RESEARCH PAPER

FORMULATION AND OPTIMIZATION OF *CALOTROPIS PROCERA* EXTRACT-LOADED NANOSPONGES USING A 3² FACTORIAL DESIGN FOR ENHANCED TOPICAL DRUG DELIVERY

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ABSTRACT

Objective: The goal of this research was to employ a 3² full factorial design to develop and optimize *Calotropis procera* extract-loaded nanosponges (CPNSs) as a topical drug delivery system. To develop an optimal formulation, the impact of significant formulation factors on the physical and chemical characteristics of the nanosponges was systematically evaluated.

Methods: By using polyvinyl alcohol as the stabilizing agent and ethyl cellulose as the polymeric carrier, CPNSs were developed using the emulsion solvent diffusion method. The effects of polymer concentration and stirring rate on particle size, entrapment efficiency, and production yield were examined using a 3² full factorial design. In order to determine its physical and chemical properties, the optimized formulation was further investigated using dynamic light scattering (DLS), zeta potential measurement, scanning electron microscopy (SEM), and X-ray diffraction (XRD).

Results: The optimized formulation demonstrated good colloidal stability with a mean particle size of 380 ± 97.83 nm, an entrapment efficiency of 93%, an 83% production yield, and a zeta potential of −34.1 ± 9.55 mV. XRD results indicate a decrease in crystallinity, showing the extract's effective incorporation into the polymeric matrix, while SEM images showed clearly defined spherical nanosponges with a porous surface. While stirring rate mostly affected particle size, statistical analysis showed that polymer concentration had a major impact on entrapment efficiency and production yield.

Conclusion: The optimized CPNS formulation demonstrated appropriate physical and chemical characteristics, a high drug-loading capacity, and good stability, indicating its potential as a nano-scale carrier for the topical delivery of *Calotropis procera* extract and enhancing its potential for use in herbal-based dermatological treatments.

Keywords: Nanosponges, Calotropis procera, Topical Drug Delivery, Factorial Design, Entrapment Efficiency, Zeta Potential

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INTRODUCTION

The application of nanotechnology in drug delivery has significantly advanced pharmaceutical research by addressing many of the challenges associated with conventional dosage forms. Limitations such as poor aqueous solubility, inadequate bioavailability, rapid degradation of active compounds, and non-specific drug distribution often reduce therapeutic effectiveness [1-5]. Nanotechnology-based delivery systems offer an effective strategy to overcome these drawbacks by improving drug stability, enhancing bioavailability, enabling controlled release, and increasing therapeutic efficiency while minimizing systemic adverse effects. Among the different nano-carrier platforms developed for pharmaceutical applications, nanosponges have attracted considerable attention because of their unique structural characteristics and versatility [6-10].

Nanosponges are porous, three-dimensional polymeric nanoparticles composed of cross-linked polymers that form interconnected cavities capable of entrapping a wide variety of therapeutic molecules, including both hydrophilic and lipophilic compounds [11–14]. Their highly porous architecture provides a large surface area for drug

loading and supports controlled and sustained drug release. In addition, their structural properties help protect encapsulated bioactive compounds from environmental degradation, thereby improving formulation stability. Because of these qualities, nanosponges are especially well suited for topical and dermatological applications, where long-term drug retention and prevention against external factors are important [15–20].

Nanosponges have a number of benefits over traditional microsponge systems that make them more suitable for topical drug delivery. Their nanoscale size allows for more homogeneous drug distribution, better penetration into superficial skin layers, and closer contact with the skin's surface. Additionally, its polymeric network offers increased physicochemical and thermal stability, which enhances formulation performance throughout production and storage. [21–23].

Topical administration is widely preferred for the treatment of localized skin disorders because it delivers therapeutic agents directly to the affected site while limiting systemic exposure. Nevertheless, conventional topical formulations often exhibit poor skin permeation, limited residence time, and uncontrolled drug release, which may reduce therapeutic effectiveness. Incorporating drugs into nanosponge-based delivery systems offers a promising approach to overcoming these limitations by enhancing skin retention, improving permeation, and providing sustained release of encapsulated compounds [24–28].

Calotropis procera is an important medicinal plant that has been extensively used in traditional medicine for the management of various skin disorders and wounds. Its leaves contain several bioactive phytoconstituents with documented anti-inflammatory, antimicrobial, antioxidant, and wound-healing activities. Despite these pharmacological benefits, the therapeutic application of *C. procera* extract remains restricted because of poor aqueous solubility, limited stability, and inadequate bioavailability. Encapsulation of the extract within a nanosponge matrix may improve its physicochemical stability, protect sensitive phytoconstituents from degradation, and facilitate controlled release, thereby enhancing its therapeutic performance following topical administration [29–35].

