RESEARCH ARTICLE

Development and Characterization of Oxiconazole Loaded Plga Nanogel

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ABSTRACT

The goal of the current study is to develop and improve polylactic co-glycolic acid (PLGA) loaded with oxiconazole nanogel for topical application. Oxiconazole Nanoprecipitation was used to create the nanoparticles method using PLGA and acetone. The point prediction method served as the foundation for the ideal oxiconazole nanoparticle composition using the Minitab®21 software, three levels two factors (3^2). The optimized composition of oxiconazole nanoparticle showed a particle size of 185 ± 0.5nm with an entrapment efficiency of 95.2 ± 0.09%. Using chitosan, a naturally occurring polymer, the ideal oxiconazole nanoparticle composition was further transformed into a gel formulation. The developed topical nano gel formulation underwent additional evaluations for antifungal, drug release, permeability, and nano gel characterization. The developed oxiconazole nanogel formulation had the ideal drug content, pH, viscosity, and spreadability. The results of the medication release and penetration investigation showed that Oxiconazole released slowly (74.06 ± 0.5%) with significantly enhanced permeation across Franz diffusion cell using membrane. The antifungal *ex-vivo* study and skin irritancy study of nanogel results would conclude higher activity of oxiconazole nano gel. According to the overall analysis of the data, oxiconazole nanogel is the best delivery method for treating fungal infections on the skin.

Keywords: Oxiconazole, PLGA-based nanoparticles, antifungal activity, nano gel.

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INTRODUCTION

Numerous regions of the natural environment are prone to fungal diseases. Fungal infections in humans occur when an invasive fungus colonizes a body part that is too big for the immune system to handle. There are two categories of fungal infections: superficial and invasive. Internal organs and other tissues can become infected with invasive fungal infections, which are caused by agents that penetrate the affected tissue or organ.[1]

Up to 20–25% of people worldwide suffer from superficial fungal infections, which disrupt everyday activities, lower quality of life, and increase health care costs. In clinical practice, superficial fungal skin disorders are the most prevalent infectious disease in humans. Candida albicans or dermatophytes are the culprits behind superficial fungal infections, which affect the skin, scalp, and nails.[2]

According to epidemiological studies on fungal infections, people of all ages are susceptible to the fungi that cause surface mycoses, which are common. Because of their broad skinfolds, obese persons are far more likely to have these kinds of illnesses, especially if the skin inside a skinfold becomes infected and breaks down. Additionally, those with diabetes are more susceptible to fungal infections.[3]

The intended location dictates the severity and duration of the infection, which varies correspondingly. The most frequent cause of infection is dermatophytes, or fungi, which act by degrading keratin, a protein that covers the skin's outer layer. The classification of fungal infections is based on the affected body part. Fungal infections can cause rashes in non-affected areas of the body in one place. An itchy, bumpy rash on the foot could result from a fungal infection on the fingertips. Usually, an allergic reaction to the fungus is the cause of this. They do not result from contact with the contaminated area.[4]

Oxiconazole active substance was selected because it has a low-medium molecular weight, high permeability, no deep irritation, high local bioavailability, and good compatibility with polymers. The chemical formula and molecular weight of Oxiconazole are $C_{18}H_{13}C_{14}N_3O$ and 429.126g/mole. It is poorly soluble in water and soluble in organic solvents such as acetone, ethanol, dichloromethane, etc.[5]

Several synthetic, semisynthetic, and natural polymers have been utilized in the preparation of nanoparticles. Among these, PLGA stands out as a commonly employed polymer due to its biodegradable and biocompatible properties. Comprising polylactic acid (PLA) and polyglycolic acid (PGA), PLGA is available in various molecular weights. It plays a significant

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role in enhancing the pharmacokinetic and pharmacodynamic profiles of numerous therapeutics, such as sildenafil citrate, tenofovir, and zaleplon. However, nanoparticles formulated as nanosuspensions often exhibit very low viscosity, making it challenging to apply them to the skin and potentially compromising their efficacy by hindering their ability to reach the targeted tissue site. To address this issue, efforts are made to increase the viscosity of the prepared nanoparticles, thereby ensuring prolonged retention of the delivery system on the skin.[6]

Chitosan and poloxamer were employed in the formulation of topical gel. Chitosan, a natural polymer, serves as a gelling agent for topical delivery. Derived from the deacetylation of chitin, it is a biological macromolecular polymer known for its biodegradability, biocompatibility, and various beneficial properties. These properties include its role as a penetration enhancer, bioadhesive, and non-toxic substance. Additionally, chitosan exhibits therapeutic properties such as wound healing enhancement, antimicrobial action, and hemostatic effects.[7]

The present study aimed to develop and characterize Oxiconazole-loaded plga-nanoparticulate-laden chitosan nano gel for topical application.

MATERIALS AND METHODS

Materials

Oxiconazole Hcl was procured from Research Lab Fine Chem Industries, polylactic co-glycolic acid 50:50 was procured from Nomisma Healthcare, Chitosan (87% deacetylation), Acetone, and Poloxamer-188 were procured from Isochem Laboratories, Kochi. The dialysis membrane was procured from Hi-Media Pharmaceuticals. All the solvents and other ingredients used are of analytical grade.

Animals

A total of 80 rabbits, divided into groups, were used for the experiment. The experiments were conducted in accordance with the guidelines and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee (IAEC) approved the procedures under IAEC No. *Invivo*/002/2024. During the 7-day acclimatization period, the rabbits were provided with unlimited access to food and water and housed in SS cage, with six rabbits per cage. The animal housing environment was maintained at an average temperature of 22°C with a 12-hour light/dark cycle.

Methods

Development of PLGA nanoparticle

Oxiconazole-loaded PLGA nanoparticles were prepared by using the nano-precipitation method. Nine batches were created by varying the concentration of PLGA and organic solvent. Oxiconazole and PLGA were dissolved in 5 ml of acetone, which acts as an organic phase. Simultaneously, the aqueous solution was prepared by the addition of Poloxamer 188 (250 mg) to 25 mL of warm water (40ºC) under constant stirring. Previously formulated organic phase was injected to the magnetically stirred (1200rpm, at 45ºC) aqueous stabilizer solution through a syringe at a constant flow rate (0.5mL/minute). Then, the prepared emulsion was kept overnight under constant magnetic stirring to completely evaporate the organic solvent. Finally, the prepared solution was centrifuged at 10000 rpm for 10 mins, then the nanoparticles were separated and lyophilized.[8]

Optimization

Oxiconazole-loaded PLGA nanogel was statistically optimized by using three levels and two factors (3^2) of complete factorial design. The optimization was performed by Minitab®21 software by varying the concentration of independent variables PLGA as X_1 (30mg-60mg), and organic solvent as X_2 (5–7.5 mL). The effect of PLGA and organic solvent influences the p.s (Y_1) and %EE (Y_2). This design depicted nine experimental runs. Lower particle size and greater entrapment efficiency were employed to determine optimized formulation composition. Later, the statistical analysis was evaluated to examine the impact of several factors (X_1 X_2) on (Y_1 Y_2).[18]

Characterization

Particle characterization

The prepared nanoparticles' particle size (PS) and polydispersity index (PDI) were measured using a particle size analyser (Malvern Zetasizer 2000MU).

