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Original Article

Development, Evaluation, and *in vitro* Anti-Acne Activity of Tretinoin Nanocrystals Gel

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ABSTRACT

Background: Tretinoin is generally used to treat acne vulgaris and photoaging. The study aimed to develop a gel composition containing tretinoin nanocrystals and characterize and compare *in vitro* anti-acne activity. **Materials and Methods:** The anti-solvent precipitation approach was used to prepare tretinoin nanocrystals. The drug nanocrystals were optimized for parameters such as particle size, drug content, and drug release. The optimized nanocrystals (TN9) were incorporated into the gel system (TNG1-TNG5) and characterized for pharmaceutical properties. The selected formulation (TNG1) was tested for anti-acne activity using *Propionibacterium acnes* and *Staphylococcus epidermidis* and compared with Doxycycline HCl as standard. **Results:** Nanocrystals (TN9) showed the lowest particle size of 114.2 nm, the least value of polydispersity of 0.232, the highest drug content of 95.24%, and higher cumulative drug release of 97.2% in 10 hr. TEM and DSC images conformed to the nano-size range and crystalline nature of nanocrystals. The prepared gel composition indicates a clear, smooth, and homogeneous nature, good spreadability, suitable viscosity, pH, and drug content. The drug release from TNG1 was found to be significant ($p < 0.0001$) and was superior to the commercial gel. Results of anti-acne activity signify that the zone of inhibition exhibited by tretinoin nanocrystals was comparable with Doxycycline HCl (standard) for both microorganisms tested. **Conclusion:** These results demonstrated that the developed tretinoin nanocrystals could be a feasible approach for effective therapy in the treatment of acne.

Keywords: Tretinoin, Nanocrystals, Gel, Drug release, Anti-acne activity, *Propionibacterium acnes*, *Staphylococcus epidermidis*.

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INTRODUCTION

Propionibacterium acnes (*P. acnes*) is an anaerobic, obligatory diphtheroid infection of the sebaceous follicles triggered by androgens that can last for years.[1] This gram-positive bacterium grows in an anaerobic environment, which is created by the oxidative stress within the pilosebaceous unit and leads to acne with inflammation.[2] *Staphylococcus epidermidis* (*S. epidermidis*), another member of the human skin flora, is an aerobic bacteria linked to superficial infections in the sebaceous units. However, *P. acnes* is the most important causative agent of inflammatory acne. The progressive spread of *P. acnes* strains that are resistant to antibiotics has been a source of growing concern over the past 20 years.[3] Over the last few years, there has been a significant surge in interest in therapies for photodamaged skin. Although several topical therapies have been used to treat photodamaged skin, many of them still need sufficient clinical evidence to confirm their efficacy or are not yet licensed.[4]

The treatment of photodamaged skin and acne vulgaris using topical tretinoin has been authorized for use in dermatology for several years. Over this time, topical tretinoin showed significant efficacy and safety in the treatment of acne and photodamaged skin.[5,6] Tretinoin has traditionally been used to treat mild to severe acne as a comedolytic drug. Tretinoin's capacity to support the natural flow of sebum, clear pores, and stimulate the creation of new cells is well demonstrated. It can be used as a stand-alone therapy or in conjunction with antibacterial treatments.[7] Tretinoin works by attaching to the α, β, and γ Receptors for Retinoic Acid

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(RARs).[5] RAR-α and RAR-β expression has been linked to the development of acute promyelocytic leukemia and squamous cell carcinoma, respectively while RAR-γ is connected to the retinoid effect on bone and mucocutaneous tissues.[8,9] According to recent studies, the success of tretinoin in treating acne is mostly a result of its capacity to alter aberrant follicular keratinization.[10,11] It has been reported that orally administered tretinoin can often cause severe side effects including cheilitis and irritation.[12] The most frequent side effects of tretinoin when applied topically are pruritus, skin discomfort, skin/subcutaneous irritation, erythema, and pharyngitis as per FDA medication labeling requirements.