To optimize nanosponge formulations, statistical experimental designs have become increasingly valuable because they enable systematic evaluation of formulation variables while reducing the number of experimental trials. Design of Experiments (DoE), particularly factorial design, allows simultaneous assessment of the individual and combined effects of formulation parameters on critical quality attributes, resulting in a more efficient and scientifically robust optimization process.

In the present investigation, *Calotropis procera* extract-loaded nanosponges were formulated using the emulsion solvent diffusion technique and optimized through a 3^2 full factorial design. Ethyl cellulose concentration and stirring rate were selected as the independent variables, while particle size, entrapment efficiency, and production yield served as the response variables. The optimized formulation was subsequently characterized to determine its physicochemical properties and to assess its suitability as a nano-scale carrier for topical delivery of *Calotropis procera* extract.

This work explores the formulation and optimization of *Calotropis procera* extract-loaded nanosponges using a 3^2 full factorial design, providing a systematic approach for developing a topical nanosponge-based herbal delivery system

MATERIALS AND METHODS

Materials

Calotropis procera leaves were gathered in Bareilly, Uttar Pradesh, India in April, 2024. Prof. Alok Shrivastav, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, authenticated the plant material (Ref. number PS/MJPRU/2024/01). Ethyl cellulose was purchased from A. B. Enterprises, Mumbai, Maharashtra, India. Polyvinyl alcohol was purchased from Labdhi Chemicals**,** Navrangpura, Ahmedabad, Gujarat, India. All chemicals used were of analytical grade.

Methods

Calotropis procera-loaded nanosponges (CPNSs) were prepared using the emulsion solvent diffusion (ESD) technique, a widely adopted method for fabricating polymeric nanosponge systems. Ethyl cellulose was selected as the polymeric carrier, whereas polyvinyl alcohol served as the stabilizer in the external aqueous phase [36].

Briefly, the organic phase was prepared by dissolving 100 mg of *Calotropis procera* extract together with ethyl cellulose at drug-to-polymer ratios of 1:4, 1:6, and 1:8 in 20 mL of ethanol. The resulting solution was stirred for approximately 10 min to obtain a clear and homogeneous mixture.

Separately, the aqueous phase was prepared by dissolving 1 g of PVA in 100 mL of distilled water with continuous heating at 60°C until complete dissolution was achieved [37–39].

The organic phase was then introduced slowly into the aqueous phase under continuous mechanical stirring. Continuous agitation was maintained for 2 h, allowing ethanol to diffuse into the aqueous medium and promoting the formation of nanosponge particles. The prepared nanosponges were subsequently separated by filtration and dried in a hot-air oven at 40°C for 24 h to obtain a free-flowing dry powder suitable for further characterization [40].

Fig.1: Different stages of preparation of C.procera Nanosponges.

Characterization of Nanosponges

The physicochemical properties of the prepared *Calotropis procera*-loaded nanosponges (CPNSs) were evaluated using a series of analytical techniques to determine their structural characteristics, particle properties, drug encapsulation efficiency, and formulation optimization.

X-ray Diffraction (XRD)

The crystalline characteristics of the *Calotropis procera* extract, ethyl cellulose, and the optimized nanosponge formulation were examined using powder X-ray diffraction (XRD) (X'Pert Pro diffractometer). Each sample was evenly spread on the sample holder, and diffraction patterns were recorded over a 2θ range of 5°–50° at a scanning speed of 0.019° s^{-1}. The resulting diffractograms were compared to identify changes in crystallinity following encapsulation and to evaluate possible interactions between the extract and polymer matrix [41,42].

Particle Size and Zeta Potential

Particle size and zeta potential were determined by dynamic light scattering (DLS) using a particle size analyzer (Thermo Fisher Scientific). Before analysis, the nanosponge suspension was diluted with distilled water in a 1:200 ratio to minimize multiple light scattering. Particle size measurements were carried out using disposable polystyrene cuvettes, whereas zeta potential was measured using an electrode cuvette under identical experimental conditions. All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation [43,44].