Zeta potential

A Malvern Zetasizer was employed to measure the nanoparticles' zeta potential at 25°C. A 1 ml sample was placed in a polystyrene cuvette, and a zeta dip cell was utilized to determine the zeta potential.

Entrapment efficiency

The entrapment efficiency was analysed by the indirect method using the ultracentrifugation process. The prepared nanoparticles were filled into the centrifugation tube and run at 10000 rpm for 30min. After centrifugation the supernatant was collected and measured the absorbance by UV-visible spectrophotometer at 215nm.[19]

$$\%EE = \frac{\text{amount of drug added in nanoparticles-amount of drug unentrapped}}{\text{amount of drug added in nanoparticles}} \times 100$$

Scanning electron microscopy

A high-resolution electron microscope was used to assess the surface morphology of the prepared nanoparticles. The sample was carried out by lightly dusting the nanoparticle powder over a double adhesive tape that was adhered to an aluminum stub at an acceleration voltage of 15kV.[20]

Table 1: List of Independent Variable and Dependent Variable

Independent variables	*Dependent variables (Response)*
X_1 = PLGA 50:50	Y_1 = Particle size (nm)
X_2 = Acetone	Y_2 = Entrapment efficiency (%)

Atomic force microscopy (AFM)

To assess the morphology of Oxiconazole-loaded PLGA nanoparticles via AFM, approximately 10 microliters of the formulation were placed on a silicon wafer and allowed to evaporate at room temperature. The resulting images were captured using a multimode scanning probe microscope (Innova SPM, Bruker, Santa Barbara, CA) in semi-contact mode.[15]

Development of PLGA nano gel

Chitosan polymer was used to prepare Oxiconazole-loaded nano gel. The measured amount of chitosan (10 mg/mL) was placed into a beaker containing acetic acid solution (1% w/v) and set aside to fully dissolve. The formaldehyde (0.002g/g of chitosan) and glycerine (0.2 g/g of chitosan) were added to the chitosan solution and stirred continuously until the formation of gel takes place. The nanoparticles (1% of Oxiconazole equivalent in gel) was incorporated in the above mixture. Then methyparaben and triethanolamine were added as a preservative and to adjust pH.[21]

Drug content

Oxiconazole-loaded nanogel was taken and dissolved in the acetone. Then the sample was sonicated for 5 mins and centrifuged for 10 min at 10000 rpm. The supernatant was collected and filtered, and drug content in the formulation was determined after dilution using a spectrophotometer.[22]

Appearance

Oxiconazole-loaded PLGA nanogel underwent visual inspection to assess their uniformity, texture, absence of phase separation, and any signs of aggregation.[23]

Measurements of pH

Here, 20 ml of distilled water was used to dilute 1g of the roughly weighed amount of Oxiconazole-loaded PLGA nanogel. Then, the prepared gel solution was immersed, or the pH meter was placed in it. Three runs of the experiment were performed, and the average value were determined.[24]

Spreadability

The evaluation of spreadability is a crucial step in determining the appropriate viscosity and uniform distribution of gels. To perform this assessment, 1-gram of the prepared gel was placed between two glass slides of the same thickness. A weight of 1-kg was then applied to the slides for one minute, allowing the gel to spread out. Once the weight was removed, the diameter of the spread area was measured in centimeters.[25]

Infrared spectroscopy

The FTIR study was used to determine how drugs and polymers interacted. To verify if the formulation composition was compatible, the drug, excipients, physical mixture, and formulation peaks were measured. The sample was created in potassium bromide discs, with a JASCO FTIR 460 plus spectrophotometer having a 400–4000 cm^{-1} resolution was used to produce the spectra.[26]

Differential scanning calorimetry

A Mettler Toledo 821e Differential Scanning Calorimeter (DSC), coupled with STARe Software, was employed for the thermal analysis of SLN gel. This analysis was conducted within a temperature range of 25–85°C, employing a heating rate of 5K/min. The objectives were to ascertain the melting point, quantify the enthalpy of melting, and determine the crystallinity index (CI) of the SLN gel.[11]

Drug release

The drug release study of oxiconazole-loaded nanoparticles and nanogel was performed using dialysis bag. The samples containing 5mg of drug were filled into a dialysis bag and both sides were tightly tied. The release media phosphate buffer (pH-5.5) was taken in a beaker and placed over a temperature-enabled magnetic stirrer at 37±0.5ºC. Both samples were immersed in the release media and stirred at 100 rpm. At specific intervals, 5ml of released aliquots were collected and simultaneously replenished with an equal volume of fresh media to uphold study conditions. Subsequently, the samples underwent filtration and triplicate analysis using a UV-visible spectrophotometer at a wavelength of 215nm. The release data for the oxiconazole nanogel were fitted to various release kinetic models. The model exhibiting the highest regression value (R^2) was utilized to select the optimal release model and characterize the release behavior.[27]

Permeation study

The *ex-vivo* permeation analysis of oxiconazole nanogel utilized freshly acquired hairless goat skin procured from a nearby slaughterhouse, ensuring use within two hours of collection. The skin was prepared by eliminating underlying fat and loose tissues, followed by thorough washing with distilled water and subsequently with 0.1 N NaOH.[9]

The membrane was positioned between the donor and acceptor compartments of a diffusion cell, each with a designated effective area and volume. The acceptor compartment was filled with phosphate buffer solution at pH 5.5, while maintaining a constant temperature of 37±0.5ºC with continuous agitation. Oxiconazole nanogel was introduced into the donor compartment. At predetermined intervals, samples from the acceptor compartment were withdrawn and substituted with an equal volume of fresh buffer. These samples were then filtered, appropriately diluted, and analyzed at 215nm. The permeation flux was determined using the slope of the calibration plot according to the following formula.[8,10]

$$ER=\frac{\text{flux of formulayion}}{\text{flux of contol}}$$

Antifungal activity

The antifungal efficacy of Oxiconazole gel and Oxiconazole-loaded PLGA nano gel was tested against *Candida albicans* using the cup plate method. *Candida albicans* was cultured in potato dextrose broth for 2-3 days at 30°C with constant shaking at 150 rpm. Potato dextrose agar medium, preinoculated with 1% fungal pathogen, was poured into sterile petri dishes and allowed to solidify. Wells of 4 mm was bored using a sterile cork borer and filled with 100 µL of the sample. The plates were left at room temperature for 30 minutes to allow proper diffusion, then incubated at 30°C for 2-3 days and observed for zones of inhibition. Antibiotic fluconazole (1 mg/ml) was used as a positive control.[16]