The primary problems with transdermal medication administration include poor penetration and inadequate bioavailability. Contrarily, because the drug activity influences the rate of diffusion rather than concentration in the donor compartment, the conventional topical administration method has limitations with poor skin penetration and limited topical bioavailability.[13] Literature indicates various physical, chemical and formulation approaches to overcome the skin barriers.[14-17] In this situation, the barriers provided by the epidermal layers restrict the percutaneous absorption of tretinoin in topical treatment.[18] In recent years, various tretinoin-loaded nanocarriers topical formulations like polymeric nanoparticles, nano-emulsions, lipid nanocarriers, liposomes, niosomes, microemulsions, nanoemulsions, etc. have been attempted.[19] However, due to their abilities like high solubility, dissolution, and greater adherence to cell skin membranes, nanocrystals are currently gaining popularity and have been shown to be superior to other nano-carrier systems in topical treatment.[20] Indeed, transdermal administration of water-insoluble medicines using nanocrystal technology seems appealing.[21] The literature showed that the nanocrystal technology considerably increased and improved the penetration and bioavailability of the transdermal administered water-insoluble medicines.[22,23] Drug nanocrystals are pure drug particles that are smaller than 1000 nm in size and are stabilized by appropriate surfactants and/or polymeric stabilizers, as opposed to other nanocarriers.[24,25] The efficiency of nanocrystals in delivering pharmaceuticals to or into cells is a result of their high drug loading and low concentration of stabilizing surfactants.[25,26] The passive diffusion of the medication through the skin is accelerated by nanocrystals because they boost the drug's saturation solubility and dissolution rate. This increases the concentration gradient between the applied formulation and the skin membrane.[27] Drug formulations using nanocrystals have been shown to boost bioavailability and skin penetration due to their higher solubility and extended retention at the site of infection.[28] Considering the significance of nanocrystals, the present study assessed the feasibility of developing gel formulations comprising tretinoin nanocrystals by utilizing an anti-solvent precipitation method and evaluated accordingly. Pluronic F-127 was utilized as a stabilizer in the preparation of tretinoin nanocrystals, which was extensively used in stabilizing nanocrystals.[29] The produced nanocrystals were optimized for drug release, drug content, and particle size. The optimized nanocrystals were made into a gel and subsequently examined for various pharmacological characteristics and anti-acne effectiveness.

MATERIALS AND METHODS

Materials

Tretinoin sample was procured from East West Pharma, Roorkee, Uttarakhand, India. Pluronic F-127 was provided by BASF, New Jersey, USA. Ethanol, carbopol 940, sodium hydroxide pellets, methylparaben, EDTA and propylene glycol (CDH Ltd., New Delhi, India) and other chemicals such as acetone and triethanolamine were provided by Qualikems Fine Chem. Pvt. Ltd., Mumbai,.

Optimization Design

In this study, tretinoin nanocrystals have been formulated by changing the amount and proportions of tretinoin (X_1) and stabilizer (Pluronic F-127, X_2). The responses were recorded on particle size (Y_1), drug content (Y_2), and drug release (Y_3). Ethanol was used as a solvent and distilled water was used as an anti-solvent.

Preparation of Tretinoin Nanocrystals

Low energy anti-solvent precipitation method described in the literature with minor modification was employed to prepare stable tretinoin nanocrystals.[30] The tretinoin solution (mg/mL), which is shown in Table 1, was made in ethanol as solvent. Using a syringe and a steady flow rate of 2-8 mL/min, this solution was injected into an anti-solvent mixture having different concentrations of Pluronic F-127 (Table 1). The anti-solvent phase in this instance was distilled water. Throughout this stage, the solution was continually agitated at 600 to 1000 rpm. After the filtration, tretinoin nanocrystals were vacuum-dried.

Characterization of Nanocrystals

Particle Size and Polydispersity Index

A Zeta sizer (Malvern Instruments Ltd., Malvern, UK) was used to quantify the Polydispersity Index (PDI) and particle size of tretinoin nanocrystals (TN1-TN9). The temperature was held constant at 25°C while the scattering angle was fixed at 173°. Before being filtered through a membrane with a 0.45 m pore size and having their particle sizes measured, each sample of tretinoin nanocrystals was diluted with deionized water. A transparent, disposable sizing cuvette was used to measure the particle size.[31]

Drug Content

Methanol (50 mL) was added to 10 mg of nanocrystal in a glass vial to thoroughly dissolve the particles. To dissolve the crystals, the solution was agitated for 15 min. The filtrate (100 µL) from this

solution was then collected and further diluted with methanol. The samples were spectrophotometrically examined (λ_{max} 348.6 nm; UV-1800, Shimadzu Corporation, Tokyo, Japan).

In vitro Drug Release

Using a Franz diffusion cell with a donor and receptor compartment divided by a dialysis membrane with a size of 0.22 mm, the *in vitro* release of tretinoin nanocrystals was performed (Himedia, Mumbai, India). One milliliter of tretinoin-nanocrystal formulation (TN1-TN9) was added to the donor compartment, and ten milliliters of phosphate buffer, pH 7.4, with 10% Tween 80, were added to the receptor compartment.[32] Franz diffusion cell receiving chamber was continually swirled (50 rpm) and maintained at 37°C to imitate physiological circumstances. 1 mL of sample was periodically removed from the diffusion cell via the sampling port, and it was then examined with a UV spectrophotometer with a maximum wavelength of 348.6 nm. The receptor compartment received an equal volume of buffer after each sample.

