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FORMULATION AND EVALUATION OF CLINDAMYCIN NANOSUSPENSION LOADED LYOPHILIZED POWDER FOR EFFICIENT TREATMENT OF AXILLARY OSMIDROSIS

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Keywords:

Axillary osmidrosis, Clindamycin phosphate, Nanosuspension, Lyophilized powder, Topical drug delivery, Antibacterial activity, Central composite design (CCD), Apocrine gland, *Staphylococcus aureus*

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ABSTRACT: The aim of the present work was to develop clindamycin phosphate-loaded nanosuspension lyophilized powder for axillary osmidrosis caused by bacterial activity in apocrine glands. The formulation prepared using the solvent/antisolvent method included Eudragit L100, PVP K30, and Tween 80. Optimization *via* central-composite design (CCD) yielded formulation with a particle size of 179 nm and viscosity of 1.35 cps. Characterization confirmed favorable sedimentation volume, drug content and zeta potential with SEM revealing a spherical and porous structure. Lyophilization improved stability and permeability, and *in-vitro* studies demonstrated 24-hour drug release. Antibacterial testing showed good efficacy against *Staphylococcus aureus* indicating potential of clindamycin nanosuspension as a promising topical delivery system with enhanced efficacy.

INTRODUCTION: The human apocrine gland releases secretions into hair follicles and has a distribution limited to the axilla, anogenital region, and breasts. They are located deep in the dermis or upper region of the hypodermis. Axillary apocrine glands are the greatest contributors to bad body odor. The bad odor produced is called axillary osmidrosis, and it is caused by the oxidation and hydrolysis of 3-methyl-2-hexenoic acid by the normal flora *Staphylococcus aureus* bacilli, which exists in the apocrine sweat gland [1]. Estimated with varying prevalence rates from160 to 400 million people globally based on the current population, this is a common disease in developing countries, and to prevent it, several measures are taken.

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The control of this disease is possible with the proper treatment. Botulinum toxin (BTX) has been the preferred treatment for focal hyperhidrosis, though it offers only temporary relief. Axillary osmidrosis management includes hygiene practices, antibacterial soaps, and antiperspirants with metals like aluminium. Energy-based devices, especially microwaves, and surgical methods like excision and curettage offer longer-lasting effects but carry potential side effects due to their invasive nature. However, they were not able to efficiently control the bad odor produced by bacteria, which led to the use of higher-end antibiotic drugs, such as clindamycin, which is used for the efficient treatment of axillary osmidrosis.

Clindamycin is a BCS class III drug with good aqueous solubility and poor membrane permeability. It suffers from a low topical bioavailability of 4-5%, which leads to less membrane permeability through the skin [2]. The necessary increase in the permeation of the

clindamycin phosphate could be achieved for therapeutic efficacy when clindamycin phosphate is applied topically; thereby, the bioavailability of the drug can be improved. Thus, researchers are attempting to improve the bioavailability of this drug using novel approaches. Many techniques have been reported in the literature toward an increase in the permeability and bioavailability of clindamycin phosphate, such as formulating nano-emulsions, solid-lipid nanoparticles, nano-liposomes, Emulgel, ethosomes, nanoparticles, etc. A lower percentage of drug loading, lower stability, high manufacturing cost, and an increased number of steps in manufacturing for the above-cited techniques limit its application in the preparation of a viable formulation of the drug at an economic level [3-6]. The present research attempts to develop a nanosuspension of clindamycin phosphate to formulate novel delivery system of nanosuspension lyophilized powder to possess improved permeability and bioavailability.

MATERIALS AND METHODS: Clindamycin phosphate was procured as gift sample from zydus life since limited (Zydus Candila health care limited) Ahmedabad, India. Eudragit L100 Otto Chemie Pvt Ltd, PVPk-30 signet chemical corporation Pvt Ltd, Tween 80 Thomas baker (chemicals) PVT. LTD. Ethanol Changsu Hong sheng Fine Chemicals Co. Ltd.

