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Formulation, Optimization And Characterization Of Hydroquinone And Tretinoin-Loaded Polymeric Nanoparticle Gel For Topical Drug Delivery

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ABSTRACT

The aim of this study was to develop and optimize polymeric nanoparticle (PNP) formulations and gel systems for the controlled and sustained delivery of hydroquinone (HYQ) and tretinoin (TRE). The nanoparticle formulations were prepared using the solvent evaporation method, where Eudragit RS 100 served as the encapsulating polymer, and polyvinyl alcohol (PVA) acted as a stabilizer. Design of Experiment (DOE) software (Design Expert Version 12.0.1.0) was used to optimize the formulation variables, including the polymer concentration, surfactant concentration, and stirring time. A total of 12 formulations were prepared with varying parameters, and particle size, entrapment efficiency (EE), and stability were evaluated. The optimized formulation, NG1, exhibited a particle size of 146.08 nm (predicted: 144.19 nm) and an entrapment efficiency of 86.77% (predicted: 88.15%). The zeta potential was -22.9 mV, indicating good stability of the nanoparticles. In-vitro drug release studies revealed that NG1 provided sustained release, with 96.13% of the drug released at 16 hours. The release kinetics of NG1 were best described by Higuchi's model ($R^2 = 0.9961$), confirming that the drug release followed a diffusion-controlled mechanism. In the stability study conducted over 3 months at 25°C, NG1 maintained a clear appearance and showed minimal changes in drug content, with hydroquinone (HYQ) and tretinoin (TRE) retaining 94.85% and 93.67% of their initial content, respectively. The pH increased slightly from 5.1 ± 0.03 to 5.5 ± 0.05, and viscosity decreased from 5628 ± 0.53 cps to 5025 ± 0.12 cps. These results demonstrate that NG1 is a stable and effective formulation for controlled drug delivery. Additionally, six gel formulations (NG1–NG6) were prepared and characterized for their physical properties, including appearance, pH, viscosity, extrudability, and spreadability. NG1 was selected as the optimized gel formulation based on its favorable characteristics, such as a pH of **5.1 ±** 0.03, viscosity of 5628 ± 0.53 cps, and excellent extrudability (+++). The gel showed a high drug content retention of 99.81% for HYQ and 98.26% for TRE at baseline. The study demonstrated that NG1 nanoparticles and the associated gel formulation offer a promising approach for the transdermal delivery of hydroquinone and tretinoin, with sustained drug release, good stability, and favorable drug permeation characteristics.

Keywords: *Polymeric Nanoparticles, Hydroquinone, Tretinoin, Nanogel, Drug Delivery, Box- Behnken Design, Entrapment Efficiency, Sustained Release, Dermatological Therapy.*

1. INTRODUCTION

The use of combination therapy, particularly in the treatment of dermatological conditions such as hyperpigmentation, acne, and photoaging, has gained significant attention in recent years. Hydroquinone and tretinoin are two active pharmaceutical ingredients (APIs) widely used for such purposes. Hydroquinone is known for its skin-lightening effects, mainly by inhibiting melanogenesis, whereas tretinoin, a derivative of Vitamin A, is used for its anti-acne and anti- aging properties due to its ability to accelerate cell turnover and reduce hyperkeratinization (Liu et al., 2018).

However, both compounds have limitations when used individually. Hydroquinone may cause skin irritation, especially in higher concentrations, while tretinoin may result in excessive dryness and peeling, which can lead to poor patient compliance (Khaled & Ahsan, 2021). Thus, combining these two agents in a single formulation could enhance their therapeutic efficacy while potentially reducing the adverse effects associated with their individual use.

To overcome the inherent limitations of these APIs and to improve their skin penetration and therapeutic performance, advanced drug delivery systems such as polymeric nanoparticles (PNPs) have been proposed. Polymeric nanoparticles, typically ranging from 10 to 1000 nm in size, offer several advantages, including enhanced bioavailability, sustained drug release, and targeted delivery to specific skin layers. These nanoparticles are also advantageous because they can protect the

drugs from degradation, improve skin permeation, and provide controlled release over an extended period, reducing the frequency of application and improving patient compliance (Agarwal et al., 2020; El-Laithy et al., 2019).

In recent years, the development of gel formulations incorporating polymeric nanoparticles has garnered attention due to their ability to provide a smooth and non-greasy texture, ideal for topical application. These gels can serve as effective carriers for hydrophilic and lipophilic drugs alike, offering a high level of stability and an appropriate vehicle for the controlled release of the active ingredients. By encapsulating hydroquinone and tretinoin in polymeric nanoparticles, these compounds can be delivered more effectively to the skin layers, optimizing their therapeutic effects in combination therapy (Singh et al., 2021).