Transmission Electron Microscopy (TEM)

Using TEM (Zeiss Microscopy), the morphology of Tretinoin Nanocrystals (TN9) was studied. One drop of a nanocrystal solution was dispersed over a copper grid with a carbon coating and a mesh size of 400, and any extra droplets were wiped away using filter paper. After adding a drop of a 2% w/v phosphor tungstic acid solution to the grid, the negatively stained sample was dried at ordinary room temperature.[33]

Differential Scanning Calorimetry (DSC)

The sample's thermogram was captured using DSC-60 (Shimadzu, Tokyo, Japan). A sample of pure tretinoin, pluronic F-127 and tretinoin nanocrystals (TN9) of 2 mg was weighed and placed in an aluminum pan while being heated at a rate of 10°C per minute and monitored between 25 and 450°C. Excipients, a physical combination, and drug were used in the test.[34]

Preparation of Nanocrystal Gel

Drug nanocrystals were prepared according to the method described before.[35] Carbopol 940 and methylparaben were dissolved slowly with continuous stirring in distilled water for 1 hr to avoid agglomeration. For appropriate swelling, the mixture was left at room temperature for 8 hr. Then, EDTA and triethanolamine were dissolved separately and stirred for 15 min and added into the carbopol base for its neutralization. In another beaker, propylene glycol was heated at 65°C temperature and 12 mL distilled water was added into this solution. The mixture of propylene glycol and distilled water were stirred for 10 min at 200 rpm. Then, pH of the carbopol base was adjusted to 6.7-6.9. The carbopol base was stirred continuously while the propylene glycol solution was added dropwise, resulting in the gel base. With a pestle and mortar, gel bases were triturated. The smooth nanocrystal gel was created by adding the nanocrystal suspension dropwise into the gel foundation and continually stirring. The formulation of tretinoin nanocrystal gel was designed according to Table 2.

Characterization of Tretinoin Nanocrystal Gel

Visual Inspection

The physical properties of tretinoin nanocrystal gels were examined by visual inspection of color, phase separation, clarity, and homogeneity.

pH-measurement

The pH of the tretinoin nanocrystal gel was measured using a digital pH meter (Hicon ®, New Delhi, India). A homogeneous solution was created by dissolving 2 g of nanocrystal gel in distilled water and stirring it consistently. The solution was then tested for pH, and the measurement was performed in triplicate.[36]

Viscosity

A Brookfield viscometer (Brookfield Inc., Middleborough, MA, USA) was used to measure the viscosity of the nanocrystal gel at a temperature of 25°C and a spindle speed of 12 rpm. A sample of nanocrystal gel between 0.5 and 1 g was added to the plate, and it was left until the cone's temperature was maintained. The nanocrystal gel's viscosity was then assessed for 2 min.[37]

Extrudability

For determination of extrudability, approximately 20 g of nanocrystal gel was added to a closed collapsible tube, force was applied at the crimped end to force out the gel and for prevention of any type of roll back, a clamp was also used on the tube. The gel was then ejected from the tube once the cap had been removed. The whole extruded amount of gel was collected and carefully weighed. The percentage of the extruded gel had been determined.[38]

Spreadability

The spreadability of nanocrystal gels was performed as described earlier with minor modifications.[39] The equipment was equipped with two sets of glass slides that had typical proportions. Two grams of nanocrystal gel were put into one of the slides before another slide was set on top of it. The gel between the two slides was then evenly compressed by the application of 50 g of weight to the upper slide, which resulted in the formation of a thin gel film. Both the surface area of distributed gel and the length of time it took for the gel to produce the thin layer were observed. The experiment was then performed three times using various weight applications after the imposed weight was withdrawn.[40] The mean of time taken to form the thin layer was used for calculation. In order to determine the spreadability, the following formula was used:

$$S = m \times l/t$$

Where, S = spreadability, m = weight placed to upper slides, l = length of the glass slide, t = time taken in sec.

In vitro Anti-Acne Activity

Well diffusion method was used to analyse anti-acne activity using two microorganisms (*P. acnes*-MTCC1951 and *S. epidermidis*-MTCC-435). In order to get about 1 × 10^8 CFU/mL, *S. epidermidis* was cultivated in nutritional broth at 37 °C for 24 hr under aerobic conditions. The inoculum was spread equally throughout the agar plates using 100 µL. Equally spaced wells were drilled into each plate using a sterile 6 mm borer. Dissolving 10 mg of tretinoin and 10 mg of doxycycline HCl (standard) in 1 mL of dimethyl sulfoxide yielded solutions. Both drugs were added into 50 µL wells. After pre-diffusion at room temperature for 30 min, the plates incubated aerobically at 37°C for 24 hr. After 48 hr at 37°C in anaerobic circumstances, *P. acnes* was adjusted to produce about 1 x 10^8 CFU/mL in a brain heart infusion medium. To inoculate the agar plates with bacteria, 100 µL was spread evenly across each surface. Each plate had a series of evenly spaced wells drilled into it using a sterile 6 mm borer. Fifty microliters of drug solutions were poured into each well. After 30 min of pre-diffusion at room temperature, the plates were placed in an anaerobic bag with a gas pack and indicator tablets, then placed in an incubator at 37°C for 72 hr. After 72 hr of the incubation, the diameter of zones of inhibition were noted.

