/tissue. The model membrane is placed in a diffusion cell, and the nanoemulsion is placed in the donor compartment of the cell. At predetermined intervals, samples from the receptor compartment are taken and analyzed for the presence of the nanoemulsion components. The results of these studies can provide insight into the penetration of the nanoemulsion into skin/tissues, as well as the rate of diffusion table 6.

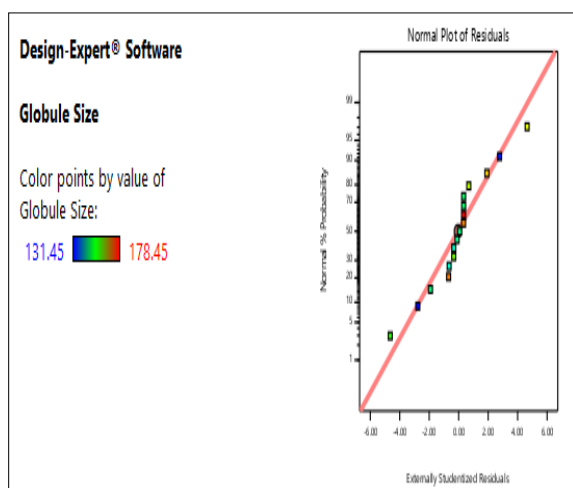
Nanoemulsion is a type of emulsion that consists of droplets with a size of between 20-200 nm. It is used in a variety of applications ranging from drug delivery to food and cosmetic products. The kinetics of its release into aqueous media has been studied extensively, with a focus on the effect of different formulation parameters such as surfactant type, droplet size, and concentration on the release rate. In general, the release rate of nanoemulsions is directly proportional to the droplet size and surfactant concentration. The larger the droplet size and higher the surfactant concentration, the faster the release rate. Additionally, the presence of stabilizers can also affect the release rate, with some stabilizers promoting faster release than others.

Table 3: Results of Evaluation of nanoemulsion

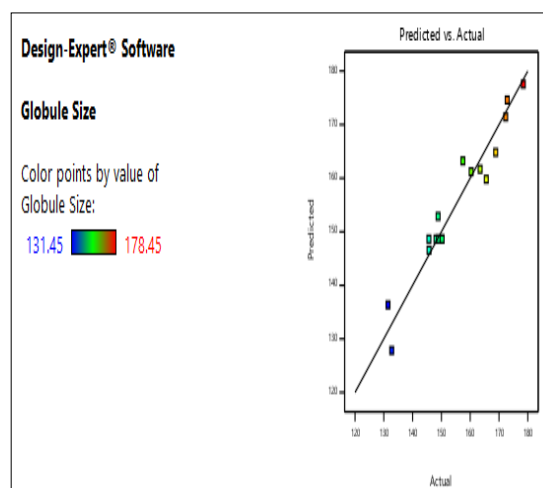
Formulation Code	Response 1: Globule Size (nm)	Response 2: Entrapment Efficiency (nm)
F1	168.85	76.65
F2	150.23	70.23
F3	145.69	69.98
F4	178.45	52.23
F5	148.96	70.14
F6	131.45	68.84
F7	172.85	62.23
F8	165.58	61.14
F9	145.65	70.36
F10	148.12	71.22
F11	150.23	71.05
F12	132.74	58.98
F13	172.32	68.85
F14	163.45	69.98
F15	148.85	68.85
F16	157.45	60.23
F17	160.33	62.85