Skin irritation study

The dermal irritation study was performed in male NW rabbits in a single dose as per OECD guideline 404. In this study, naïve NW rabbits were taken. Dorsal areas (approximately 6 cm^2) of the animals were shaved, and after 24 hours, a dose of 100 mg oxiconazole-loaded PLGA nano gel formulation F8 and plain oxiconazole gel used was applied with the help of a gauze patch. After 4 hours, traces of test substance were removed, and skin was observed for 14 days. Animals were housed under standard air-conditioned laboratory conditions (temperature: maximum 24ºC and minimum 23ºC). The test substance was applied as uniformly as possible over the exposed area of dorsal/flank skin. The test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 4 hours exposed period. During the 4 hours exposed period, animals were caged individually in order to avoid oral ingestion of the test chemical by other animals in the cage.[17]

RESULT AND DISCUSSION

Optimization

The study conducted involved the preparation of Oxiconazole nanoparticles using the emulsification sonication method, followed by statistical optimization using Minitab®21 software. Nine formulation runs were designed, and their results indicated significant differences in particle size (PS), encapsulation efficiency (EE), and zeta potential (ZP) of the prepared nanoparticles. The responses, Y_1 and Y_2, corresponding to PS and EE, respectively, were fitted into different design models, with the quadratic model being identified as the best-fit model due to its maximum regression value compared to other models. The lack of fit for each model was assessed and found to be well-fitted. The quadratic model for each response was further validated using ANOVA, where the sum of squares, degrees of freedom, F-value, and p-value of each factor for each response was calculated. Additionally, the effects of individual and combined independent variables on each response were elucidated using 3D-surface plots. The closeness between the actual and predicted values of each response for all formulations (F1-F9) was depicted, showing that the findings of the study are well accepted with the method used. This was evidenced by the linear proximity of points representing all factors on the graph, indicating agreement between the observed and predicted outcomes.[29]

Particle Size

The Malvern apparatus was utilized to assess the particle size (p.s) and polydispersity index (PDI) of Oxiconazole-loaded PLGA nanogel formulations. It was observed that the particle sizes of all formulations fell within the range of 185 to 400nm. The particle size distribution is illustrated in the Table 2 and graph provided in Figures 1-3. Additionally, all formulations exhibited PDI values below 1, indicating a uniform distribution of particle sizes within each formulation. Moreover, it was discovered that the PLGA and acetone concentrations affected the nano gel formulations' particle sizes. Particle size was specifically found to decrease with increasing acetone levels and to rise with greater PLGA concentrations significantly. This relationship is depicted in the response plot of particle size against PLGA and acetone.

Regression Equation

Particle Size = 208.37 + 17.30 PLGA_10 + 2.63 PLGA_20 - 19.93 PLGA_30 - 26.70 Org: Aq_2.5- 12.27 Org: Aq_5.0 + 38.97 Org: Aq_7.5

Zeta Potential

The zeta potential distribution graph for all nine nanogel formulations was obtained and is presented in Table 2 and Figures 4-6. Across all formulations, the zeta potential values varied from -15.3 to -23.2 mV. These values indicate that the nanogels possess a neutral zeta potential, likely attributed

Table 2: Observed responses from 3^2 factorial designs of nano-gel formulations

Batch code	*Drug (mg)*	*Independent Variables*		*Dependent Variables*		
		PLGA (mg) X_1	*Acetone (ml) X_2*	*Particle size Y_1*	*Zeta potential Y2*	*Entrapment efficiency Y_3*
1	30	10	2.5	190 ± 1.5	-21.4 ± 2.3	83.1 ± 3.05
2	30	10	5	205 ± 2.5	-16.6 ± 1.9	85.3 ± 2.11
3	30	10	7.5	282 ± 1.7	-15.3 ± 2.8	86.2 ± 2.02
4	30	20	2.5	182 ± 1.1	-22.1 ± 3.5	87.8 ± 1.01
5	30	20	5	198 ± 1.7	-19.8 ± 2.17	89.2 ± 217
6	30	20	7.5	253 ± 0.5	-17.7 ± .1.2	90.3 ± 1.06
7	30	30	2.5	173 ± 1.1	-23.2 ± 0.9	91.7 ± 2.01
8	30	30	5	185 ± 3.6	-20.7 ± 1.6	95.2 ± 2.09
9	30	30	7.5	207 ± 1.1	-17.9 ± 1.3	93.8 ± 3.05

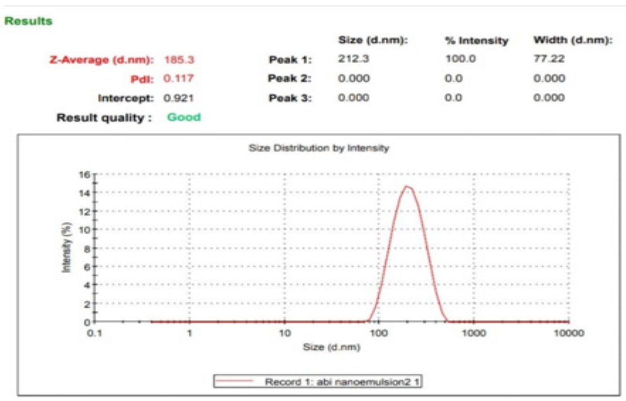

Figure 1: Particle size distribution of Nanogel formulation F(8)

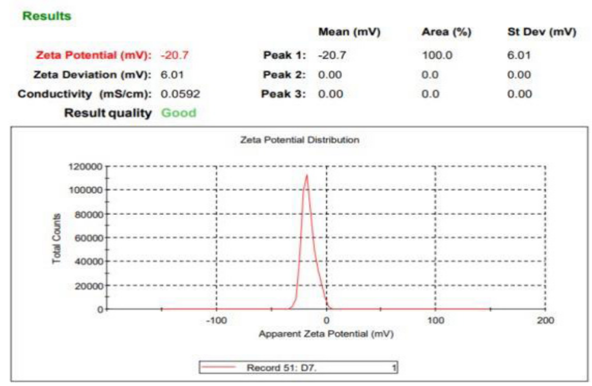

Figure 4: Zeta potential distribution of Nanogel formulation (F8)