Stability

Analytical drug content measurement and visual control were both created for the stability examination of nanocrystal gel. In this study, tretinoin nanocrystal gel was kept in a chamber for three months with long-term settings at room temperature of 25°C and 60±5% relative humidity. This gel of nanoparticles was placed in an amber container that had a lid. The drug content was evaluated spectrophotometrically at the beginning and end of the three months at a maximum wavelength of 348.6 nm.[41]

RESULTS AND DISCUSSION

Characterization of Nanocrystals

Particle Size

According to Table 3, the mean particle diameters of tretinoin nanocrystals (TN1–TN9) ranged between 114.2 to 183.1 nm, with PDI values between 0.232 and 0.293. The results showed that the drug and stabilizer concentrations significantly affected the size of the generated nanocrystal. The most crucial factors for drug delivery via topical route are uniform size distribution and drug particle size. The formulation (TN9) has a low PDI value of 0.232 and the ideal particle size for topical application of 114.2 nm (Figure 1). The ratio of drug to stabilizer (Pluronic F-127) also had a significant impact on nanocrystal size. Use of less amount of stabilizer in TN1, TN6, and TN9 produced smaller size nanocrystals as the stabilizers helps in producing adequate particle-particle repulsion, while the nanocrystals were

Table 1: Formulation design for tretinoin nanocrystals.

Code	Tretinoin (mg/mL)	Pluronic F-127 (%, w/v)	Solvent	Anti-solvent
TN1	15(+1)	1.0(-1)	Ethanol	Distilled Water
TN2	15(+1)	1.5(0)	Ethanol	Distilled Water
TN3	05(-1)	2.0(+1)	Ethanol	Distilled Water
TN4	05(-1)	1.5(0)	Ethanol	Distilled Water
TN5	10(0)	2.0(+1)	Ethanol	Distilled Water
TN6	10(0)	1.0(-1)	Ethanol	Distilled Water
TN7	10(0)	1.5(0)	Ethanol	Distilled Water
TN8	15(+1)	2.0(+1)	Ethanol	Distilled Water
TN9	05(-1)	1.0(-1)	Ethanol	Distilled Water

Table 2: Formulation design of tretinoin nanocrystal gel as carrier system (TNG1- TNG5).

Formulation code	Carbopol-940 (mg)	Methylparaben (mg)	Propylene glycol (mL)	Triethanolamine (mL)	EDTA (mg)	Water (mL)
TNG1	0.150	0.004	5	2	0.034	q.s.
TNG2	0.250	0.004	5	2	0.034	q.s.
TNG3	0.350	0.004	5	2	0.034	q.s.
TNG4	0.450	0.004	5	2	0.034	q.s.
TNG5	0.550	0.004	5	2	0.034	q.s.

larger in size when the stabilizer amount was high. Similarly, the formulation (TN9) displayed the lowest PDI value, suggesting a limited and mono-disperse pattern, and the best particle size range of nanocrystals for topical distribution out of all the formulations (TN1-TN9). A quadratic equation was created to represent the influence of independent variables on mean nanocrystal size, which is as follows:

$$Y_1 = +54.01667+1.37667X_1+53.6333X_2$$

This analysis shows the model is significant as described in Table 4 (F value = 201.61, and $p < 0.0001$). The above equation suggests that the size of the nanocrystal is influenced by both independent variables, while a greater effect was noticed by the stabilizer in the current experimental conditions.

Drug Content

According to the results in Table 3, the drug content of nanocrystals ranged from 47.13±0.54% to 95.24±0.24% and is highly influenced by the variable chosen. Nanocrystals prepared with low content of stabilizer exhibited higher drug concentration than the nanocrystals prepared using a higher stabilizer. The influence of the independent variable is illustrated in the below equation:

$$Y_2 = +79.03-7.01X_1 -13.03 X_2-7.04 X_1X_2$$

The model for the drug content is found significant [F value = 18.06 and $p < 0.005$). The above equation suggests that the drug content in nanocrystals had a negative effect on selected independent variables in the current experimental conditions.