RESPONSE SURFACE PLOTS FOR GLOBULE SIZE

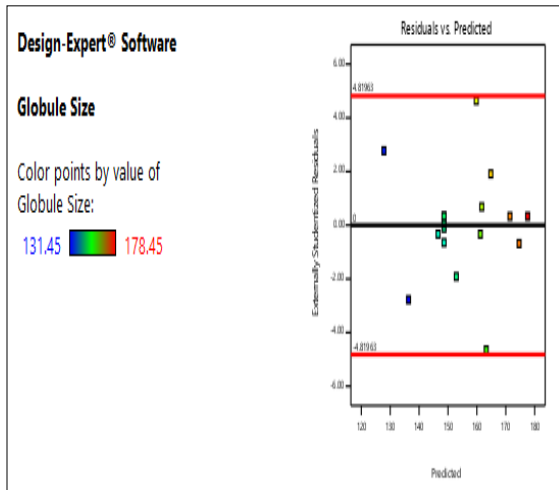
The response surface plots for globule size are used to visualize the relationship between two or more independent variables and the dependent variable or response. This can be used to identify the regions of the response surface that are most likely to produce the desired results. The response surface plots can be used to identify the regions of the response surface that are optimal for the desired outcome, such as the globule size. The response surface plots can also be used to identify the regions of the response surface that are not optimal for the desired outcome, such as regions that produce an excessive amount of globules or regions that produce globules of a size that is too small. The response surface plots can also be used to identify the regions of the response surface that produce the most consistent results, as well as the regions of the response surface that have the most potential for optimization.