The present study aims to prepare and evaluate a polymeric nanoparticle gel containing both hydroquinone and tretinoin, intended for the treatment of hyperpigmentation and acne, two common dermatological issues that frequently require combination therapy. This formulation is expected to enhance the efficacy and stability of both drugs while minimizing their individual adverse effects (Thakur et al., 2020).

2. MATERIAL AND METHODS MATERIAL

The following chemicals were used in the preparation of the polymeric nanoparticle gel formulation: propylene glycol, methyl paraben, triethanolamine, petroleum ether, glacial acetic acid, sodium hydroxide, tretinoin, hydroquinone, methanol, ethanol, DCM, chloroform, nitroprusside, concentrated H_2SO_4 and HCl, 95% alcohol, ammonia, magnesium, copper sulfate solution, Eudragit RS 100, and PVA. These chemicals were sourced from suppliers including Loba Chemie, Merck, Research lab, Fizmerck, Molychem, Clorofiltind, Himedia, and Evonik Operations GmbH to ensure high-quality materials for the formulation process.

Methods

Optimization of formulation by design of expert (DOE) software

The dependent variables' values and their respective ratios are displayed in Table. This component mixture was used in the experiment, which produced 12 different batches of nanoparticle formulation. Numerous nanoparticle lots were made and thereafter evaluated for every reaction, as mentioned. After fitting the observed responses to 12 runs trails, it was found that the linear model best fit the two dependent variables. Analysis of variance (ANOVA) is used to determine the model's significance when compared to other models. Every response was recorded for 12 runs trails, and the table shows the relationship between the independent and dependent variables.

Formulation of Polymeric Nanoparticle using Eudragit RS 100 Polymer

The solvent evaporation method was employed for the preparation of hydroquinone and tretinoin nanoparticles.

In this process, hydroquinone (2.0%) and tretinoin (0.025%) were dissolved in a solvent mixture of acetone and methanol. Under constant stirring with a magnetic stirrer, the organic solution was carefully added to the aqueous phase containing polyvinyl alcohol, which acted as a stabilizing agent. The resulting emulsion was subjected to sonication using a probe sonicator for 6 minutes, ensuring the reduction of droplet size to achieve nanoscale dimensions. Subsequently, the organic solvent was evaporated through continuous stirring on a magnetic stirrer for approximately 4 to 5 hours. This step facilitated the formation of nanoparticles. Afterward, the nanoparticle suspension was centrifuged at 10,000 rpm for 30 minutes to separate and collect the nanoparticles efficiently.

To enhance the stability and preservation of the nanoparticles, the prepared emulsion was subjected to lyophilization (freeze-drying) for 48 hours. The polymer used for this formulation was Eudragit RS 100, which provided the necessary matrix for encapsulating the drugs and ensuring controlled release properties (Saharan *et al.*, 2019).

Design of experiment

Using Design Expert (Version 12.0.1.0) software, the experiment was designed for the polymeric nanoparticle formulation. In this case, the second-order polynomial model represented the quadratic response surfaces. Tables 1 display the independent and dependent variables that were chosen.

Table 1: Limits of Variables (Constraints)

Name	Lower Limit	Upper Limit	Importance
A: Polymer	50	300	3
B: Surfactant	0.1	0.5	3
C: Stirring time	10	60	3

Particle size	131.2	684.5	3
EE	60.9	91.2	3

Table 2: Formulation trials as per Box–Behnken design

S. No	F. code	Factor 1 Polymer Eudragit RS 100 (mg) X1	Factor 2 Surfactant PVA (%) X2	Drug HYQ and Tretinoin (%)	Factor 3 Stirring time (Min.) X3	Response 1 Particle size (nm) Y1	Response 2 Entrapment efficiency (%) Y2
1	NP 1	175	0.5	2.0- 0.025	10	684.5	71.6
2	NP 2	300	0.1	2.0- 0.025	35	440.8	91.2
3	NP 3	175	0.5	2.0- 0.025	60	131.2	73.5
4	NP 4	50	0.3	2.0- 0.025	10	652.8	70.3
5	NP 5	175	0.1	2.0- 0.025	60	155.7	81.7
6	NP 6	50	0.5	2.0- 0.025	35	374	62.1
7	NP 7	300	0.3	2.0- 0.025	10	595.4	88.2
8	NP 8	300	0.5	2.0- 0.025	35	304.4	87.7
9	NP 9	300	0.3	2.0- 0.025	60	161.0	89.5
10	NP 10	50	0.3	2.0- 0.025	60	159.2	60.9
11	NP 11	175	0.1	2.0- 0.025	10	521.2	85.2
12	NP 12	50	0.1	2.0- 0.025	35	306.1	68.9