In vitro Drug Release

According to *in vitro* drug release profiles, the percentage of drugs that were cumulatively released over the course of 10 hr ranged from 61.20% to 97.22% (Table 3). The drug release variation could have been influenced by the drug-stabilizer weight ratio, drug content, particle size and PDI of nanocrystals. Since they may contain a high drug content and small particle size, formulation, TN9 demonstrated the greatest % cumulative drug release with 97.22% (Figure 2). The outcomes of the *in vitro* release profiles were indicated using a variety of kinetic models. The prepared nanocrystals (TN1-TN9) were best fitted into the Higuchi model (Table 3), which revealed that the drug released from the nanocrystals was consistent and was not much influenced by the drug's concentration. It is vital to observe that the cumulative drug release of TN9 was found to be completely released in 10 hr and that the Higuchi model provided the best

Table 3: Characterization of tretinoin nanocrystals.

Formulation code	Particle size (nm)	Polydispersity index	% Drug content ± (SD)	% Drug release ± (SD)	Best fit model	r^2 value
TN1	123.9±0.12	0.256±0.39	91.18±0.54	67.10±3.36	Higuchi	0.9865
TN2	159.5±0.33	0.274±0.14	79.92±0.21	65.79±3.29	Higuchi	0.9833
TN3	168.4±0.11	0.273±0.17	79.36±0.51	61.20±3.06	Higuchi	0.9834
TN4	142.6±0.13	0.293±0.39	85.67±0.28	71.40±3.57	Higuchi	0.9854
TN5	170.2±0.27	0.245±0.22	67.49±0.24	74.20±3.71	Higuchi	0.9855
TN6	122.7±0.16	0.257±0.24	85.76±0.26	89.15±4.46	Higuchi	0.9954
TN7	149.5±0.23	0.276±0.32	79.53±0.21	73.43±3.67	Higuchi	0.9834
TN8	183.1±0.25	0.258±0.18	47.13±0.46	84.41±4.22	Higuchi	0.9916
TN9	114.2±0.38	0.232±0.16	95.24±0.32	97.22±3.96	Higuchi	0.9980

Table 4: Results of the lack of fit test statistical analysis and model summary for mean particle size, drug content, and drug release.

Dependent variables	Y_1 = Particle size					Y_2 = Drug content					Y_3 = Drug release				
Source of variation	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Model	2	4599.08	2299.54	201.61	<0.0001[a]	3	1512.15	504.05	18.06	0.0041[a]	5	995.78	199.16	29.87	0.0092[a]
X_1	1	284.28	284.28	24.92	0.0025	1	294.56	294.56	10.55	0.0227	1	16.14	16.14	2.42	0.2176
X_2	1	4314.8	4314.8	378.29	<0.0001	1	1019.21	1019.21	36.52	0.0018	1	158.72	158.72	22.81	0.0165
X_1X_2						1	198.39	198.39	7.11	0.0446	1	639.08	639.08	95.86	0.0023
X_1^2											1	46.59	46.59	6.99	0.0774
X_2^2											1	135.25	135.25	20.29	0.0204

DF-Degree of freedom; SS- Sum of squares; MS- Mean of squares; F- F-value; P- P-value. [a]Statistically significant.

fit. The influence of independent variables on drug release was illustrated in the below equation:

$$Y_3 = +222.67111 - 4.05067X_1 - 159.52667X_2 + 5.05600X_1X_2 - 0.193067X_1^2 + 32.89333X_2^2$$

The model for the drug release is significant [F value = 29.87 ($p < 0.01$). The above equation suggests that the drug release from the nanocrystal had a negative effect on selected independent variables, while a greater effect was noticed by the stabilizer in the current experimental conditions.

Response-Surface Analysis and Selection of Formulation with Optimum Features

Figure 3 shows the three-dimensional response surface plots of the tretinoin nanocrystal optimization. It is apparent from Figure 3A that the amount of drug and stabilizer was less, the nanocrystal size was small. Even in Figure 3B it also demonstrated the nanocrystals prepared with low content of stabilizers exhibited higher drug content. The effect of the stabilizer as well as the drug content showed an effect on the cumulative drug release (Figure 3C). Based on particle size, drug content and the *in vitro* release pattern exhibited by the prepared nanocrystals, the optimum value was exhibited by TN9 and was selected as the optimized formulation.

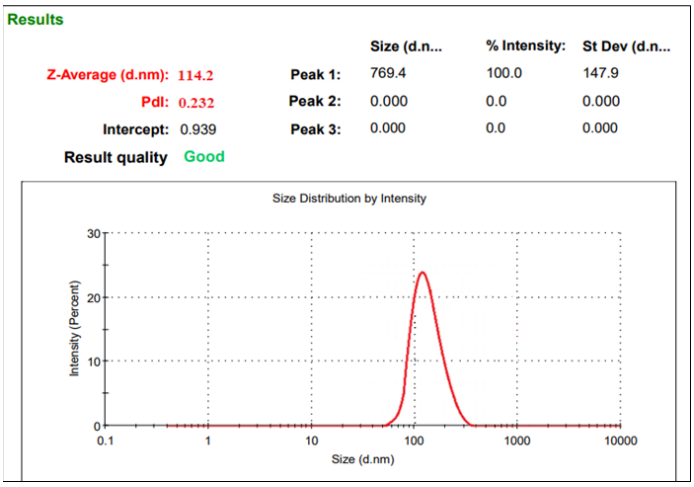

Figure 1: Particle size and polydispersity index of TN9.