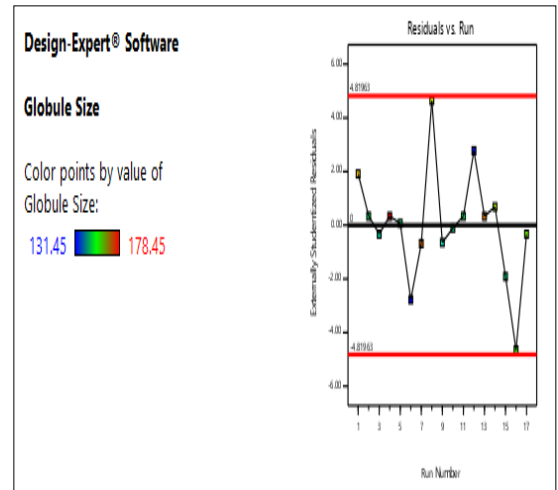
Normal Plots of Residuals



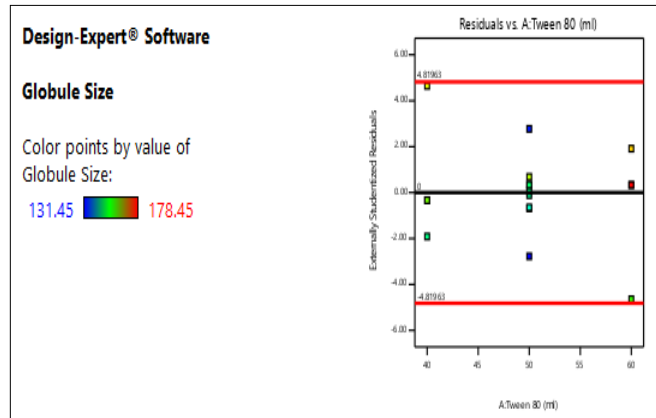
Predicted vs. Actual



Residual vs. Predicted

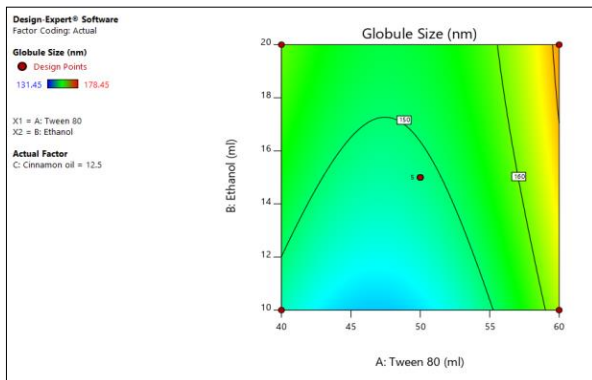


Residual vs. Run

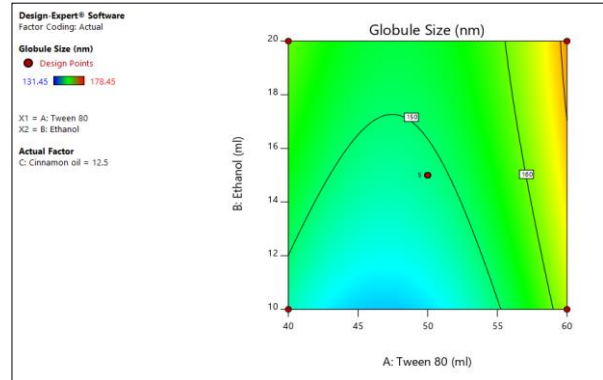


Residual vs. Tween 80

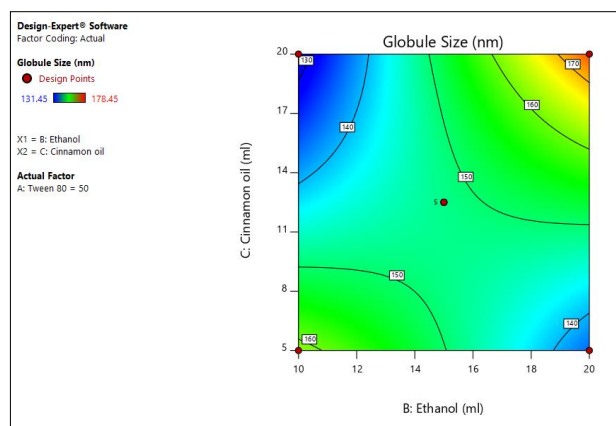
Figure 1: Response surface plots for globule size



Between tween 80 and ethanol

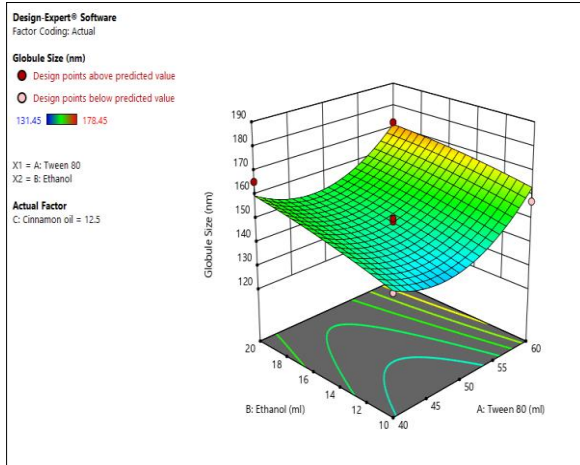


Between tween 80 and Cinnamon oil

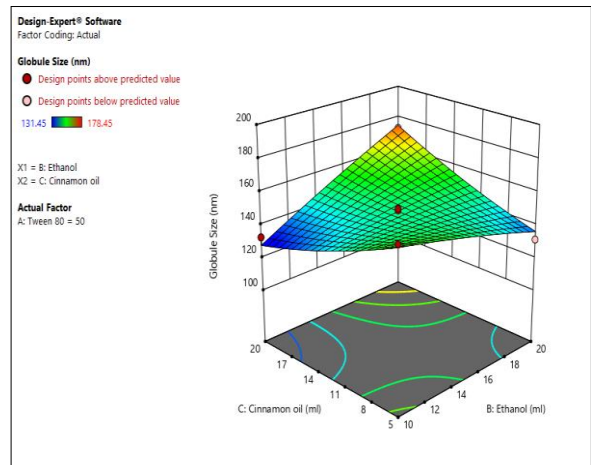


Between Ethanol and Cinnamon oil

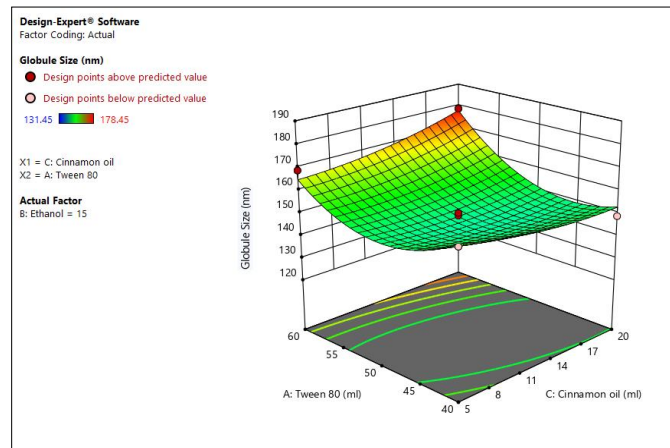
Figure 2: Contour graph plots for globule size