TABLE 1: COMPOSITION OF DIFFERENT FORMULATED COMPLEXES OF CLINDAMYCIN PHOSPHATE

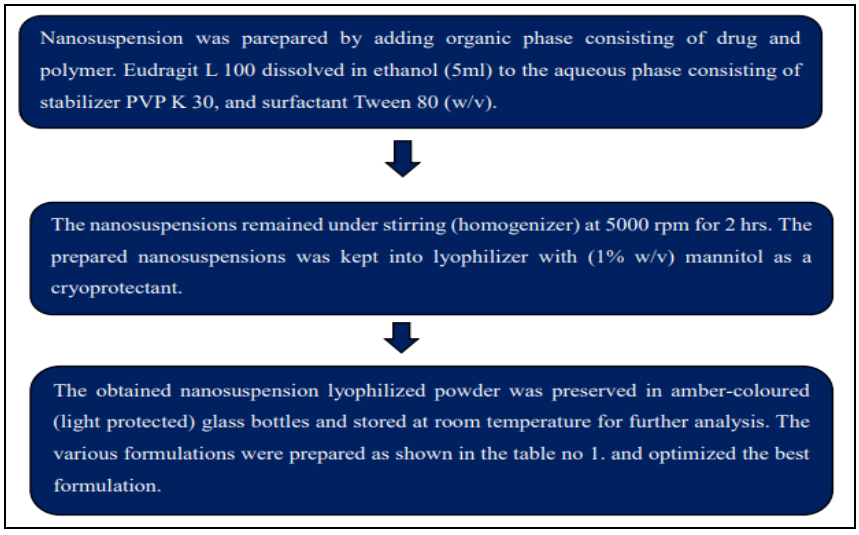
Sl. no.	Drug (mg)	Conc. Of Eudragit L 100 (mg)	Conc. of Tween80 (% v/v)	PVP K30 (1gm)	Ethanol (5ml)	Distilled water (100ml)	Homogeni zation speed (5000rpm)
1	10	50	0.5	1	5	100	5000
2	10	150	0.5	1	5	100	5000
3	10	50	1.5	1	5	100	5000
4	10	150	1.5	1	5	100	5000
5	10	50	1	1	5	100	5000
6	10	150	1	1	5	100	5000
7	10	100	0.5	1	5	100	5000
8	10	100	1.5	1	5	100	5000
9	10	100	1	1	5	100	5000

Experimental Methods:

Solubility Studies:

Selection of Solvent for Clindamycin Phosphate: The selection of a suitable solvent system for the preparation of nanosuspension was carried out by analyzing the solubility of clindamycin phosphate in different solvent systems. Thus, the solubility of LUF was determined in methanol, ethanol, distilled water, pH 7.4, and pH 6.8 phosphate buffer [7].

Method of Preparation of Nano Suspension Containing Clindamycin Phosphate [8]: The clindamycin phosphate loaded Nanosuspension formulations were prepared by Solvent/ anti-solvent method.



Lyophilization Process for Converting Nanosuspension into Stable Powder: 100 ml of the nanosuspension was prepared by adding 1% w/v of a cryoprotectant, such as mannitol to protect the nanosized particles during the freezing and drying stages. Prepared nanosuspension were transferred into glass vials maintaining the low temperature -40°C and - 80°C.

Primary drying (sublimation) places the frozen vials in the lyophilizer, where the chamber pressure is reduced to initiate the sublimation of ice directly into vapor. Set shelf temperatures between -20°C and -40°C initially, then gradually increase as sublimation progresses. The condenser temperature should be maintained at around -80°C to trap water vapor. Continue primary drying until no visible ice remains. Secondary drying (desorption) gradually raises the shelf temperature to approximately 20°C to 25°C to remove any remaining residual moisture bound to the particles. The duration of secondary drying may vary but typically lasts 1-3 hours, after which the lyophilized nanosuspension powder is obtained and ready for storage and use.

Optimization of Nanosuspension Formulation: Central Composite Design (CCD) was employed using Design Expert software version 13 to optimize the formulation by evaluating main effects and interactions among factors, identifying significant variables. The independent variables were polymer concentration (Eudragit L 100) and surfactant concentration (Tween 80), with particle size and viscosity as dependent variables. The high and low levels of these factors were selected based on previous literature, allowing a systematic analysis of their effects on formulation characteristics. The CCD facilitated efficient formulation optimization by generating a range of experimental runs. For optimization, equation of mathematical model linking independent variables and their interactions for different quantified responses derived for 32 factorial design is:

$$Y = b_o+b_1x_1+b_2x_2+b_{12}x1x^2+b_{22}x^2_2$$

Where, Y is the value of dependent variable, b0 is overall coefficient, and b_1 and b_2 are the coefficient of X_1 and X_2. Response surface plots were plotted to establish the relationship between independent and dependent variables.