TEM

TEM is a crucial technique to get high-resolution images of nanocrystals which can provide morphological properties like size and shape. The TEM image of the selected nanocrystal formulation (TN9) shows that the particles are nano in size range and have a constrained size distribution (Figure 4). Moreover, the size noticed here was comparable with the values noticed with the zeta sizer.

DSC

The DSC patterns of tretinoin, pluronic F-127, and Tretinoin Nanocrystals (TN9) are shown in Figure 5. The thermogram of raw tretinoin powder revealed a characteristic peak at 178.86°C that corresponded to the drug melting endotherm,[42] pluronic F 127 displayed an endothermic peak at 54.31°C, and in case of

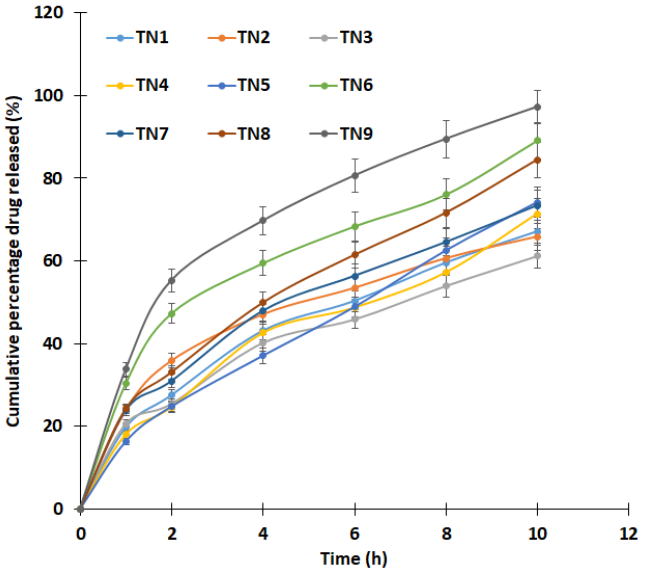

Figure 2: Comparison of *in vitro* drug release profiles of tretinoin nanocrystals (TN1-TN9).

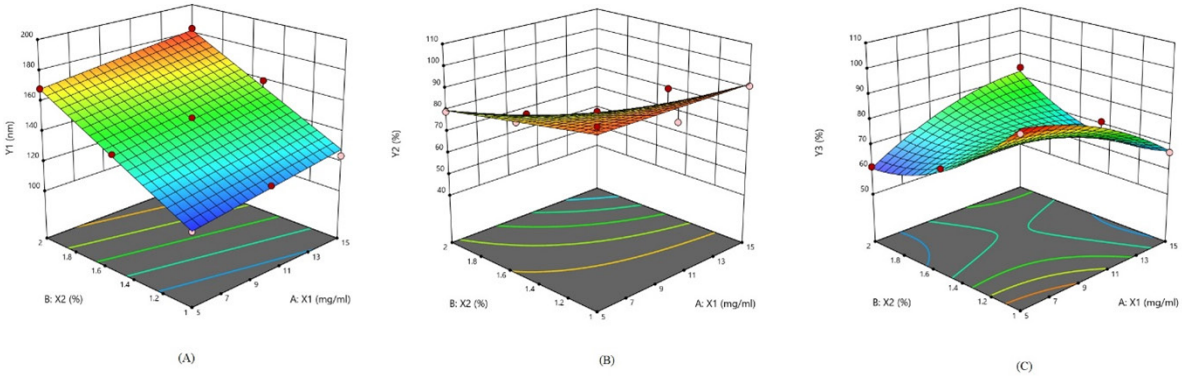

Figure 3: Response surface plot showing the effect of drug (X_1) and stabilizer (X_2) on particle size, drug content, and *in vitro* release.

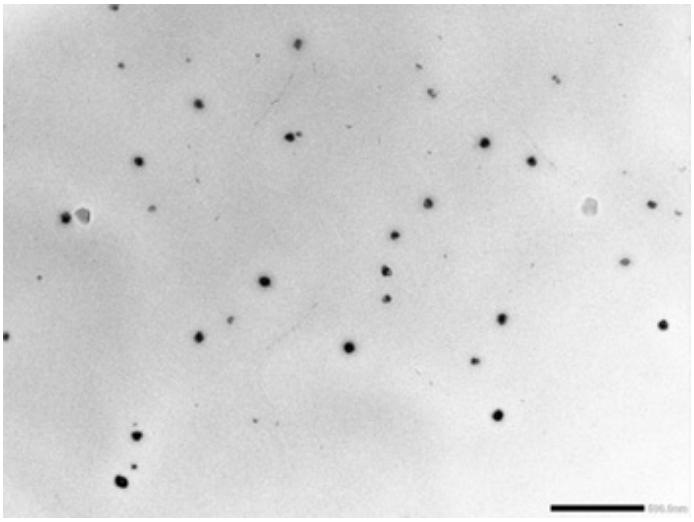

Figure 4: TEM image of optimized tretinoin nanocrystals TN9.