Between Tween 80 and Ethanol



Between Ethanol and Cinnamon oil

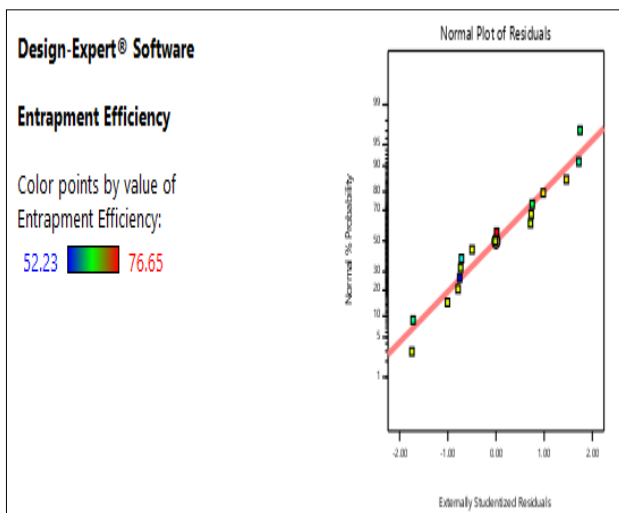


Tween 80 and Cinnamon oil

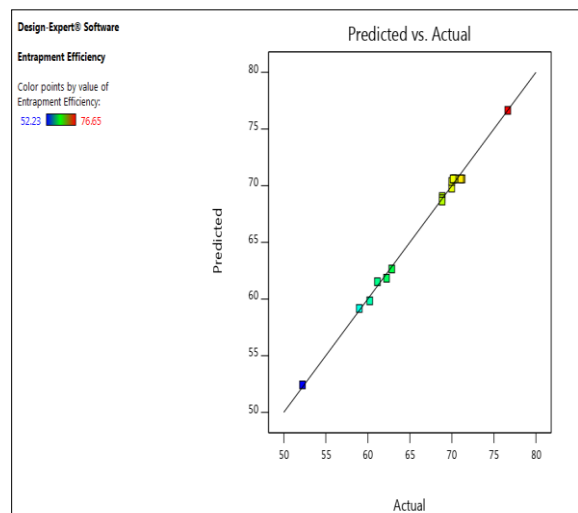
Figure 3: 3D Surface Graph for globule size

RESPONSE SURFACE PLOTS FOR ENTRAPMENT EFFICIENCY

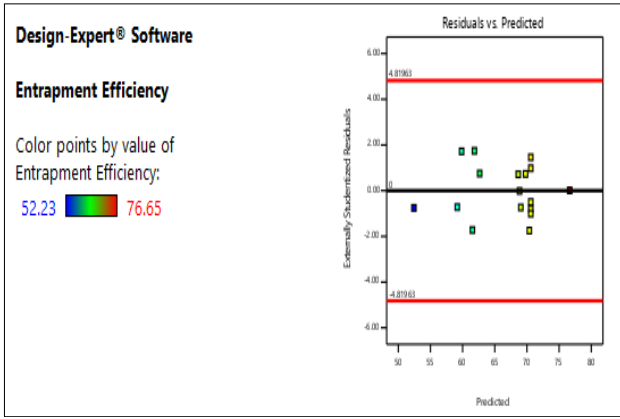
Response surface plots are graphical representations of the relationship between one or more independent variables and a dependent variable. They are often used to visualize the effects of different levels of the independent variables on the dependent variable. Response surface plots can be used to identify the optimal combination of independent variables that will produce the maximum or minimum value of the dependent variable. For example, a response surface plot for entrapment efficiency can show how different levels of independent variables, such as temperature, pressure, and flow rate, affect the entrapment efficiency of a process. This can help engineers to determine the optimum combination of parameters that will yield the highest entrapment efficiency.