Evaluation of Nano Suspension:

Sedimentation Volume: To assess nano suspension stability using sedimentation volume, start by thoroughly mixing the sample, then pouring it into a graduated cylinder and recording the initial volume (V0). Let it sit undisturbed for 24 hours, then measure the volume of the settled sediment (Vu). The sedimentation volume (F) is calculated by the below equation.

$$F = Vu/ Vo$$

Re-dispersibility Assessment: 20 mL of the suspensions or redispersed lyophilized suspension after reconstitution were allowed to free settle in a measuring 25 mL cylinder for 24 h. Cylinders' mouths were closed, inverted 180 degrees, and the number of inversions necessary for 100% redispersion was determined for each formulation. If consistency is achieved in one inversion, then it has 100% re-dispersibility. Any extra inversion reduces the percentage of simplicity of re-dispersibility by 5%. Experiments were conducted in triplicate.

Viscosity Measurement: The viscosity of 20 mL of ready prepared and reconstituted nanosuspension preparations was determined at 25 ℃ using an Ostwald viscometer. Results were taken as an average of three measurements ± SD. Calculating by the below formula [6].

$$\eta1=\eta2 \times (t1)\,(d1) / (t2)\,(d2)$$

Drug Content: The amount of clindamycin phosphate present in formulated dosage form was determined by UV Spectroscopy method. An estimated 1 ml of nanosuspension was dissolved in pH 6.75 phosphate buffer. The solution was then filtered, suitably diluted, and the resulting solution was analyzed at 210 nm.

Analysis of Zeta Potential: The zeta potential of the optimized clindamycin phosphate-loaded nanosuspension formulation was measured using the nanosuspension zeta sizer Malvern.

Particle size: The mean particle size of the nanosuspension was measured by Malvern Zetasizer (Malvern Instrument Ltd.). The dispersion was diluted with Millipore-filtered water to an appropriate scattering intensity at 25℃, and

the sample was placed in a disposable sizing cuvette, and the particle size was analyzed.

Percentage Yield: The percentage yield of the nanosuspension lyophilized powder is calculated accurately by determining the initial weight of the raw material and the last weight of the nanosuspension lyophilized powder obtained.

$$\text{Percentage yield} = \textit{Practical weight of nanosponges} / \textit{Theoretical weight} \times 100$$

Angle of Repose (θ): An accurately weighed amount of lyophilized nanosuspension powder was permitted to flow freely through a funnel. The funnel tip height (θ) was adjusted to 2 cm from a plane surface. When the apex of the flowing powder touches the funnel tip, the radius (r) of the formed powder cone is measured.

The angle of repose (tan θ) was calculated as follows:

$$\text{Tan } \theta = h / r$$

Hausner's ratio: Hausner's ratio (HR) is the ratio of the tapped bulk density (pt) to the loose bulk density (pb) as follows:

$$\text{HR} = pt / pd$$

Where pt is the aerated density obtained after freely pouring the lyophilized nanosuspension powder into a 10 mL cylinder (calculated by dividing the powder weight by its aerated volume) and pb is the constant density obtained during tapping until no further volume changes occur (calculated by dividing the powder weight by its tapped volume).

Carr's Compressibility Index [9]: Carr's index (%) was calculated by using the following equation:

$$carr'sindex\ (\%) = \text{pt - pd} / pt \times 100$$

Scanning Electron Microscope (SEM): The shape and surface morphology of lyophilized nanosuspension powder was analyzed using a TESCAN VEGA 3 LMU scanning electron microscope. Approximately 40µl of the lyophilized powder was dried overnight on an aluminium stub at room temperature. The dried sample was then coated with a thin layer of gold-palladium using a Leica EM ACE200 fine coat ion sputter in a high-vacuum evaporator under an argon atmosphere, providing a conductive surface for imaging. The coated sample was scanned at an acceleration voltage of 10 kV, and high-resolution photomicrographs were captured to observe the surface morphology and confirm the nanoscale characteristics of the powder [10].