Entrapment Efficiency

Drug entrapment efficiency was determined by the ultracentrifugation method. The nanosponge suspension was centrifuged at 10,000 rpm for 30 min using a Remi C-24 ultracentrifuge (Mumbai, India). Following centrifugation, the supernatant containing the unentrapped extract was carefully separated and appropriately diluted. The concentration of free extract was quantified using a UV–Visible spectrophotometer at 389 nm. Entrapment efficiency was calculated using the following equation 1 [45,46]:

$$\%EE = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Scanning Electron Microscopy (SEM) Analysis

The surface morphology of the optimized nanosponges was examined using scanning electron microscopy (SEM) (Thermo Fisher Scientific). The dried samples were mounted onto aluminium stubs with double-sided conductive adhesive tape and coated with a thin gold–palladium layer under an argon atmosphere to improve electrical conductivity. Micrographs were recorded at an accelerating voltage of 20 kV at different magnifications to examine particle shape, surface characteristics, and porosity [47,48].

Factorial Design and Optimization

Optimization of the nanosponge formulation was carried out using a 3² full factorial design. Ethyl cellulose concentration (X_1) and stirring rate (X_2) were selected as the independent variables, whereas particle size, entrapment efficiency, and production yield served as the response variables. Nine experimental formulations representing different combinations of the selected factor levels were prepared to evaluate the influence of formulation variables on the quality attributes of the nanosponges [49–51].

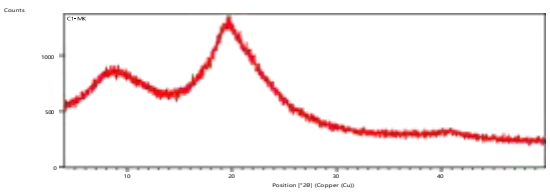
Statistical Analysis

The experimental design, optimization process, and statistical evaluation were performed using Design-Expert® software. Response Surface Methodology (RSM) was applied to investigate the individual as well as interactive effects of the independent variables on the selected responses. All experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical significance was established at $p < 0.05$.

RESULTS

X-ray Diffraction (XRD) Analysis

The XRD patterns of *Calotropis procera* extract, ethyl cellulose, and the optimized *Calotropis procera*-loaded nanosponges (CPNSs) are shown in Figure 2. The pure extract exhibited a characteristic peak at 19–20° (2θ), indicating partial crystallinity, whereas ethyl cellulose displayed a broad halo pattern, confirming its amorphous nature. The optimized CPNSs showed a marked reduction in the intensity of the extract's crystalline peaks along with the appearance of a broad amorphous halo. A weak peak around 31–32° (2θ) suggested the presence of minimal residual crystallinity. These findings indicate successful encapsulation of the extract within the polymer matrix and its partial conversion to an amorphous form, which may improve solubility and topical drug delivery. The absence of additional diffraction peaks also suggests good compatibility between the extract and ethyl cellulose.



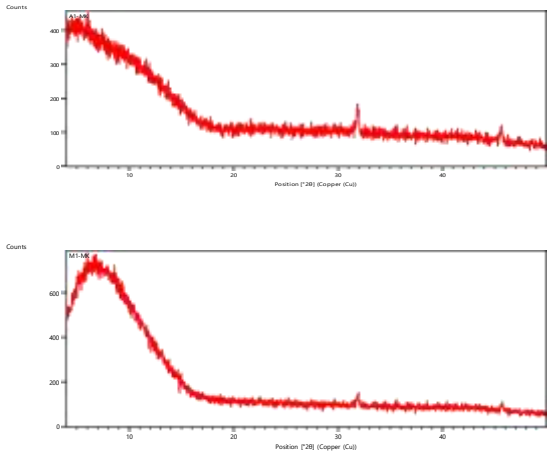
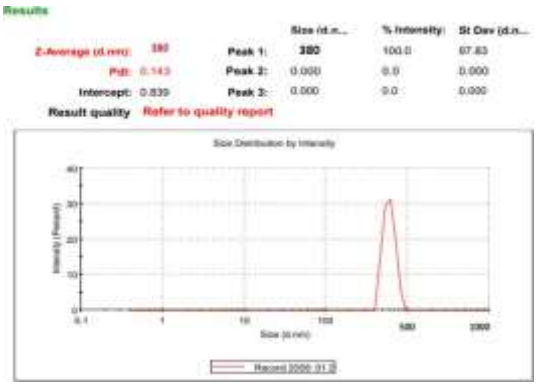