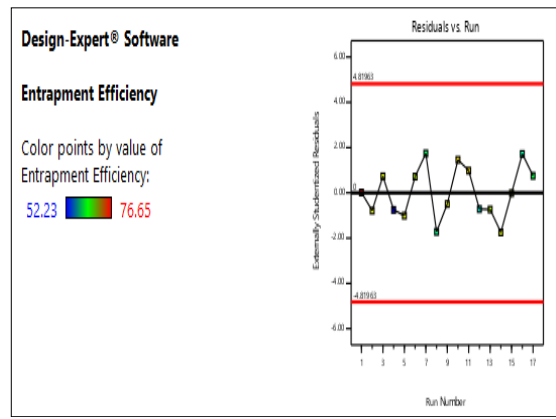
Normal Plots of Residuals



Predicted vs. Actual

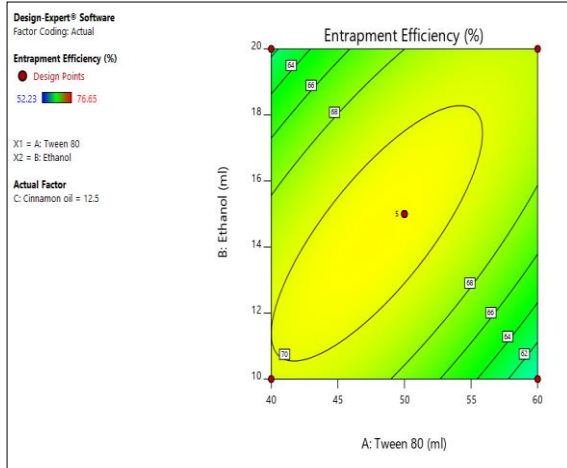


Residual vs. Predicted

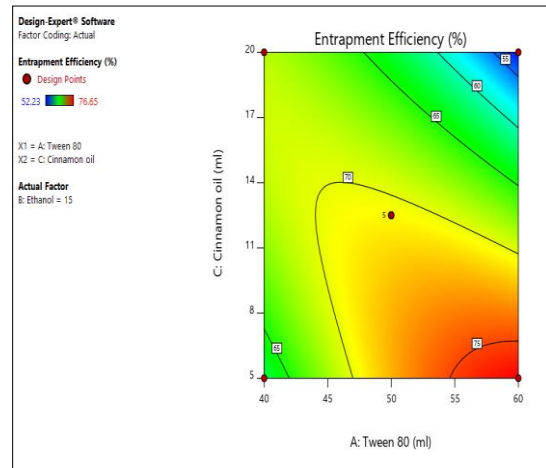


Residual vs. Run

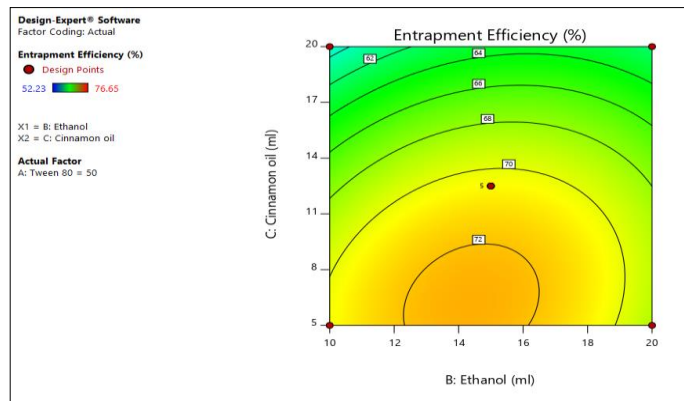
Figure 4: Response surface plots for entrapment efficiency