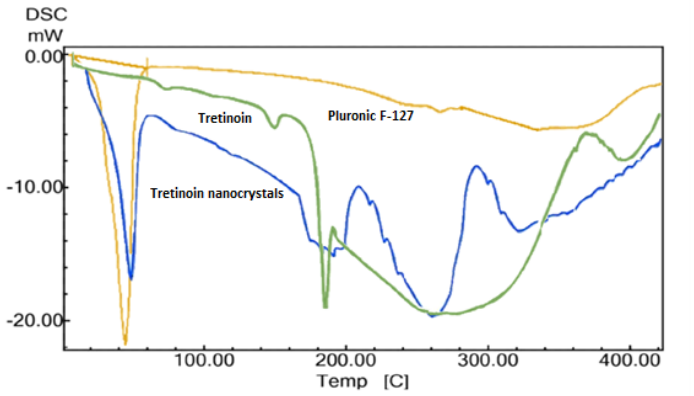

Figure 5: DSC thermogram of tretinoin, pluronic F-127 and Tretinoin Nanocrystals (TN9).

TN9, tretinoin nanocrystal peaks were obtained at 54.91°C and 178.86°C that revealed an endothermic peak that was slightly less intense than that of the raw drug powder but still exists as crystalline.

Preparation of Nanocrystal Gel

The nanocrystal formulation selected for the preparation of topical nanocrystal gel was TN9 and this optimized batch was further integrated into a pH-controlled carbopol 940 topical gelling system in order to obtain more improved drug release from the gelling system. The dispersion method was selected for the formulation of topical gel. Formulations TNG1-TNG5 were prepared using the formulation design as shown in Table 2.

Characterization of Tretinoin Nanocrystal Gel

Various physical properties such as clarity, spreadability, homogeneity, pH, viscosity and visual assessment of tretinoin nanocrystal gels were evaluated. All TNG1-TNG5 formulations were clear in color with no phase separation (Table 5). All formulated TNG1-TNG5 gels were observed to be clear, smooth and homogeneous in nature. There was no presence of any kind

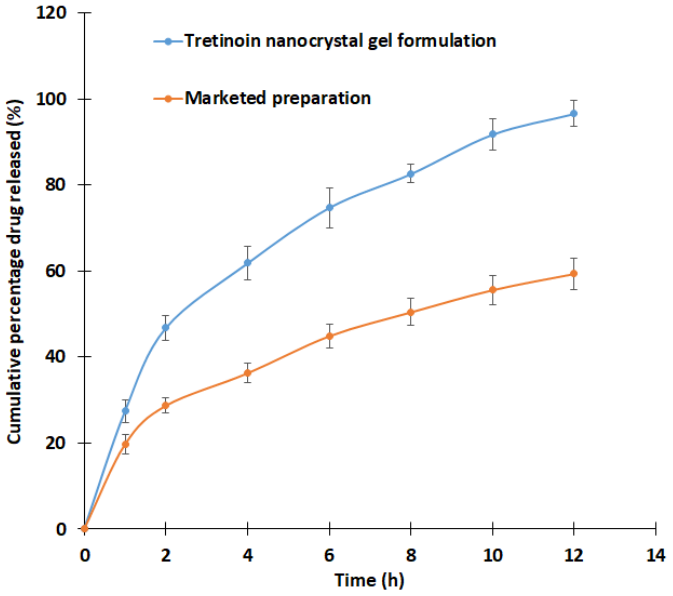

Figure 6: Comparison of *in vitro* drug release profiles of Tretinoin Nanocrystal Gel formulation (TNG1) and marketed preparation of tretinoin.