Fig. 2: XRD Patterns of (A) CP Extract, (B) Ethyl Cellulose, and (C) CPNSs

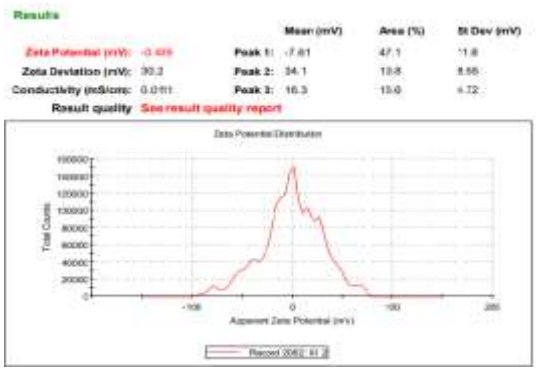
Particle Size and Zeta Potential

Particle size analysis showed that all formulations were within the nano-scale range, with slight variations depending on polymer concentration and stirring rate. The optimized formulation exhibited a mean particle size of 380 ± 97.83 nm, indicating its suitability for topical drug delivery due to improved skin penetration and uniform distribution.

The optimized CPNSs exhibited a zeta potential of 34.1 ± 9.55 mV, reflecting good colloidal stability. The high absolute zeta potential value provides sufficient electrostatic repulsion between particles, minimizing aggregation and contributing to the physical stability of the nanosponge formulation.



A



B

Fig.3: (A) Particle Size Distribution of CPNSs (B) Zeta Potential of CPNSs

Entrapment Efficiency (%EE)

The entrapment efficiency of the formulated nanosponges ranged from 55% to 93%**,** demonstrating effective incorporation of *Calotropis procera* extract within the polymeric matrix. An increase in ethyl cellulose concentration resulted in higher entrapment efficiency, likely due to the availability of a larger polymer network that enhanced encapsulation and reduced drug loss during formulation.

Table 1: Entrapment Efficiency of CPNSs

S. No.	Entrapment efficiency (%)
1.	55±2.0
2.	64±2.0
3.	83±1.5
4.	59±2.5
5.	69±3.3
6.	91±1.0
7.	79±3.0
8.	81±3.2
9.	93±2.5

Scanning Electron Microscopy (SEM) Analysis

The SEM images Figure 4 showed that the optimized CPNSs were predominantly spherical with a porous surface morphology, a characteristic feature of nanosponge systems. No visible crystalline particles of the extract were detected on the particle surface, indicating successful encapsulation and uniform distribution within the polymer matrix. The porous architecture is expected to facilitate high drug loading and promote controlled, sustained release of the encapsulated extract.

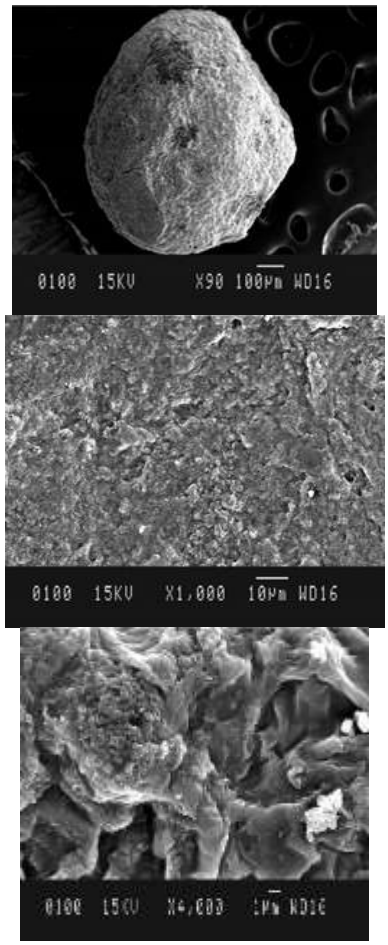


Fig.4: SEM micrographs showing the spherical porous morphology of optimized *Calotropis procera*-loaded nanosponges at different magnifications

Factorial Design and Optimization

A 3² full factorial design was applied to optimize the formulation by evaluating the effects of ethyl cellulose concentration (X_1) and stirring rate (X_2) on particle size, entrapment efficiency, and production yield**.** Nine formulations representing different combinations of the selected variables were prepared and analyzed, as summarized in Table 2. All results are expressed as mean ± standard deviation (SD, n = 3)**.**

Table 2: Effect of Formulation Variables on CPNSs

Batches	Factors		Responses		
	Calotropis procera: Ethyl	Stirring Rate Rpm	Particle Size nm	Entrapment Efficiency %	Production Yield %

	cellulose mg				
F1	100:400	600	379±2.24	55±2.00	61±2.12
F2	100:600	600	383±2.25	83±1.52	70±1.27
F3	100:800	600	383±2.28	59±2.52	76±2.94
F4	100:400	1000	384±2.26	91±1.03	67±2.51
F5	100:600	1000	382±2.24	79±3.02	79±1.86
F6	100:800	1000	380±2.10	81±3.22	81±3.12
F7	100:400	1400	383±2.21	93±2.52	72±1.27
F8	100:600	1400	381±2.20		82±2.21
F9	100:800	1400	380±3.12		83±1.98