Evaluation parameter of drug loaded Nanoparticle

Particle size

The size of particles is maintained during polymerization for the formation of free following powders having fine aesthetic attributes. The cumulative graph is maintained or plotted as particle size against time to study effect of particle size on drug release. Using a Malvern Instruments limited, at a fixed angle at 25°, photon correlation spectroscopy (PCS) was used to measure the average particle size of nanoparticles. The samples were examined for particle size after being diluted ten times with distilled water (Sharma *et al.*, 2011).

Zeta potential

In order to ascertain the particle charge and movement velocity of the particles in an electric field, the zeta potential was measured. In the current study, Zetasizer Malvern instruments to examine the nanoparticle after diluting them 10 times with pure water (Swaminathan *et al.*, 2010).

Scanning Electron Microscopy (SEM)

The electron beam from a scanning electron microscope was used to acquire the morphological characteristics of the drug-loaded polymeric nanoparticle. Under vacuum, a sputter coater

applied a thin layer (2–20 nm) of metal (such as platinum, palladium, or gold). An electron beam was then used to assault the pre-treatment specimen, producing secondary electrons called augers. Only the electrons scattered at a 90° angle were

selected from this interaction between the electron beam and the specimen's atoms, and they were then processed further using Rutherford and Kramer's Law to produce surface topography images (Ahmed *et al.*, 2020).

% Entrapment efficiency

A vortex mixer was used to precisely weigh 10 mg of nanoparticle and 5 ml of methanol in a volumetric flask, which was then shaken for one minute to determine the entrapment effectiveness. The 10 ml volume was added. After filtering and diluting the mixture, the concentration of tretinoin and hydroquinone were measured using spectrophotometry at a wavelength of 305.0 nm (Swetha *et al.*, 2011).

$$\text{Loading Efficiency} = \frac{\text{Theoretical Drug Content}}{\text{Actual Drug Content in Nanoparticle}} \times 100$$

In-vitro Drug release study

In order to determine the % drug release and the release kinetics of an optimal formulation, raw data from in vitro release tests was evaluated in the current study and fitted to several equations and kinetics models (Kumar *et al.*, 2020, Khoshbakht *et al.*, 2020, Li *et al.*, 2017).

Preparation of Polymeric nanoparticle loaded Nano-Gel (NG)

Simple hydration method was used for preparation of gel base. Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr. and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container Carboxymethyl cellulose and methyl paraben was added into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then tri-ethanol amine (Drop wise) was added to neutralize the pH and nanoparticle of optimized formulation was incorporated into the dispersion to obtained Gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps (Abbas *et al.*, 2019).

Table 3: Composition of Nanogel (NG) Formulations

F. Code	Carbopol 934 (gm)	Carboxymethyl cellulose (CMC) (gm)	Propylene glycol (ml)	Methyl paraben (ml)	Polymeric Nanoparticle (mg)	Tri-ethanolamine (ml)
NG1	1.00	0.63	1.00	0.2	100	1.0
NG2	0.63	0.25	1.00	0.2	100	1.0
NG3	0.25	0.63	1.00	0.2	100	1.0
NG4	0.63	0.25	1.00	0.2	100	1.0
NG5	0.63	1.00	1.00	0.2	100	1.0
NG6	1.00	0.50	1.00	0.2	100	1.0

Characterization of Polymeric Nanoparticle loaded Nanogel Physical appearance

The prepared Gel formulation was evaluated for appearance, Clear, and homogeneity by visual observation (Kumar and Eswaraiah 2020).

Determination of pH

pH of the formulation was determined by using Digital pH meter (EI). The two readings should agree to within the accuracy limits of the meter. The samples were analyzed in triplicate. If slight deviations in pH were noted, it was adjusted to skin pH using drop wise addition of tri-ethanolamine solution (McGlynn, W. 2003).

Viscosity

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 61 at 100 rpm at the temperature of 25^0C (Monica and Gautami 2014).

Spreadability

An ideal topical gel should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was

evaluated by placing about 1g of formulation on a glass slide. Another glass slide of the same length was placed above that, and a mass of 50 mg was put on the glass slide so that the gel gets sandwiched between the two glass slides and spreads at a certain distance. The time taken for the gel to travel the distance from the place of its position was noted down. Spreadability was determined by the following formula

S= M×L/T

Where, S-Spreadability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T-Time for spreading gel in sec (Sandeep, D. S. 2020).