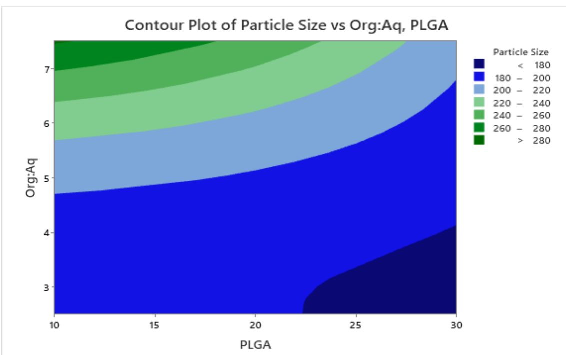

Figure 2: Effects of Acetone and PLGA on particle size are depicted in a contour plot

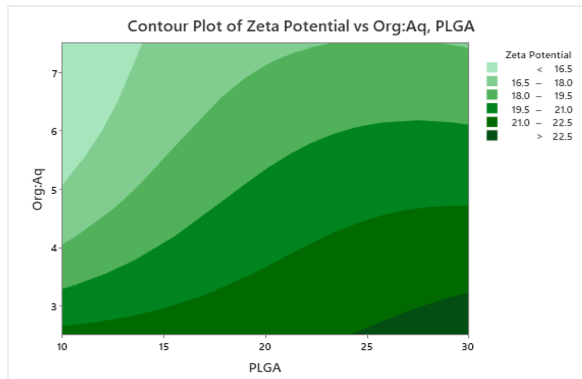

Figure 5: Effects of Acetone and PLGA on Zeta Potential are depicted in a contour plot

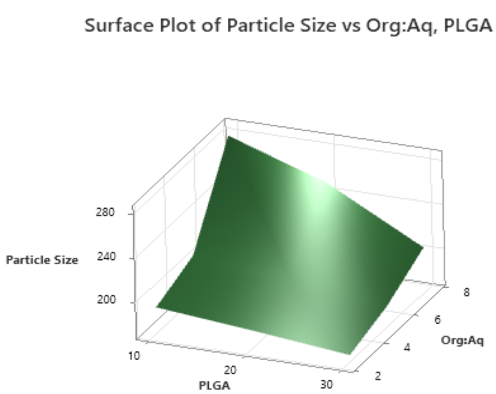

Figure 3: Effects of Acetone and PLGA on particle size are depicted in surface plot

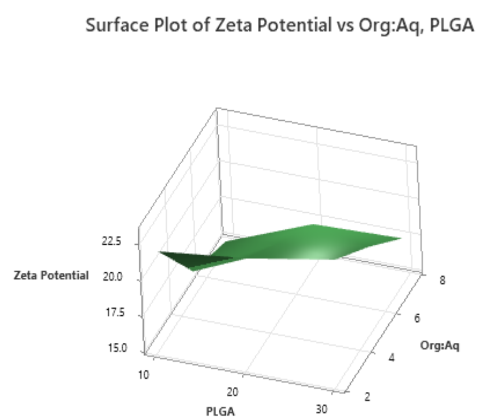

Figure 6: Effects of Acetone and PLGA on Zeta Potential are depicted in surface plot

to the usage of PLGA and acetone during formulation. It's important to note that the high zeta potential values contribute to preventing the aggregation of particles by inducing electric repulsion forces among them. This characteristic is crucial for maintaining the stability of the nano gel formulations.

Regression Equation

Zeta Potential = 19.411 - 1.644 PLGA_10 + 0.456 PLGA_20 ÷ 1.189 PLGA_30 + 2.822 Org: Aq_2.5- 0.378 Org: Aq_5.0 - 2.444 Org: Aq_7.5

Entrapment Efficiency

Oxiconazole-loaded PLGA nanogels have demonstrated entrapment efficiencies ranging from 83 to 95%. This efficiency is directly influenced by the levels of PLGA and acetone, primarily due to enhanced solubilization of the lipophilic drug. Notably, nano gel formulation (F8), containing 30 mg of PLGA and 5 ml of acetone, exhibited a higher entrapment efficiency compared to other nano gel formulations. This suggests that the specific combination of PLGA and acetone levels in formulation-8 optimally facilitates drug entrapment.

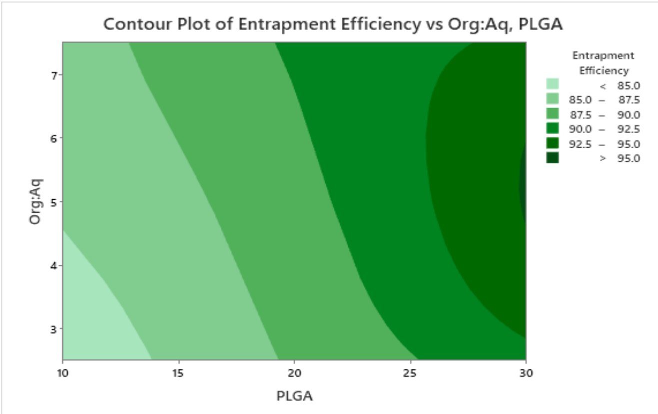

Figure 7: Effects of Acetone and PLGA on entrapment efficiency are depicted in a contour plot

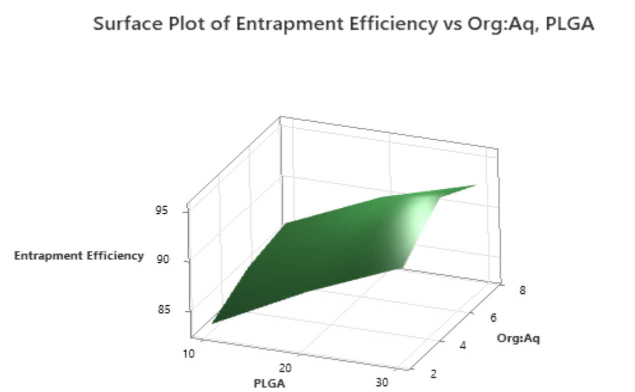

Figure 8: Effects of Acetone and PLGA on entrapment efficiency are depicted in surface plot

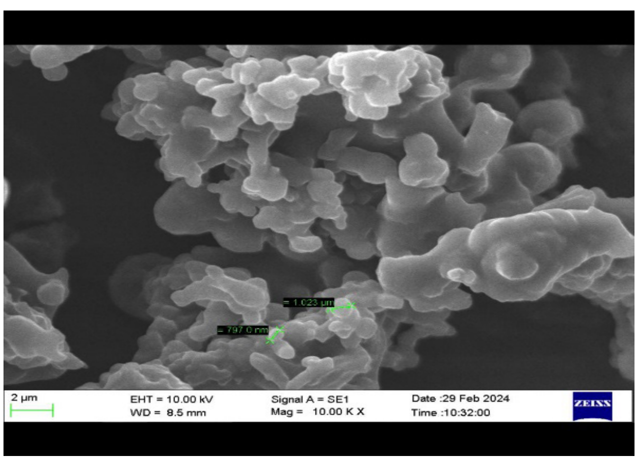

Figure 9: SEM image of Oxiconazole loaded PLGA nano gel formulation (F8)

Figures 7 and 8 and Table 2 illustrate the entrapment efficiency graph, further highlighting the performance of formulation 8 in this regard.