Comparative Study of *In-vitro* Drug Release Study: An *in-vitro* drug release study for pure drug, nanosuspension, and lyophilized nanosuspension powder was conducted using Franz diffusion cells with a dialysis membrane. The dialysis membrane was carefully positioned between the donor and receiver chambers of the cell. Phosphate buffer with a pH of 6.75 served as the diffusion medium in the receiver chamber. The entire setup was placed on a magnetic stirrer, set to 100 rpm, ensuring consistent agitation throughout the process. One gram of the nanosuspension lyophilized powder was evenly distributed in the donor chamber above the membrane. Samples from the receiver solution were carefully withdrawn at pre-determined intervals, starting at the 1st hour and continuing at each subsequent hour (2nd through 7th), with a final sample at 24 hours. After each withdrawal, the volume in the receiver compartment was replenished to its marked level with fresh buffer solution to maintain consistency. Great care was taken during solution addition to avoid air entrapment in the receiver compartment, which could impact diffusion rates. The collected samples were filtered, and the percentage of drug release was determined by measuring the absorbance of each sample at 200 nm, the drug's λmax [11].

Antimicrobial Activity: To determine the effectiveness of drug-loaded nanosuspension lyophilized powder. The antimicrobial activity was assessed using the agar well diffusion method. For culture media preparation, an agar solution with nutrient agar was sterilized at 121°C, poured into Petri dishes, and allowed to solidify. In subculture preparation, nutrient agar was poured into test tubes, sterilized, and slanted to increase surface area.

Using a sterile inoculation loop, a pure culture was transferred onto the solidified agar and incubated for 24–48 hours to confirm growth. To prepare standard antibiotics, a stock solution of 1 mg/ml is

made by dissolving 50 mg of the antibiotic in 50 ml of water; 0.3 ml of this stock is then diluted to create a 30 µg/ml solution. For testing, a small amount of organism suspension is spread over prepared media plates, wells are bored, and the test sample is added. After refrigeration for 1 hour, plates are incubated for 18-24 hours, and inhibition zones around the wells are measured to assess antimicrobial effectiveness [12].

RESULTS AND DISCUSSION:

Solubility Study: The solubility profile of clindamycin phosphate in various media were carried out and the results are showed below.

TABLE 2: SOLUBILITY PROFILE OF CLINDAMYCIN PHOSPHATE IN VARIOUS MEDIA

Sl. no.	Solvent	Solubility (mg/ml)
1	Ethanol	1.822±0.136
2	Methanol	1.672±0.009
3	DMSO	1.585±0.018
4	pH 6.75 phosphate buffer	1.401±0.0753
5	pH 7.4 phosphate buffer	1.151±0.012
6	Purified water	1.087±0.111

The solubility studies of clindamycin phosphate were performed in various media such as methanol, ethanol, DMSO, purified water, alkaline buffer pH 6.75, and phosphate buffer pH 7.4. Results showed that the solubility of clindamycin phosphate is high in methanol, i.e., 1.822±0.136 mg/ml, as shown in the table.

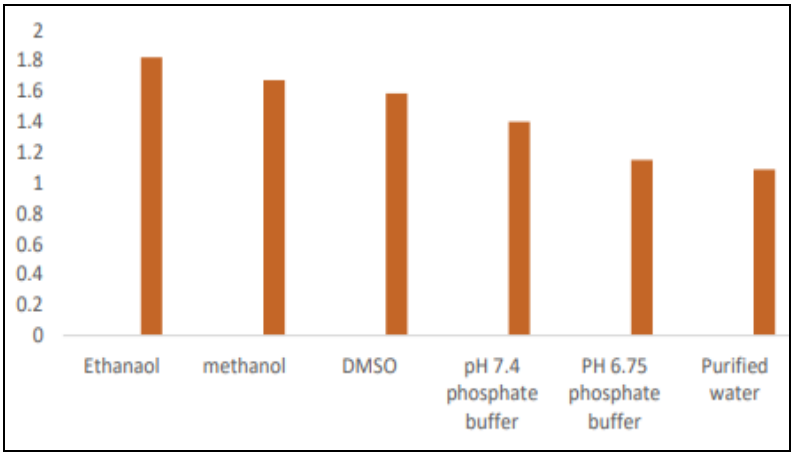


FIG. 1: SOLUBILITY GRAPH OF CLINDAMYCIN PHOSPHATE

Measurement of Particle Size and PDI: The mean particle size, PDI, and zeta potential of the optimized nanosuspension were measured by the dynamic light scattering technique using the Malvern Zeta sizer (Malvern Instrument Ltd).