Extrudability study

The nanogel formulations were filled into collapsible metal tubes or aluminum collapsible tubes.

The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

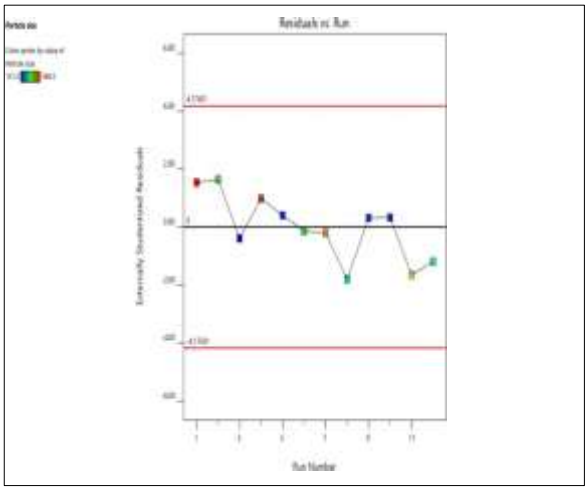
***In vitro* drug permeation study**

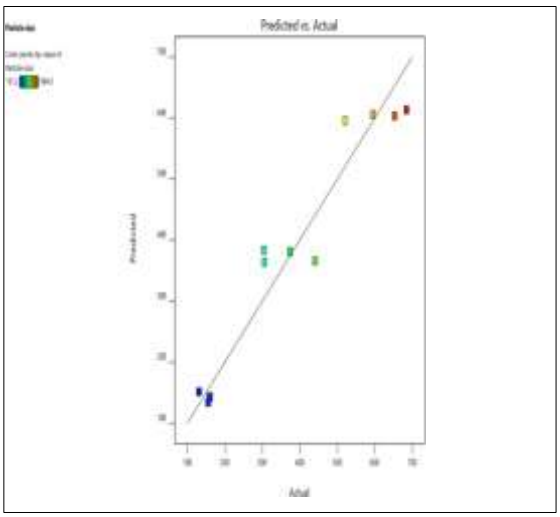
In Franz-type diffusion cells, the permeabilization of a nanoparticle gel containing tretinoin and hydroquinone through a membrane was carried out. Phosphate buffer (pH 6.8) was used as the receptor medium, and it was continuously shaken at 100 rpm using a tiny magnetic bar. A recirculating water jacket kept the receiver compartment at 37±0.2◦C. In the donor compartment, a quantity of nanoparticle equal to the required dosage of medication was added. At appropriate dilutions were taken out of the receptor compartment using the sampling portion. For this investigation, experiments were carried out in triplicate, and sink conditions were consistently upheld. Sample was examined using UV spectrophotometry at λ max 294.5 nm and 336.0 nm of medication to determine the amount of Tretinoin and Hydroquinone (Rahman *et al.*, 2015).

Stability

The short-term stability study of the prepared formulation (NG1) was conducted by storing the Formulation packs at 40º ± 2ºC and 65% relative humidity, in line with ICH guidelines. The Stability was assessed over three months at intervals of 1, 2, and 3 months, evaluating changes in Appearance, pH, drug content, and viscosity. The formulation was visually checked for any physical Changes, the pH was measured using a pH meter, drug content was analyzed using appropriate Analytical methods such as UV-spectrophotometry, and viscosity was determined using a viscometer. All data were analyzed to determine any significant changes over the testing period.