Regression Equation

Entrapment Efficiency = 89.089 - 4.222 PLGA_10 + 0.011 PLGA_20 + 4.211 PLGA_ 30 1.556 Org: Aq_2.5 + 0.811 Org: Aq_5.0 + 0.744 Org: Aq_7.5

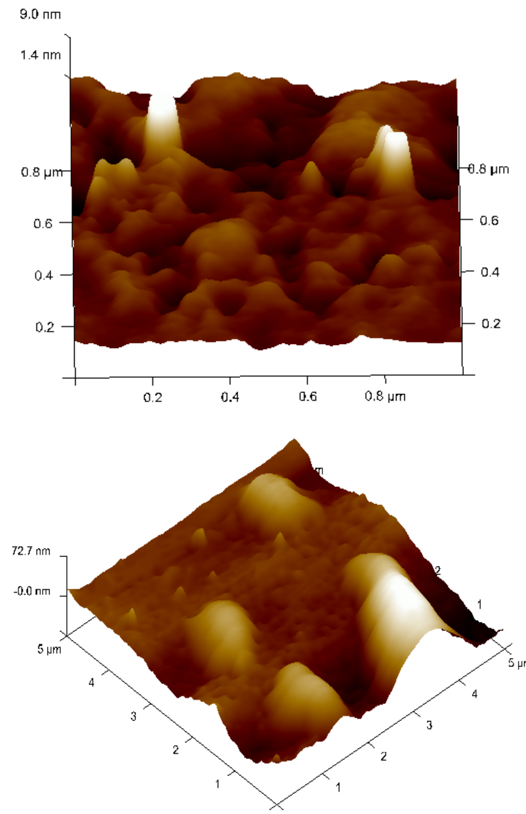

Figure 10: AFM image of Oxiconazole loaded PLGA nanogel formulation (F8)

Scanning Electron Microscope

The prepared oxiconazole nanoparticles has been evaluated for surface morphology using a scanning electron microscope (SEM). The image showed scattered particles with a smooth surface. There was no aggregation of particles observed, as shown in Figure 9.[30]

Atomic Force Microscopy

The nanogel formulation (F8) exhibited a particle size of 72.7 nm, as determined from AFM images. The topographic AFM images showed that the particles were spherical (Figure 10) with smooth surfaces, indicating a stable morphology.[31]

Development of Oxiconazole Nano Gel

Oxiconazole nanoparticle was successfully dispersed into chitosan polymer gel (10mg/ml) and prepared oxiconazole nano gel containing 1% of Oxiconazole. The gelling agent was optimized by taking different concentrations (5, 10, and 15 mg) of chitosan to select the optimum concentration. The gelling agent at (10mg/ml) concentration showed an ideal spreadability value than different formulations.

Characterization of Gel

The pH of the oxiconazole nanogel was determined to be 6.8 ± 0.3, an ideal range for topical formulations that typically does not cause irritation[10]. Additionally, the viscosity of the gel was measured at 16970 ± 14 cps, indicating its flow properties. This viscosity value is optimal for better adherence to the skin and

Table 3: Drug content and drug release of oxiconazole-loaded PLGA nano gel

Formulation code	*Drug Content ±SD (n = 3)*	*% Drug Release*
F1	69.63 ± 1.04	54.56 ± 1.01
F2	87.19 ± 1.07	51.19 ± 2.21
F3	60.85 ± 2.04	69.56 ± 1.2
F4	71.69 ± 2.5	59.27 ± 2.2
F5	85.93 ± 1.07	70.67 ± 1.5
F6	81.71 ± 1.03	65.71 ± 2.4
F7	72.62 ± 2.6	68.89 ± 2.09
F8	96.83 ± 1.14	74.06 ± 1.5
F9	80.05 ± 1.02	56.48 ± 1.6

spreadability. It is worth noting that the viscosity of the gel is influenced by factors such as particle size and polydispersity index (PDI), with larger particle sizes typically resulting in higher viscosity gels.[11] The spreadability of the oxiconazole nanogel was also assessed and found to be 14.75 ± 1.78 g/cm². This result indicates good spreadability, facilitated by the nanogels optimal viscosity. The combination of optimum viscosity and spreadability ensures that the topical gel can be easily applied to the skin, forming a thin, uniform layer.[12]

Drug Content

The drug content analysis of the prepared oxiconazole nano gel revealed a concentration of 96.83 ± 1.14% (Table 3). This indicates that the maximum amount of Oxiconazole was uniformly dispersed within the gel matrix, reflecting a desirable characteristic of the formulated product. The high drug content serves as evidence supporting the efficacy and authenticity of the method employed in the preparation of the gel formulations.

Appearance

The developed oxiconazole-loaded PLGA nanogel exhibited excellent uniformity, smooth texture, and a translucent appearance. Its tactile sensation was slippery, and there were no discernible lumps, indicating even distribution of the drug and PLGA suspension within the nanogel.

Measurements of pH

The pH of Oxiconazole loaded PLGA nano gel formulations was measured in the ranged from 6.1±0.15 to 6.8±0.3, aligning with human skin pH. Absence of skin irritation signs upon application of a gel. These values were sufficient to acquire a good viscosity and clarity of the gels.

Spreadability

Achieving optimal properties for the prepared Oxiconazole-loaded PLGA nanogel depends on attaining favourable spreadability. Spreadability values reflect the gel's application behavior and influence its therapeutic effectiveness. For formulation F8 nanogel, the measured spreadability value was (14.75 ± 1.78). These results indicate easy application and prolonged contact at the site without leakage, ensuring improved therapeutic outcomes.