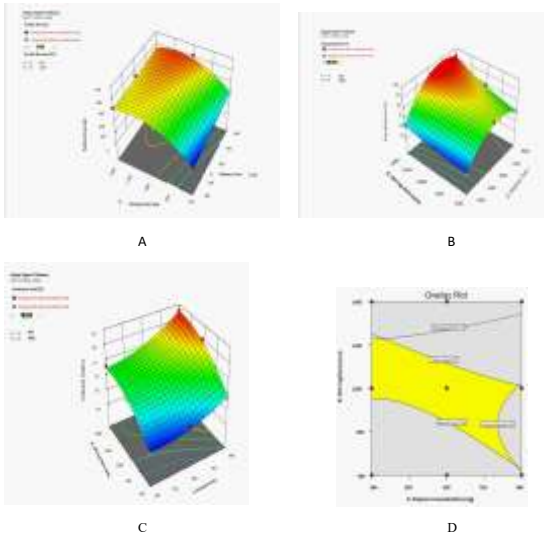


Fig.5: Response Surface Plots for (A) EE, (B) Particle Size, (C) Yield, and (D) Desirability

Increasing the concentration of ethyl cellulose improved both entrapment efficiency and production yield, likely due to the formation of a larger polymer matrix that enhanced drug encapsulation. In contrast, stirring rate had a greater influence on particle size than on entrapment efficiency. Higher stirring speeds generally produced smaller particles as a result of increased shear forces; however, their effect on drug entrapment was inconsistent, indicating a possible interaction between the selected formulation variables.

DISCUSSION

The present study successfully developed and optimized *Calotropis procera*-loaded nanosponges (CPNSs) using the emulsion solvent diffusion method and a 3² factorial design. The optimized formulation demonstrated its potential for use as a topical delivery system for herbal medicines by exhibiting good physicochemical characteristics.

The extract's effective incorporation into the polymeric matrix was verified by XRD analysis, which showed a decrease in crystallinity and a partial transformation into an amorphous form that could enhance solubility and drug release. Spherical nanosponges with a porous surface were seen in SEM images, demonstrating effective drug loading with sustained release behaviour and verifying the formulation's distinctive morphology.

The mean particle size of the optimized formulation was 380 ± 97.83 nm, which is suitable for topical use and could improve uniform distribution and skin penetration. Good colloidal stability was shown by the zeta potential of 34.1 ± 9.55 mV, which suggested no particle aggregation during storage. Higher ethyl cellulose concentrations improved drug encapsulation by creating an expanded polymeric matrix; entrapment efficiency varied from 55% to 93%.

The impact of formulation variables on particle size, entrapment efficiency, and production yield was successfully determined by the 3² factorial design. While increasing the stirring rate generally decreased particle size, polymer concentration had the greatest beneficial impact on drug loading and production yield. Because F9 combined high entrapment efficiency (93%), production yield (83%), and a suitable particle size, it was chosen as the optimized batch out of all the formulations.

Overall, by enhancing encapsulation, stability, and controlled release, the created CPNSs present a potential approach for topical delivery of *Calotropis procera*. The potential use of nanosponge-based systems to improve the therapeutic efficacy of herbal formulations in wound healing and other dermatological conditions is supported by these findings.

CONCLUSION

The emulsion solvent diffusion technique and a 3² factorial design were effectively used to develop and optimize *Calotropis procera*-loaded nanosponges. With a particle size of 380 ± 97.83 nm, 93% entrapment efficiency, 83% production yield, and high colloidal stability, the optimized

formulation demonstrated its appropriateness for topical application. The extract was successfully encapsulated within a porous polymeric matrix, supporting controlled drug release and enhanced formulation stability, according to XRD and SEM analysis.

The study demonstrated that ethyl cellulose concentration was the primary factor influencing entrapment efficiency and production yield, while stirring rate mainly affected particle size. With the potential to enhance the stability, skin retention, and therapeutic efficacy of herbal extracts, the optimized nanosponge formulation offers a suitable carrier for topical delivery of *Calotropis procera*.

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None

CONFLICT OF INTERESTS

The author's declare no conflict of interest.

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