3. RESULTS AND DISCUSSION





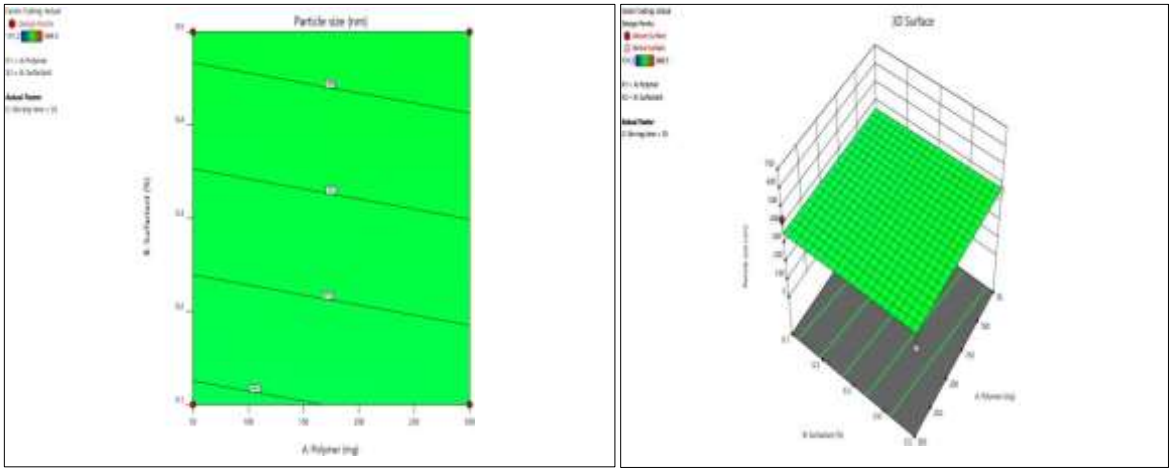


Figure 1: Response Surface Plot Showing Combined Effect of Polymer on Particle Size of Nanoparticle Formulation

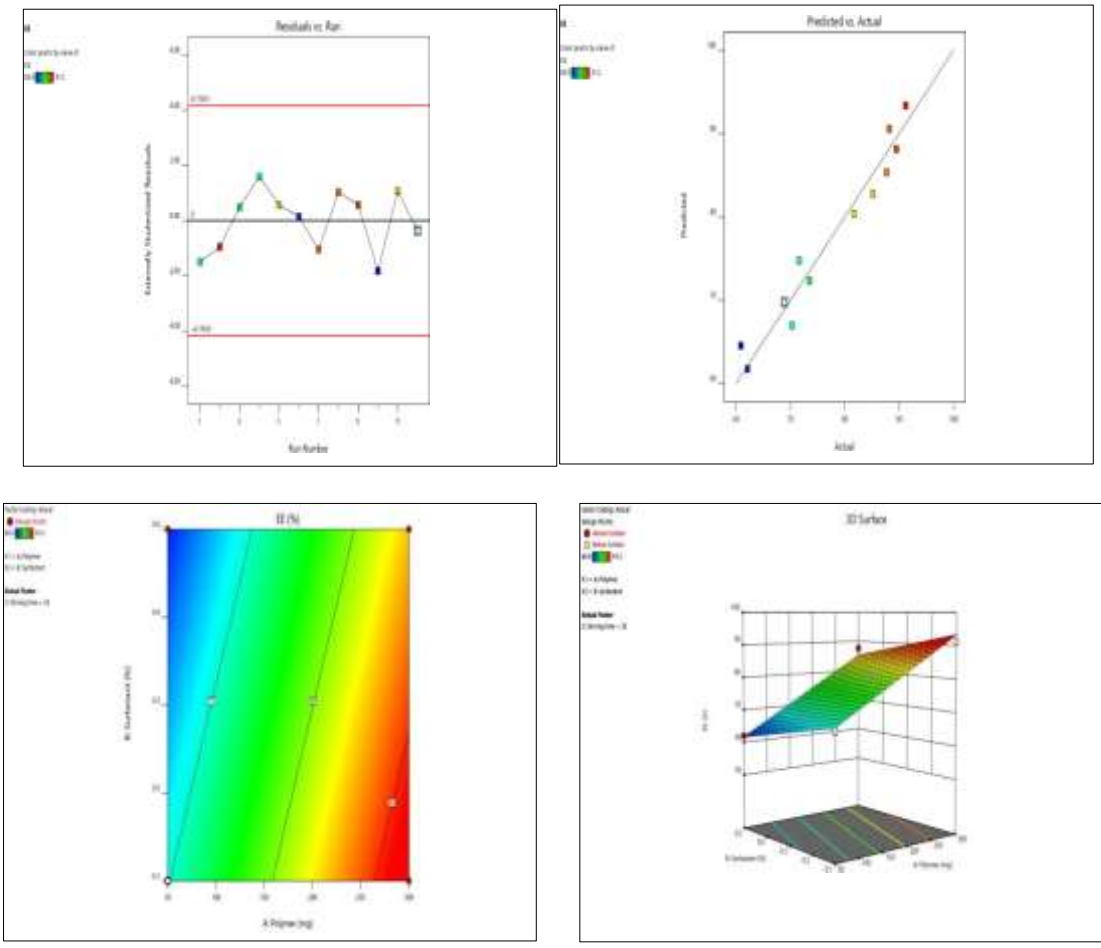


Figure 2: Response Surface Plot Showing Combined Effect of Polymer and Surfactant on % Entrapment Efficiency of Nanoparticle Formulation