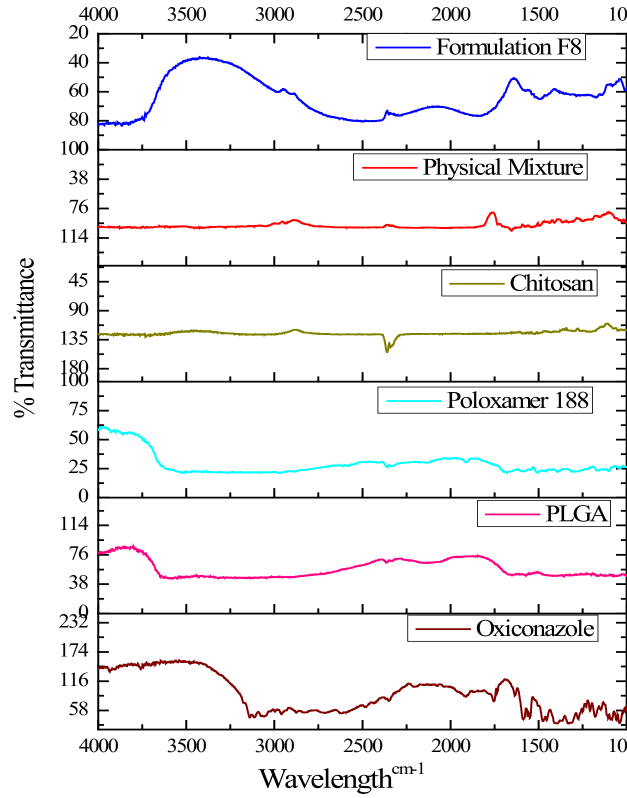

Figure 11: FT-IR image

Infrared Spectroscopy(FTIR)

FTIR study of the pure oxiconazole drug, PLGA, and physical mixture was performed to check confirmational changes in the characteristic peaks. The pure drug oxiconazole showed a sharp stretching C-H aromatic and aliphatic peak at 3139.54cm^{-1}, C-O carbonyl at 1229.4cm^{-1}, and C=C alkenes at 1634.38cm^{-1} respectively. The polymer PLGA showed a sharp stretching C=O carbonyl at 1652.7cm^{-1}, C-O ether at 1077.05cm^{-1}, and C-H aliphatic at 3576.34cm^{-1} respectively. The physical mixture exhibited the C-H aromatic and aliphatic at 3139.54cm^{-1}, C-O carbonyl at 1229.4cm^{-1}, and C=C alkenes at 1634.38cm^{-1} respectively showing no interaction as shown in Figure 11.[32]

Differential Scanning Calorimetry

DSC study of pure Oxiconazole, PLGA, Physical mixture and Formulation (F8) DSC was performed. The endothermic sharp peak for Oxiconazole and PLGA was observed at 148.1ºC and 174.66ºC, which was unaffected in the physical mixture, as shown in Figure 12. Formulation (F8) showed a peak at 177.08ºC.[33]

Drug Release Study

The release data was obtained from *in-vitro* studies of nano gel formulations F1 to F9 using Phosphate buffer pH 5.5. The drug release profiles of oxiconazole nanoparticles and oxiconazole nanogels in the comparative drug release study and the amount of Oxiconazole released over the tested period was evaluated.

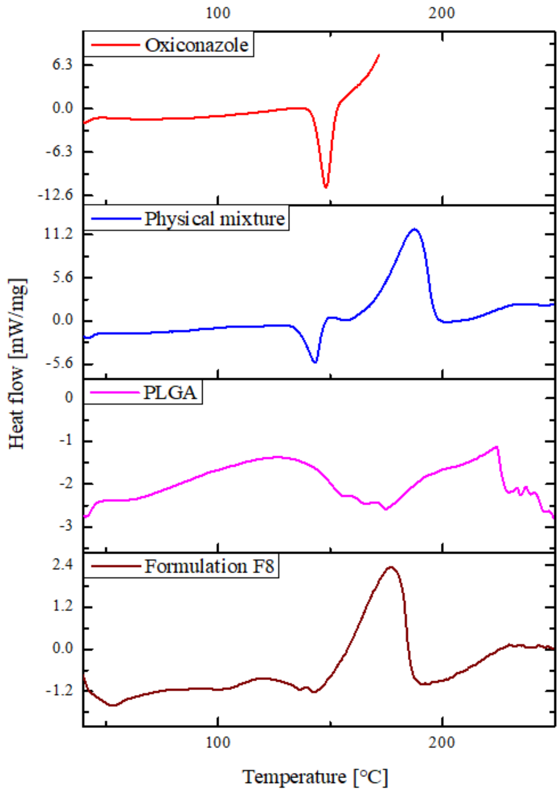

Figure 12: DSC image

Results indicated that oxiconazole nanoparticles exhibited a release of 85.9%, whereas oxiconazole nanogels showed a release of 74% within the 48-hour study period. This release pattern demonstrated sustained drug release characteristics. Initially, only a small fraction (10%) of Oxiconazole was released within the first 5 hours, with a gradual increase observed in subsequent stages. This sustained release pattern is attributed to the reported slow drug release property of PLGA, which is ideal for topical formulations. The slow release of the drug allows for a gradual increase in drug concentration at the target site over time. Comparatively, oxiconazole nanogels exhibited a significantly slower release rate than oxiconazole nanoparticles. This delay in drug release from the gel formulation is attributed to the slow diffusion of Oxiconazole from the chitosan gel matrix as shown in Figure 13 and Table 4 indicates different release kinetics for the formulation F8.[34]

Permeation Study

Goat skin was used for the permeation study due to its structural similarity to human skin. The amount of Oxiconazole diffused through the skin was compared between the selected formulation (F8) and Oxiconazole nanoparticles. The finalized formulation showed that 57.3% of the drug permeated from the Oxiconazole-loaded PLGA nano gel over 24 hours, outperforming the Oxiconazole nanoparticles. The polymer (PLGA) used in the preparation acted as a permeation enhancer. The amount of Oxiconazole permeated from the finalized formulation (F8) and the drug suspension are depicted in the Figure 14. Due to this structural similarity, nanogels can easily penetrate the skin, enhancing drug bioavailability. Additionally, acetone, used as a solvent, increased the solubility and permeability of the drug.[35]

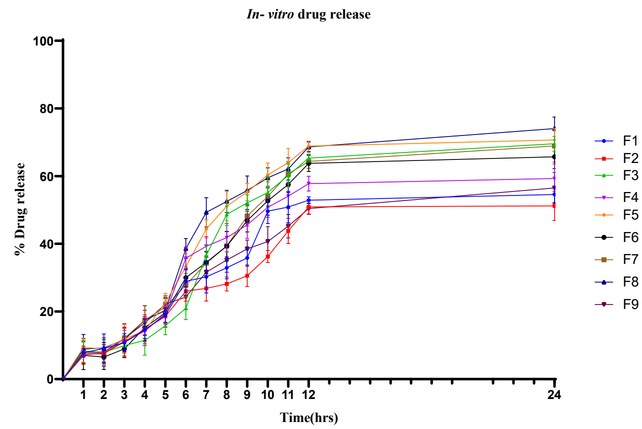

Figure 13: *In-vitro* release profile of Nanogel formulation (F1-F9) for 24 hours