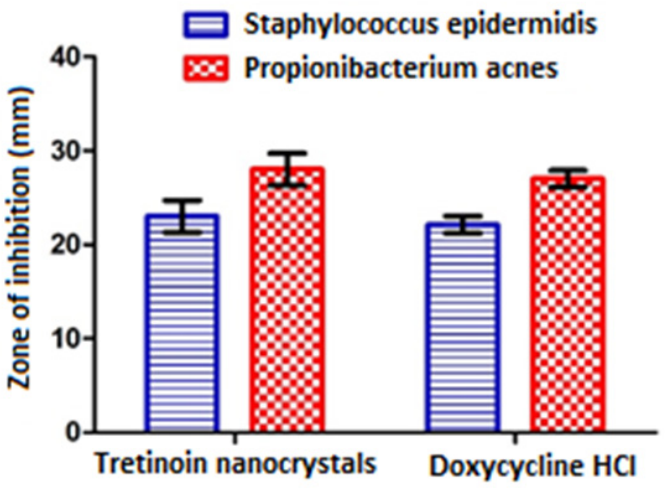

Figure 7: Anti-acne activity of tretinoin nanocrystals and doxycycline HCl (standard).

of contamination on visual inspection in all formulations tested (TNG1-TNG5). The stickiness of the gel was used to determine its spreadability, and this stickiness was determined by spreading an aliquot of gel on blank glass slides. All the formulations of gel i.e., TNG1-TNG5 were found to be easily spreadable. The viscosity of the topical formulation is an essential characteristic that influences the rate of drug release. Increased gel viscosity causes rigidity as well as a reduction in the drug release rate. The viscosity of various topical gel formulations i.e. TNG1-TNG5 was found in the range of 7479 to 9427 cP. The pH of the gel is an essential parameter for topical drug delivery. If the pH of the prepared nanocrystal gels is maintained, it will not cause skin irritation and will be easily absorbed through the skin.[43] To preserve the gel's consistency and stability, the pH of the gel should be maintained for an effective topical gelling system. The

Table 5: Characterization of tretinoin nanocrystal gel (TNG1-TNG5).

Formulation code	Clarity	Viscosity (cP)	pH	Drug content
TNG1	Clear	9427±112	6.9±0.11	98.33±2.24
TNG2	Clear	8748±98	6.9±0.15	86.26±3.56
TNG3	Clear	7847±103	6.7±0.22	93.15±1.67
TNG4	Clear	7479±157	6.7±0.24	92.18±2.34
TNG5	Clear	7856±118	6.8±0.17	88.55±2.78

pH of all formulations TNG1-TNG5 was maintained between pH 6.7 to pH 6.9. The drug content of prepared nanocrystal gel (TNG1-TNG5) was in the range of 86.26 to 98.3. The formulation with higher drug content was used for further evaluation, such as TNG1 as shown in (Table 5).

Drug Release From Tretinoin Nanocrystal Gel

The drug release from TNG1 was compared with the marketed formulation and was depicted in Figure 6. It is apparent that the release profiles were distinct and statistically significant ($p < 0.0001$). In case of TNG1, the release was comparatively steady throughout the study period (12 h) and was complete. When compared to TN9 (nanocrystals without gel, Figure 2), the release of tretinoin from TNG1 was relatively slow though statistically insignificant. On the other hand, the marketed product could only achieve a release of ~60% in 12 hr. Thus, it was demonstrated that the prepared nanocrystal containing gel is superior to the commercial gel.

In vitro Anti-Acne Activity

Measurements of the diameters of zones of inhibition were used to determine the efficacy of the samples against two microorganisms namely *P. acnes* and *S. epidermidis*. The measured zone of inhibition for tretinoin nanocrystals and doxycycline against both organisms are shown in Figure 7. The data here signifies the zone of inhibition exhibited by tretinoin nanocrystals was comparable with the standard for both microorganisms tested. The zone of inhibition observed against *P. acnes* and *S. epidermidis* with tretinoin nanocrystals (23±1.7 and 28±1.9 mm) and doxycycline (22.1±0.9 and 27.1±1.2 mm), respectively. The data observed here is also comparable with the other reported in the literature.[44] The effect observed here demonstrate the good therapeutic efficacy of prepared tretinoin nanocrystals and could be effective in topical treatment of acne.

Stability Study

The nanocrystal gel formulation developed was tested for stability for a period of three months. The results signified that the amount of tretinoin in the formulation didn't alter significantly and was found to have 93.56±0.64% after three months, showing no chemical degradation. Similarly, there was no phase separation or sedimentation of particles found during the stability investigation. Overall, the results here indicate the tretinoin nanocrystals were stable for three months.

CONCLUSION

Nanocrystals of tretinoin were successfully developed by anti-solvent precipitation methods. The chosen approach was discovered to be extremely straightforward, affordable, and repeatable. Effective production methods were used to prduce the nano sized tretinoin nanocrystals. Optimization of nanocrystals was done by assessing the particle size, drug content, and drug release. The selected nanocrystals were incorporated into five gel systems and evaluated for various pharmaceutical properties. The ideal tretinoin nanocrystal gel had good drug content, sustained drug release, ideal pH as well as viscosity in addition to good anti-acne activity indicating its prospective for topical therapy in acne. Considering the various advantages of the nanocrystal system, it is likely that the prepared tretinoin nanocrystals may produce better results for treating anti-acne than other formulations. Further investigations are necessary to assess the skin permeation potential and the clinical efficacy in humans.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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