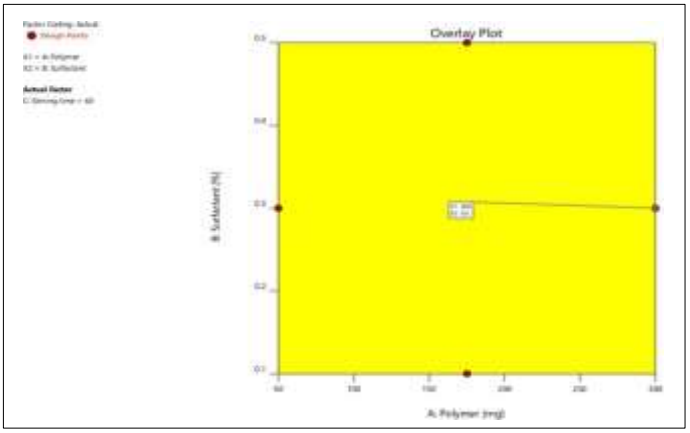


Figure 3: Overlay Plot of Nanoparticle Formulation for Optimization (Affecting Polymer and Surfactant)

The formulation of hydroquinone and tretinoin-loaded polymeric nanoparticles was optimized using the Design Expert software (Version 12.0.1.0) by applying a design of experiments (DOE) approach. The solvent evaporation method enabled the successful preparation of nanoparticles, with Eudragit RS 100 serving as the encapsulating polymer and PVA as the stabilizer. Optimization involved 12 experimental runs, where a linear model best fit the two dependent variables: particle size and entrapment efficiency (EE). ANOVA confirmed the model's significance, validating the influence of formulation factors. The response surface plots (Figures 1 and 2) clearly demonstrated how polymer concentration and surfactant level significantly impacted both particle size and EE.

Specifically, increased polymer concentrations resulted in larger particle sizes, while a balanced ratio of polymer and surfactant enhanced EE. Stirring time also played a crucial role in achieving nanoscale dimensions. The overlay plot (Figure 3) further aided in identifying the optimal formulation window where the desired particle size and EE were simultaneously achieved. Overall, this systematic DOE approach provided a robust method for fine-tuning formulation parameters, ensuring efficient drug encapsulation and stable nanoparticle production.

Table 4: Results of Predicted and Actual Value of Entrapment Efficiency and Particle Size

Formulation code	Actual Value of Entrapment efficiency	Predicted Value of Entrapment efficiency	Actual Value of Particle size	Predicted Value of particle size
NPs 1	78.25	76.09	684.50	613.50
NPs 2	74.02	84.89	440.80	366.26
NPs 3	78.63	77.25	131.20	151.80
NPs 4	66.35	69.62	652.80	603.52
NPs 5	85.63	86.05	155.70	134.22
NPs 6	62.30	68.45	374.00	381.46
NPs 7	81.24	87.26	595.40	605.90
NPs 8	86.25	76.05	304.40	383.83
NPs 9	61.08	68.41	161.00	144.20
NPs 10	83.74	78.42	159.20	141.82
NPs 11	86.79	78.46	521.20	595.92

NPs 12	89.52	86.09	306.10	363.88

The comparison between actual and predicted values of entrapment efficiency and particle size demonstrates good agreement, confirming the reliability of the DOE model. Minor deviations were observed, likely due to experimental conditions, but the overall trend was consistent. The highest EE (89.52%) was seen in NPs 12, showing effective formulation. The model proved effective in predicting outcomes and optimizing nanoparticle properties, supporting its use in formulation development.

Table 5: Results of Evaluation of optimized nanoparticles formulation

Formulation (F3)	**Particle size (Predicted value)**	**Particle size (Actual value)**	**Entrapment efficacy at (305 nm) (Predicted value)**	**Entrapment efficacy at (305 nm) (Actual value)**	**Zeta potential (mV)**
Nanoparticle	144.19 nm	146.08 nm	88.15 %	86.77 %	- 22.9

The optimized formulation (F3) showed strong agreement between predicted and actual values, confirming the model's accuracy. The actual particle size was 146.08 nm, close to the predicted 144.19 nm, indicating good control over size distribution. Similarly, the actual entrapment efficiency (86.77%) was in line with the predicted value (88.15%), reflecting effective drug loading. The zeta potential of -22.9 mV suggests moderate stability of the nanoparticles due to sufficient surface charge, which helps prevent aggregation. Overall, F3 demonstrated favorable characteristics for stable and efficient drug delivery.