Antifungal Activity

The antifungal study demonstrates that both Oxiconazole gel and Oxiconazole-loaded PLGA nano gel exhibit significant antifungal activity against *Candida albicans*. The Oxiconazole loaded PLGA nano gel showed a larger zone of inhibition (22±1.5 mm) compared to the gel (20±0.1 mm), suggesting enhanced efficacy due to improved drug delivery. Using Oxiconazole as a positive control confirmed these findings, indicating that the nano gel formulation could be a more effective treatment option as shown in Figure 15.[36]

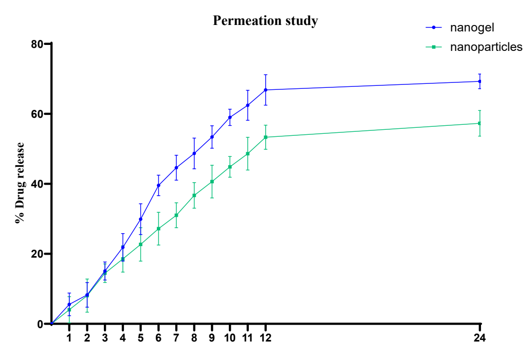

Figure 14: Permeation of Oxiconazole nanoparticles and nano gel

Table 4: Different release kinetics of formulation F8

Formulation	*Models*				
	Zero order	*First order*	*Higuchi*	*Korsmeyer-peppas*	*Hixson-crowell*
F8	0.6805	0.9198	0.8293	0.8453	0.9099

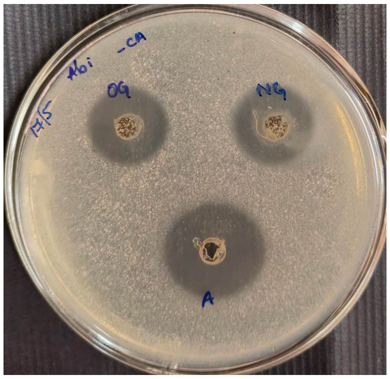

Figure 15: Antifungal activity of Oxiconazole gel and Oxiconazole loaded PLGA nano gel

Figure 16: Skin irritation study in animal 1

Figure 17: Skin irritation study in animal 2

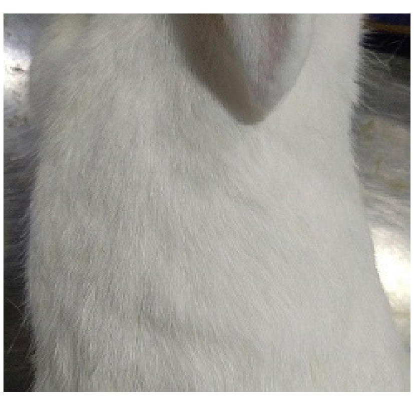

Figure 18: Skin irritation study in animal 3

Figure 19: Skin irritation study in animal 1

Figure 20: Skin irritation study in animal 2

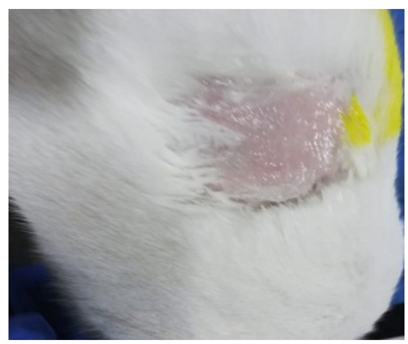

Figure 21: Skin irritation study in animal 3

Skin Irritation Study

Before application

After application

The animals showed no signs of erythema/inflammation or adverse skin reactions. Gross pathology also indicated no adverse changes in the skin.

Throughout a 14-day observation period, none of the dosing groups experienced erythema, skin toxicity, gross pathological changes, morbidity, or mortality. Therefore Nanogel of Oxiconazole acts as non-irritant and non-toxic to the skin was shown in Figure 16-21.[28]

CONCLUSION

Oxiconazole-loaded PLGA nanoparticles were synthesized through the nanoprecipitation method, with formulation optimization conducted using Minitab®21 software, considering PLGA and acetone as key factors. Both individual and combined effects of these factors were Particle size and entrapment efficiency were investigated. Chitosan, a mucoadhesive polymer, was then added to the optimized mixture to turn it into a gel. The gel formulation was characterized in order to determine the ideal values for factors such as pH, spreadability, viscosity, and medication content. The gel formulation produced sustained drug release, and permeation tests showed that Oxiconazole was more porous and retained in the gel than in oxiconazole-loaded PLGA nano gel or nanoparticles. This indicates the potential of oxiconazole-loaded PLGA nano gel for topical delivery, offering advantages in terms of sustained release and improved permeation for enhanced antifungal activity.