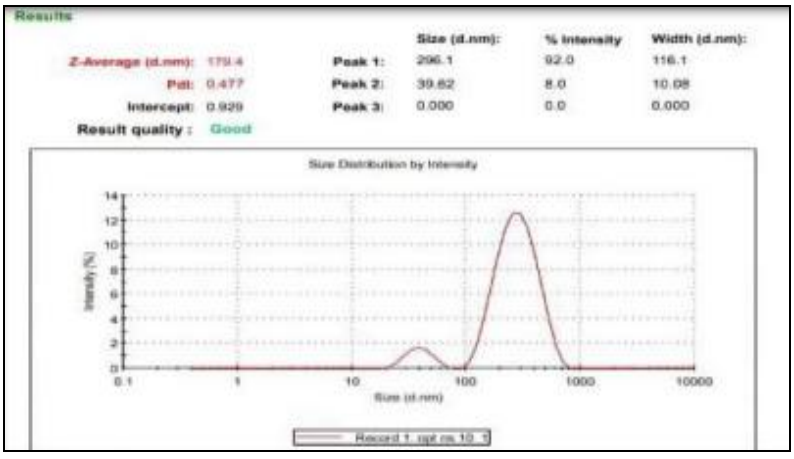


FIG 2: MEASUREMENT OF PARTICLE SIZE AND PDI

Zeta Potential: The mean particle size and zeta potential of optimized nanosuspension were measured by dynamic light scattering technique using Malvern Zeta sizer (Malvern Instrument Ltd) and were found to be 179.4 nm, respectively. Zeta potential indicates the charge on the surface of the particle, and it can impact the stability of the nanosuspension formulations. Zeta potential was found to be -33.7 mV, indicating high stability of the formulation.

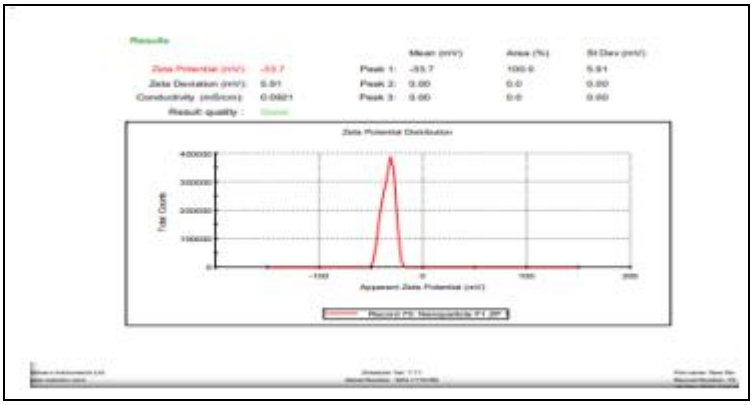


FIG. 3: ZETA POTENTIAL REPORT

Scanning Electron Microscopy: The morphology of the Nanosuspension lyophilized powder prepared by the solvent/anti solvent method, i.e., was investigated by SEM. The representative SEM photographs of the Nanosuspension lyophilized powder shown in **Fig. 4** images showed that the Nanosuspension lyophilized powder was porous and spherical in nature.