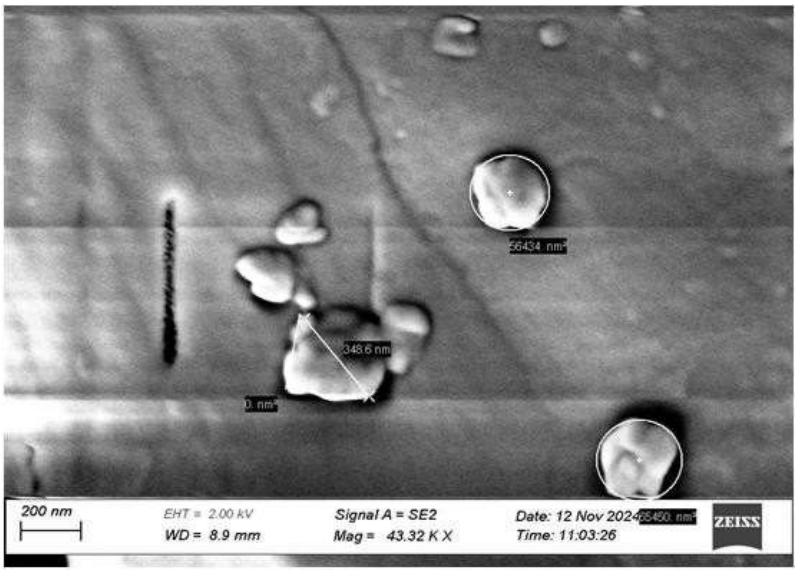


Figure 7: Scanning electron microscope (SEM) (F3)

Table 6: In-vitro drug release of PNPs (F3)

Serial No.	**Time (Hour)**	**Cumulative % drug released**		
		PNPs (F3)		**Suspension of both drugs Hydroquinone and tretinoin**
		Hydroquinone	**Tretinoin**	
1	0	0	0	0

2	2	23.18	18.23	38.56
3	4	36.09	30.77	56.23
4	6	43.63	39.85	69.27
5	8	61.23	52.66	79.65
6	10	78.56	65.23	89.56
7	12	90.56	70.26	99.56

The in-vitro release study of optimized PNPs (F3) demonstrated a sustained drug release profile compared to the drug suspension. Hydroquinone and tretinoin from PNPs showed gradual release, reaching 90.56% and 70.26% at 12 hours, respectively, whereas the suspension released almost 100% much earlier. This sustained release indicates effective encapsulation and controlled drug delivery from the nanoparticles, which can enhance therapeutic efficacy and reduce dosing frequency.

Table 7: Characterization of gel formulations (Physical appearance)

S. No.	Formulation	Color and Texture	Homogeneity	Odor
1	NG1	Light yellow colored Gel	+++	Odorless
2	NG2	Light yellow colored Gel	+++	Odorless
3	NG3	Light yellow colored Gel	+++	Odorless
4	NG4	Light yellow colored Gel	+++	Odorless
5	NG5	Light yellow colored Gel	+++	Odorless

All gel formulations (NG1–NG5) exhibited uniform physical characteristics, appearing as light yellow gels with smooth texture. Each formulation showed excellent homogeneity (+++), indicating consistent mixing and uniform distribution of ingredients. Additionally, all gels were odorless, enhancing their suitability for topical application and improving patient compliance. These results confirm the physical stability and aesthetic acceptability of the gel formulations.

Table 8: Characterization of gel formulations (Viscosity, pH, Extrudability, Spreadability)

Formulations	Viscosity (cps) at 100 rpm	pH at (25-30^{0}C)	Extrudability	Spreadability (g.cm/s)
NG1	30256 ± 0.23	5.1 ± 0.03	+++	12.47 ± 0.12
NG2	55235 ± 0.45	4.5 ± 0.05	++	09.23 ± 1.23
NG3	35265 ± 0.36	4.8 ± 0.06	+++	10.98 ± 2.32
NG4	40789 ± 0.78	5.0 ± 0.08	+++	11.75 ± 1.45
NG5	32456 ± 0.23	5.2 ± 0.03	++	09.85 ± 2.15

The gel formulations (NG1–NG5) showed acceptable viscosity levels, with NG2 having the highest viscosity (55235 cps), which slightly reduced its extrudability and spreadability. Most formulations maintained a skin-friendly pH (4.5–5.2), suitable for topical application without causing irritation. NG1 and NG4 demonstrated optimal balance with good extrudability (+++) and high spreadability, making them ideal candidates for smooth application and user comfort. Overall, all gels were within acceptable physicochemical limits for topical formulations.