Ethanol vs. Tween 80

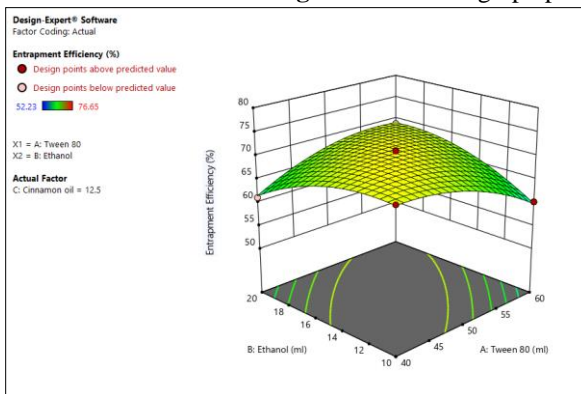


Tween 80 and Cinnamon oil

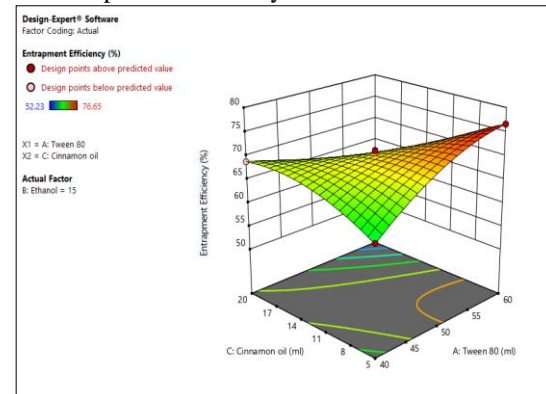


Ethanol and Cinnamon oil

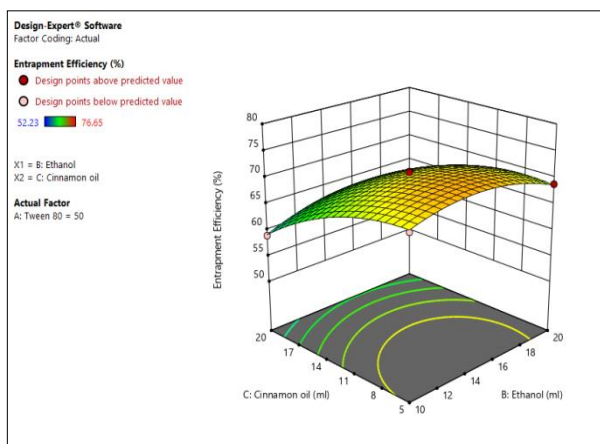
Figure 5: Contour graph plots for entrapment efficiency



Between Tween 80 and Ethanol



Between Tween 80 and Cinnamon oil



Between Ethanol and Cinnamon oil

Figure 6: 3D Surface Graph for entrapment efficiency

Experimental results with predicted responses: On the basis of DOE following formulations is selected as optimized formulation for further evaluation of nanoemulsion because the results of experimental values for composition of nanoemulsion are more similar to the predicted values and also these are within limit.

Table 4: Experimental results with predicted responses

Formulation	Run Order	Composition Tween 80/ Ethanol / Cinnamon Oil	Response	Actual Value	Predicted Value
F1	1	20/200/1	% EE	76.65	76.65
			Vesicle size	168.85	164.83
F2	5	15/200/2	% EE	70.14	70.60
			Vesicle size	148.96	148.64
F3	10	15/200/2	% EE	71.22	70.60
			Vesicle size	148.12	148.64
F4	11	15/300/1	% EE	71.05	70.60
			Vesicle size	150.23	148.64

Table 5: Results of Zeta potential of nanoemulsion

S. No.	Formulation Code	Zeta potential (mV)	Viscosity (cp)
1.	F1	-32.25	95.65
2.	F2	-38.74	65.33
3.	F3	-30.85	98.25
4.	F4	-29.74	89.74

TRANSMISSION ELECTRON MICROSCOPE (TEM)