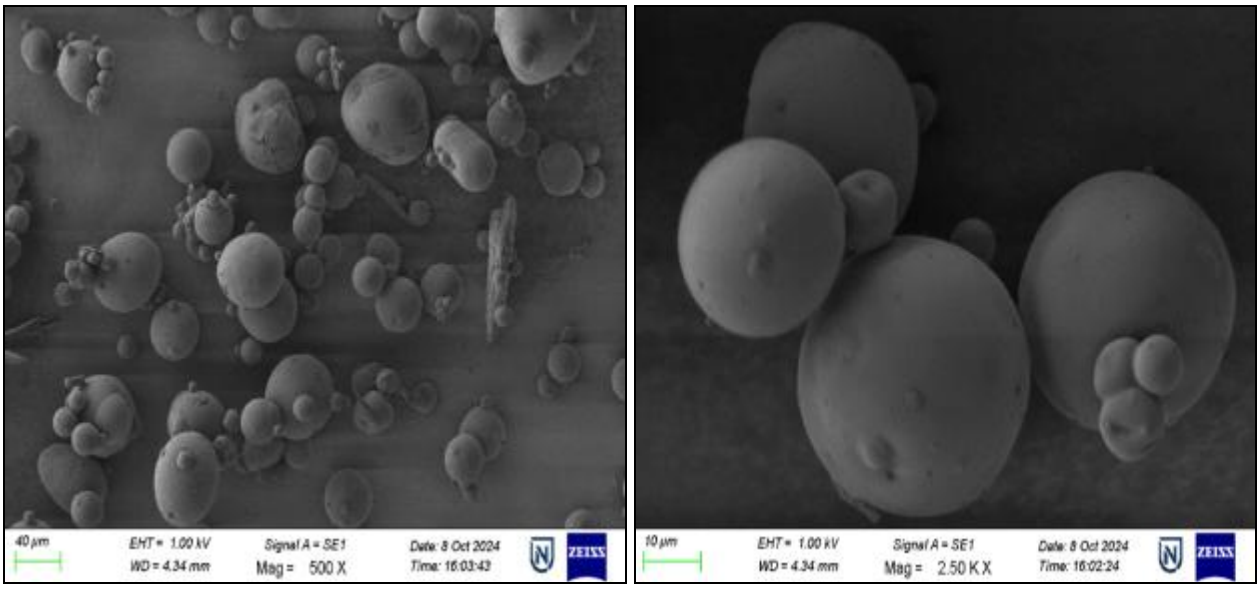
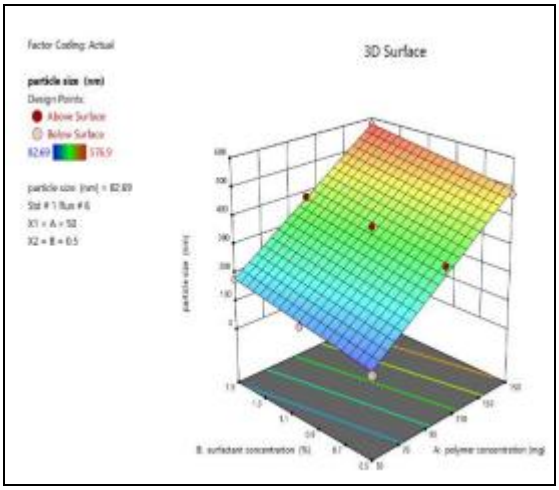
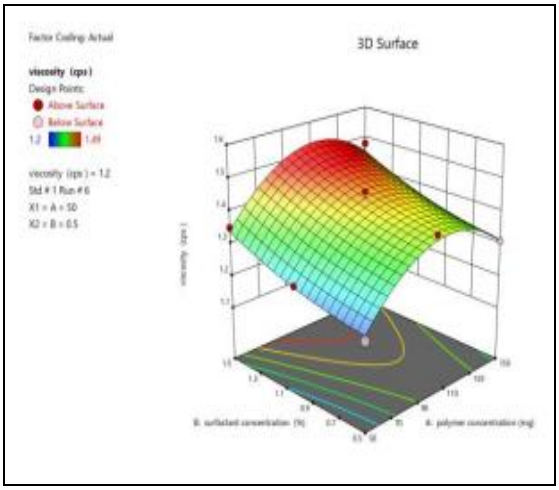


FIG. 4: SEM IMAGES OF DRUG LOADED NANOSUSPENSION LYOPHILIZED POWDER

Preparation of Nanosuspension: Finally, the nanosuspension of clindamycin phosphate was prepared using the solvent antisolvent technique as described earlier. The nanosuspension experiment was designed as per the 32 full factorial design as per DoE (Table). The result of the experimental runs is presented in the table.

Optimization of Critical Factors using CCD: In the optimization experiments using CCD, a total of 9 independent runs were generated to study the main effect, interaction effect, and quadratic effect of the three critical factors on particle size and viscosity. Nanosuspension was prepared independently, in triplicate, based on the details of the actual composition of each factor experimental run (after randomization of the run order) and characterized for particle size (Y1) and viscosity (Y2).





Polynomial Equation: polynomial equation for predicting the particle size (*Y*1) of Nanosuspension in original units of measure is given below:

Particle size (Y1) = - 144.86+3.966*A+85.23*B

Polynomial Equation: Polynomial equation: polynomial equation for predicting the viscosity (*Y*2) of Nanosuspension in original units of measure is given below:

Viscosity (Y2) = +0.7807+0.0112*A–
0.0493*B+0.002*A+B–0.00005*A^2+0.0813*B^2

TABLE 3: FORMULATION DESIGN FOR PREPARATION OF CLINDAMYCIN PHOSPHATE NANO SUSPENSION

STD	Runs	Polymer concentration (mg)	Surfactant concentration%	Particle size(nm)	Viscosity (cps)
3	1	50	1.5	179.4	1.35
5	2	50	1	122.7	1.26
6	3	150	1	522	1.339
4	4	150	1.5	576.9	1.48
7	5	100	0.5	325.4	1.41
1	6	50	0.5	82.69	1.2
9	7	100	1	364.8	1.46
2	8	150	0.5	475.7	1.31
8	9	100	1.5	383.2	1.49

Evaluation of Re-dispersed LY-NS:

TABLE 4: FORMULATION DESIGN FOR PREPARATION OF CLINDAMYCIN PHOSPHATE NANO SUSPENSION

Parameter	Results
%Sedimentation volume ± SD%	91.833± 0.289
Re-dispersibility assessment	95
Viscosity (cp) ± SD	1.35cps
Drug content (%)	95.76%
Percentage yield	88%
Particle size	179.4nm
Measurement of Zeta potential	-33.7

TABLE 5: EVALUATION RESULTS NANOSUSPENSION LYOPHILIZED POWDER

Parameter	Results
Angle of repose	23.5°
Hausner's ratio	1.08
Carr's index	7.4%

Drug-excipient Compatibility Studies:

ATR Studies:

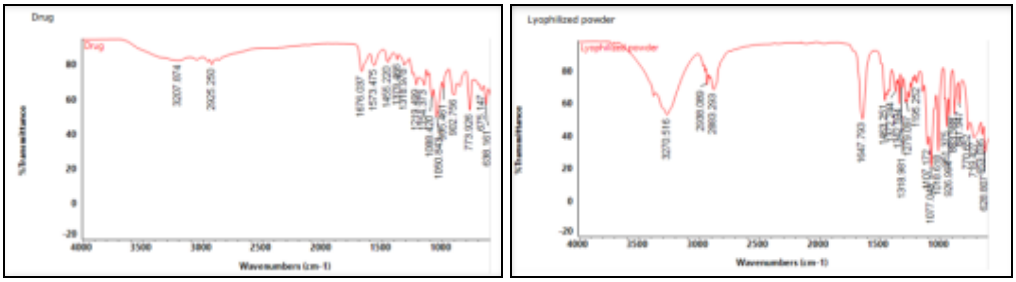


FIG. 5: ATR STUDIES OF PURE DRUG AND NANOSUSPENSION LYOPHILIZED POWDER

ATR was used to study the physical and chemical interactions between the drug and excipients. It was observed that there were no major shifts in the spectral values of the drug, indicating no chemical interaction. Hence, it can be concluded that there is compatibility between the drug and the excipients used. It was concluded that the drug maintains its identity without undergoing any interaction with the excipients used.

Comparative Study of *In-vitro* Drug Release Study: The *in-vitro* drug release of pure drug nanosuspension, optimized drug-loaded nano-suspension formulation, and optimized nanosuspension lyophilized powder in pH 6.75 phosphate buffer was found to be 38.66%, 57.531%, and 69.733% (fig) at the end of 24 hours, respectively. The pure drug, drug-loaded nanosuspension, and lyophilized powder formulation. Hence, the drug release of nanosuspension and lyophilized powder formulation can successfully aid the delivery of the drug topically.

TABLE 6: COMPARATIVE STUDY OF IN-VITRO DRUG RELEASE STUDY

Sl. no.	Time (hours)	Pure drug	Nanosuspension	Nanosuspension lyophilized powder
1	1	8.685	10.577	14.1889
2	2	11.969	13.925	22.832
3	3	13.747	16.701	29.213
4	4	15.381	19.786	36.954
5	5	17.043	21.086	44.577
6	6	19.76	22.409	46.811
7	7	20.016	26.326	48.834
8	24	38.66	57.531	69.733

Comparative Study Pure Drug and Formulations Chart:

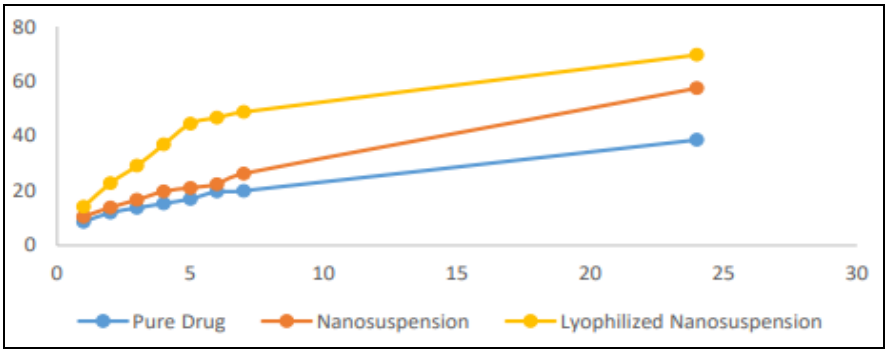


FIG. 6: COMPARATIVE STUDY DRUG AND FORMULATION

Microbiological Studies: The antibacterial efficacy of the optimized clindamycin phosphate-loaded nanosuspension lyophilized powder was evaluated against Staphylococcus aureus using a saline and nutrient agar medium. After a 24-hour incubation, the nanosuspension powder formulation (F1) showed a significant inhibition zone of 48 mm, outperforming both the standard and blank solutions. This strong antibacterial activity suggests the formulation's potential for effectively reducing bacterial growth associated with skin conditions like axillary osmidrosis, likely due to its enhanced bioavailability and prolonged release properties, making it a promising candidate for topical antibacterial applications.

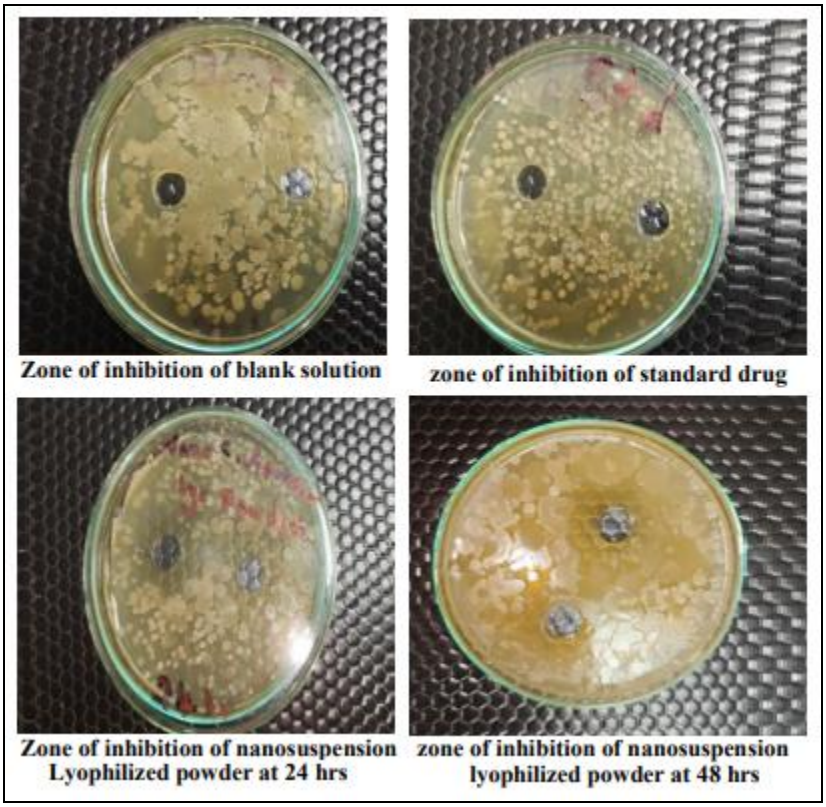


FIG. 7: ZONE OF INHIBITION OF MICROBIOLOGICAL STUDIES

CONCLUSION: This study aimed to design, develop, and evaluate a clindamycin phosphate-loaded nanosuspension lyophilized powder for controlled topical drug delivery, targeting improved local bioavailability and extended release. Clindamycin phosphate, a BCS Class III broad-spectrum antibiotic, has low permeability, warranting the development of a formulation that could achieve prolonged release. Using the solvent/antisolvent method, the formulation incorporated Eudragit L 100, PVP K30, Tween 80, ethanol, and water. Compatibility studies by ATR showed no interaction between the drug and excipients, with SEM results indicating spherical and porous nanoparticles. The optimized F1 formulation demonstrated favorable characteristics, including a particle size of 172.11 nm, good sedimentation volume, high redispersibility, low viscosity, and satisfactory drug content. It exhibited prolonged drug release over 24 hours and antibacterial efficacy, with a larger zone of inhibition than the standard and blank formulations. Supporting its potential as a novel topical nanosuspension for clindamycin phosphate.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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