Table 9: In-vitro drug permeation studies of formulation NG1-NG6

S. No.	Time (Hour)	Cumulative % drug released					
		NG1	NG2	NG3	NG4	NG5	NG6
1	0	0	0	0	0	0	0
2	1	23.18	12.85	15.51	14.23	13.56	11.12
3	2	36.09	25.63	23.65	25.68	26.54	22.30
4	4	43.63	39.46	35.62	36.78	38.25	32.51
5	6	57.60	45.89	42.59	48.96	42.85	39.96
6	8	66.13	59.32	49.85	56.26	49.81	42.32
7	10	77.56	61.25	69.56	70.45	65.48	59.36
8	12	82.45	81.23	79.26	73.56	72.65	73.69
9	16	96.13	90.23	89.23	87.23	88.23	81.85

Among all formulations (NG1–NG6), NG1 showed the highest cumulative drug permeation, reaching 96.13% at 16 hours. It demonstrated a faster and more sustained release profile compared to others, especially in the initial hours, indicating efficient drug delivery through the skin. Other formulations showed relatively lower permeation, with NG6 having the slowest release. Based on its superior permeation performance, NG1 was selected as the optimized formulation for further development.

Table 10: Release Kinetics Study of Nano Gel NG1 Formulation

Time (Hr)	% CDR	% Drug remaining	Square root time	Log % CDR Remaining	Log time	Log % CDR
0	0	100	0.000	2.000	0.000	0.000
1	23.18	76.82	1.000	1.885	0.000	1.365
2	36.09	63.91	1.414	1.806	0.301	1.557
4	43.63	56.37	2.000	1.751	0.602	1.640
6	57.6	42.4	2.449	1.627	0.778	1.760
8	66.13	33.87	2.828	1.530	0.903	1.820
10	77.56	22.44	3.162	1.351	1.000	1.890
12	82.45	17.55	3.464	1.244	1.079	1.916
16	96.13	3.87	4.000	0.588	1.204	1.983

The release kinetics data of NG1 indicate a sustained drug release over time. The gradual increase in log % CDR and square root of time suggests that the drug follows **Higuchi kinetics**, implying diffusion-controlled release from the gel matrix. The linearity in the log % drug remaining vs. time also points toward possible **first-order kinetics.** Formulation NG1 exhibits controlled and predictable drug release behavior, making it suitable for prolonged topical therapy.

Table 11: Correlation value (R^2 value)

Formulation	Model	Kinetic parameter values (R^2)
Gel	Zero Order	0.9253
	First Order	0.8512
	Higuchi	0.9961
	Korsmeyer Peppas	0.6015

The release kinetics of the NG1 nano gel formulation best fit the **Higuchi model** with the highest R^2 value of **0.9961**, indicating that drug release is primarily governed by a diffusion-controlled mechanism. The zero-order model also showed a moderate fit ($R^2 = 0.9253$), suggesting a steady release to some extent. However, lower R^2 values for the first-order (0.8512) and Korsmeyer-Peppas (0.6015) models further confirm that diffusion is the dominant release mechanism for NG1.

Table 12: Stability Study of Formulation (NG1)

F. Code.	Months	Appearance	pH (At 25^{0}C)	Drug Content (%)		Viscosity
				HYQ	TRE	
NG1	0	Clear	5.1 ± 0.03	99.81	98.26	5628 ± 0.53
	1	Clear	5.5 ± 0.05	97.56	96.35	5364 ± 0.32
	2	Clear	5.6 ± 0.02	96.44	95.45	5289 ± 0.66
	3	Clear	5.5 ± 0.05	94.85	93.67	5025 ± 0.12

The stability study of NG1 formulation demonstrated that the product remained clear and visually stable over the 3-month period. The pH showed only a minor increase from 5.1 to 5.6, which is within the acceptable range for topical applications. Drug content for both hydroquinone (HYQ) and tretinoin (TRE) decreased gradually, with HYQ and TRE retaining 94.85% and 93.67% of their initial content, respectively, after 3 months. The viscosity also slightly decreased, indicating a minor reduction in gel consistency, but it remained functional. NG1 showed good stability under the storage conditions, with minimal degradation of both the drug content and physical properties.

4. CONCLUSION

The study successfully formulated and characterized a polymeric nanoparticle gel containing hydroquinone and tretinoin for combination therapy. The optimized nanoparticle formulation (F3) showed excellent stability, drug entrapment efficiency, and particle size, while the gel formulations exhibited desirable physicochemical properties, including appropriate viscosity, pH, and spreadability. The in-vitro drug permeation studies demonstrated sustained drug permeation , which is crucial for effective treatment. The results suggest that this formulation has significant potential for improving the therapeutic efficacy of hydroquinone and tretinoin in dermatological applications.

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