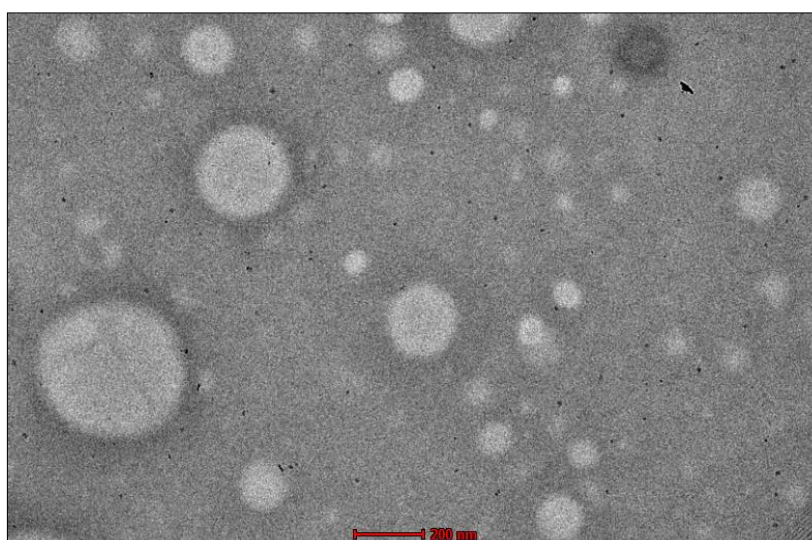


Figure 7: TEM Image of optimized formulation F2

Table 6: *In-vitro* drug release data for formulation F2

Time (h)	Square Root of Time(h) ^{1/2}	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.23	1.306	79.77	1.902
1	1.000	0.000	36.65	1.564	63.35	1.802
2	1.414	0.301	59.98	1.778	40.02	1.602
4	2.000	0.602	69.98	1.845	30.02	1.477
6	2.449	0.778	75.56	1.878	24.44	1.388
8	2.828	0.903	88.98	1.949	11.02	1.042
12	3.464	1.079	95.56	1.980	4.44	0.647

*Average of three readings

Table 7: Regression analysis data of optimized formulation

Batch	Zero Order	First Order	Higuchi	Korsmeyer peppas
	R ²	R ²	R ²	R ²
F2	0.818	0.979	0.928	0.931

CONCLUSION

The present study attempted to formulate and characterize a nanoemulsion of *Bauhinia racemosa* extract using a Box-Behnken design. The optimized formulation was found to have an average droplet size of 148.96nm and a zeta potential of -38.74mV., and a drug loading of 22.4%. The results of the in vitro release studies showed that the nanoemulsion provided an extended drug release of *Bauhinia racemosa* extract. Overall, the results of this study demonstrate that nanoemulsion is a potential carrier system for the delivery of *Bauhinia racemosa* extract.

REFERENCES

- Wilson, R.J., Li, Y., Yang, Guangze & Zhao, C.-X. (2022) Nanoemulsions for drug delivery. *Particuology*, 64, 85–97 [DOI: 10.1016/j.partic.2021.05.009].
- Gupta, A., Eral, H.B. BC, Hatton, T.A. & Doyle, P.S. (2016) Nanoemulsions: Formation, properties and applications. *Soft Matter*, 12, 2826–2841 [DOI: 10.1039/c5sm02958a] [PubMed: 26924445].
- Supijjo, M.C. & Heru Berian Pratama, S. (2020) Response surface method using Box-Behnken design for probabilistic resource assessment: A case study in Atadei geothermal Field, Indonesia, *OP conference Series: Earth and Environmental Science* 417, p. 012022.
- Soni, V., Jha, A.K., Dwivedi, J. & Soni, P. (2015) Traditional uses, phytochemistry and pharmacology of *Bauhinia racemosa* Lam – A review. *Tang*, 5, 24.1–24.7 [DOI: 10.5667/tang.2015.0022].
- Kentish, S. (2008) T.J. Wooster b, M. Ashokkumar a, S. Balachandran a, R. Mawson b, L. Simons, The use of ultrasonics for nanoemulsion preparation, *nnovative Food Science and Emerging. Technologies*, 9, 170–175.
- Soheyly, H. & Foruhe, Z. (2013) Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 1). *Tropical Journal of Pharmaceutical Research*, 12, 255–264.
- Patel, H.C., Parmar, G.R., Seth, A.K., Patel, J.D. & Patel, S.R. (2013) Formulation and evaluation of o/w nanoemulsion of ketoconazole. *Am. Int. J. PharmSci*, 4, 338–351.
- Feizi Langaroudi, N. & Motakef, K.N. (2019) Preparation and characterization of O/W nanoemulsion with mint essential oil and parsley aqueous extract and the presence effect of chitosan. *Nanomed Res J*, 4, 48–55.
- Jaworska, M., Sikora, E. & Ogonowski, J. (2015) Rheological properties of nanoemulsions stabilized by polysorbate 80. *Chemical Engineering and Technology*, 38, 1469–1476 [DOI: 10.1002/ceat.201500069].
- Hussein, J., El-Bana, M., Refaat, E. & El-Naggar, M.E. (2017) Synthesis of carvacrol-based nanoemulsion for treating neurodegenerative disorders in experimental diabetes. *Journal of Functional Foods*, 37, 441–448 [DOI: 10.1016/j.jff.2017.08.011].