REFERENCES

1. Akhtar N, Verma A, Pathak K. Topical delivery of drugs for the effective treatment of fungal infections of skin. Current pharmaceutical design. 2015 Jun 1;21(20): 2892-913. https://doi.org/10.2174/1381612821666150428150456
2. Zhang T, Yang R, Yang S, Guan J, Zhang D, Ma Y, Liu H. Research progress of self-assembled nanogel and hybrid hydrogel systems based on pullulan derivatives. Drug Delivery. 2018 Jan 1;25(1):278-92.
3. Maddiboyina B, Desu PK, Vasam M, Challa VT, Surendra AV, Rao RS, Alagarsamy S, Jhawat V. An insight of nanogels as novel drug delivery system with potential hybrid nanogel applications. Journal of Biomaterials Science, Polymer Edition. 2022 Jan 24;33(2): 262-78.
4. Hay R. Therapy of skin, hair and nail fungal infections. Journal of Fungi. 2018 Aug 20;4(3): 99. https://doi.org/10.3390/jof4030099
5. Arslan A, Ozkan CK, Sig AK, Dogan E, Esim O, Cetinkaya S, Atalay F, Tas C, Savaser A, Ozkan Y. Evaluation of a novel oxiconazole nitrate formulation: The thermosensitive gel. Saudi pharmaceutical journal. 2018 Jul 1;26(5):665-72.
6. Ghasemian E, Vatanara A, Rouholamini Najafabadi A, Rouini MR, Gilani K, Darabi M. Preparation, characterization and optimization of sildenafil citrate loaded PLGA nanoparticles by statistical factorial design. DARU Journal of Pharmaceutical Sciences. 2013 Dec;21:1-0.
7. Yenilmez E, Başaran EB, Arslan R, Berkman MS, Güven UM, Bayçu C, Yazan Y. Chitosan gel formulations containing egg yolk oil and epidermal growth factor for dermal burn treatment. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2015 Feb 2;70(2):67-73.
8. Alshehri S, Imam SS. Formulation and evaluation of butenafine loaded PLGA-nanoparticulate laden chitosan nano gel. Drug Delivery. 2021 Jan 1;28(1):2348-60.
9. Pillai AB, Nair JV, Gupta NK, Gupta S. Microemulsion-loaded hydrogel formulation of butenafine hydrochloride for improved topical delivery. Archives of dermatological research. 2015 Sep;307:625-33.
10. Kumar L, Verma R. In vitro evaluation of topical gel prepared using natural polymer. International journal of drug delivery. 2010 Jan 1;2(1).
11. Khurana S, Bedi PM, Jain NK. Preparation and evaluation of solid lipid nanoparticles based nanogel for dermal delivery of meloxicam. Chemistry and physics of lipids. 2013 Oct 1;175:65-72.
12. Riaz A, Hendricks S, Elbrink K, Guy C, Maes L, Ahmed N, Kiekens F, Khan GM. Preparation and characterization of nanostructured lipid carriers for improved topical drug delivery: evaluation in cutaneous leishmaniasis and vaginal candidiasis animal models. AAPS PharmSciTech. 2020 Jul;21:1-4.
13. Koca HD, Doganay S, Turgut A, Tavman IH, Saidur R, Mahbubul IM. Effect of particle size on the viscosity of nanofluids: A review. Renewable and Sustainable Energy Reviews. 2018 Feb 1;82:1664-74.
14. Motawea A, Borg T, Abd El-Gawad AE. Topical phenytoin nanostructured lipid carriers: design and development. Drug development and industrial pharmacy. 2018 Jan 2;44(1):144-57.
15. Tetik G, Rencber S, ÖZOĞLU ET, Yapar EA, Karavana SY, Özer Ö. Design and Evaluation of Topical Antioxidant Nanogel Formulations. Journal of cosmetic science. 2021 Jan 1;72(1).
16. Pereira P, Pedrosa SS, Correia A, Lima CF, Olmedo MP, González-Fernández Á, Vilanova M, Gama FM. Biocompatibility of a self-assembled glycol chitosan nanogel. Toxicology in Vitro. 2015 Apr 1;29(3):638-46.
17. Weil CS, Scala RA. Study of intra-and interlaboratory variability in the results of rabbit eye and skin irritation tests. Toxicology and Applied Pharmacology. 1971 Jun 1;19(2):276-360.
18. Avasatthi V, Pawar H, Dora CP, Bansod P, Gill MS, Suresh S. A novel nanogel formulation of methotrexate for topical treatment of psoriasis: optimization, in vitro and in vivo evaluation. Pharmaceutical development and technology. 2016 Jul 3;21(5):554-62.
19. Patel J, Patel B, Banwait H, Parmar K, Patel M. Formulation and evaluation of topical aceclofenac gel using different gelling agent. Int J Drug Dev Res. 2011 Jan;3(1):156-64.
20. Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. The Journal of Strength & Conditioning Research. 2005 Feb 1;19(1):231-40.
21. Wang H, Qian J, Ding F. Recent advances in engineered chitosan-based nanogels for biomedical applications. Journal of materials chemistry B. 2017;5(34):6986-7007.
22. Sharma A, Garg T, Aman A, Panchal K, Sharma R, Kumar S, Markandeywar T. Nanogel—an advanced drug delivery tool: Current and future. Artificial cells, nanomedicine, and biotechnology. 2016 Jan 2;44(1):165-77.
23. Pedrosa SS, Gonçalves C, David L, Gama M. A novel crosslinked hyaluronic acid nanogel for drug delivery. Macromolecular Bioscience. 2014 Nov;14(11):1556-68.
24. Zhou T, Xiao C, Fan J, Chen S, Shen J, Wu W, Zhou S. A nanogel of on-site tunable pH-response for efficient anticancer drug delivery. Acta biomaterialia. 2013 Jan 1;9(1):4546-57.
25. Nnamani PO, Ugwu AA, Nnadi OH, Kenechukwu FC, Ofokansi KC, Attama AA, Lehr CM. Formulation and evaluation of transdermal nanogel for delivery of artemether. Drug Delivery and Translational Research. 2021 Aug;11:1655-74.
26. Ferrer MC, Dastgheyb S, Hickok NJ, Eckmann DM, Composto RJ. Designing nanogel carriers for antibacterial applications. Acta biomaterialia. 2014 May 1;10(5):2105-11.
27. Ahmed S, Alhareth K, Mignet N. Advancement in nanogel formulations provides controlled drug release. International Journal of Pharmaceutics. 2020 Jun 30;584:119435.
28. PATIL A, KONTAMWAR P. Formulation and evaluation of antifungal nanogel for topical drug delivery system. Asian J Pharm Clin Res. 2021;14(10):127-34.
29. Pal R, Pandey P, Jha D, Dutta P, Sahoo S, Gupta R, Rizwan M, Keskar MS, Kumar V, Chawra HS. The Utilization of

32Full Factorial Design (FFD) for Optimization of Lincomycin Hydrochloride (LNH) Loaded Nanogel Involving; Design of Experiments (DoE) an Advanced Approach. Advances in Research. 2023 Dec 21;24(6):272-81.

30. Kuckling D, Vo CD, Wohlrab SE. Preparation of nanogels with temperature-responsive core and pH-responsive arms by photo-cross-linking. Langmuir. 2002 May 28;18(11):4263-9.
31. Huang X, Misra GP, Vaish A, Flanagan JM, Sutermaster B, Lowe TL. Novel nanogels with both thermoresponsive and hydrolytically degradable properties. Macromolecules. 2008 Nov 25;41(22):8339-45.
32. Jayakumar R, Nair A, Rejinold NS, Maya S, Nair SV. Doxorubicin-loaded pH-responsive chitin nanogels for drug delivery to cancer cells. Carbohydrate Polymers. 2012 Feb 14;87(3):2352-6.
33. Chatap V, Choudhari G, Jain P, Bhat MR. Synthesis and Characterization of Hydroxypropyl Sesbania Galactamannan Seed Gum for Pharmaceutical Application. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):303-9.Tan JP, Goh CH, Tam KC. Comparative drug release studies of two cationic drugs from pH-responsive nanogels. European Journal of Pharmaceutical Sciences. 2007 Dec 1;32(4-5):340-8.
34. Liu R, Sun L, Fang S, Wang S, Chen J, Xiao X, Liu C. Thermosensitive in situ nanogel as ophthalmic delivery system of curcumin: development, characterization, in vitro permeation and in vivo pharmacokinetic studies. Pharmaceutical development and technology. 2016 Jul 3;21(5):576-82.
35. Farooq U, Rasul A, Sher M, Qadir MI, Nazir I, Mehmood Y, Riaz H, Shah PA, Jamil QA, Khan BA. Development, characterization and evaluation of antifungal activity of miconazole based nanogel prepared from biodegradable polymer. Pakistan Journal of Pharmaceutical Sciences. 2020 Jan 2;33.