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# Tretinoin loaded NLCs-based sunscreen cream; preparation and characterization

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**Abstract**: FDA has recognized tretinoin as the gold standard for skin anti-aging products but it has the disadvantage of being a strong irritant and unstable in light. Nanostructured lipid carriers (NLCs) are advanced, second-generation solid lipid nanoparticles with the intriguing feature of acquiring the properties of physical sunscreens in terms of UV ray scattering. Thus, this study was designed to formulate a tretinoin-loaded NLCs-based sunscreen cream to potentiate the photo-stability of tretinoin. Tretinoin-loaded NLCs were prepared using micro-emulsion method and the Box-Benkhen design was utilized to optimize the formulations. The optimized tretinoin-loaded formulations were characterized based on particle size, PDI, zeta potential and entrapment efficiency. Moreover, the physicochemical parameters of optimized tretinoin-loaded NLCs, including morphology, FTIR studies, DSC analysis and stability studies and release profile were also examined. The results revealed that tretinoin-loaded NLCs-based sunscreen formulation was stable in all aspects and showed better photo-stability and sun protection with prolonged release profile. In conclusion, the present study affirms the NLCs formulation as a viable carrier for topically administering tretinoin, reducing its photosensitivity and suggesting that incorporated sunscreens may provide prolonged protective effects against harmful UV radiations.

**Keywords**: NLCs, tretinoin, UV radiations, sunscreen, photo-protection.

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## INTRODUCTION

Naturally occurring acid of retinol and retinaldehyde "all-trans retinoic acid", sometimes termed as tretinoin, is a vitamin-A active metabolite. It has many therapeutic benefits for treating a variety of dermatological ailments, including psoriasis (MACDONALD and FRY, 1972) and acne vulgaris (Thielitz and Gollnick, 2008), as well as UV-induced skin aging (Gilchrest, 1997). In the past, tretinoin was prescribed for the treatment of moderate to severe cases of acne. It can be used either independently or together with antibacterial drugs due to its well-established capability to maintain the normal flow of sebum, unclog pores and trigger the production of new cells (Schmidt and Gans, 2011). Tretinoin first becomes ineffective owing to photo-degradation and then the treated region becomes photosensitive because of the harmful effects of the products that cause photo-degradation on the skin. To improve tretinoin photostability and lessens its therapeutic side effects, it is important to manufacture a photo-protective formulation (Asfour *et al*., 2019).

Nanostructured lipid carriers (NLCs) are advanced, second-generation of solid lipid nanoparticles (SLNs) (Viegas *et al*., 2023). Chemically, NLCs contain liquid lipid and solid lipid, in the oily phase that crystallizes at 25ºC to create a nano-sized solid lipid matrix. As a result, an amorphous lipidic core is assembled. The deformity in the internal structure assists in more accommodation of drugs (Elmowafy and Al-Sanea, 2021). NLCs have an intriguing feature of acquiring the properties of physical sunscreens in terms of UV rays scattering. Thus, they act an important function in enhancing photoprotective impact of both chemical and physical sunscreen agents (Abdel-Salam *et al*., 2017). Although liquid lipid fatty acid chains fluctuate in density, NLCs can maintain a less defined system than SLNs, which results in a higher drug-loading capacity (Mall *et al*., 2024, Saupe *et al*., 2006). It is effective for delivering irritant agents at high doses while inhibiting the systemic absorption of the medications (Selvamuthukumar and Velmurugan, 2012). After applying topically, the lipid film increases the skin hydration effectiveness, thus providing occlusive property. Because of the amorphous structure, NLCs have the benefit of scattering light and can be used to incorporate light sensitive drugs (Almousallam *et al*., 2015).

The protection of skin from harmful ultraviolent rays is the use of sunscreening agents. (Touitou and Godin, 2008). By reflecting UV rays at the skin's surface, sunscreens are another first-line defense against DNA deterioration. Organic sunscreens, such as oxybenzone and methoxycinnamate, on the basis of their structure, can absorb UV rays of different wavelengths. Inorganic sunscreens, such as zinc oxide (ZnO) and titanium dioxide ($TiO_2$), on the other hand, may reflect and scatter UV light. Inorganic sunscreens offer several benefits, including

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broad-spectrum protection, photostability, irritation potential and lack of skin sensitivity. Furthermore, because they do not permeate the skin, physical sunscreen agents are superior to chemical sunscreens agents in term of safety (Leite-Silva *et al*., 2016, Sadrieh *et al*., 2010). Based on these assumptions, this study seeks to fabricate and optimize topically applied tretinoin loaded NLCs-based inorganic sunscreen to protect skin against UV rays and treat photoaging.

## MATERIALS AND METHODS

### *Materials*

Tretinoin was provided by Wilshire Pharma (Pvt.) Ltd., Lahore, Pakistan. $TiO_2$, ZnO, Tween-80, Oleic acid and Lauric acid, were purchased from Sigma-Aldrich, USA. Cetostearyl alcohol, span-60, soft paraffin, liquid paraffin, propylene glycol, methyl and propylparaben were purchased from Merck. All the solvents, chemicals and reagents, used in present study were of analytical grade.

### *Fabrication and optimization of tretinoin-loaded NLCs*

Tretinoin-loaded NLCs were fabricated by micro-emulsion method with slight adjustments. In the organic phase, the drug and lipids were weighed and melted at 65ºC on a magnetic stirrer. Simultaneously, the aqueous phase, comprising distilled water and tween-80, was maintained at the same temperature on another stirrer. The aqueous phase was combined with the melted lipid phase at 65ºC and mixed for approximately 1 hour at 1000-1200 rpm. The formulation was homogenized for 3 minutes at 10,000 rpm using a homogenizer. NLCs were produced by incorporating the warm micro-emulsion (65ºC) into cold distilled water (2-3ºC) at a 1:9 pre-emulsion to water ratio (v/v), followed by stirring (12000 rpm) for 15 minutes (Khan *et al*., 2021). The resulting NLCs were lyophilized for protection. Mannitol (5% w/v) was added to the tretinoin-loaded NLCs dispersion as a cryoprotectant, gently mixed and stored in the refrigerator. Lyophilization of the samples for 24 hours was performed using a lyophilizer (Soni *et al*., 2018). The Box-Behnken design model was utilized to optimize tretinoin-loaded NLCs formulation using Design-Expert® Software V. 12. To estimate the prediction variance more precisely over the whole design space, this design predicted 17 experimental runs with 5 consecutive centre points (Lee *et al*., 2014). An evaluation of the impact of factors on several responses was conducted using this three-level experimental design.

### *Evaluation of tretinoin-loaded NLCs*

The fabricated formulations were examined to determine particle size, polydispersity index (PDI), zeta potential (ZP). Fourier transform infrared spectroscopy (FTIR) was performed to check the compatibility between the tretinoin and excipients. Differential scanning calorimetry and X-ray diffraction analysis were also conducted to assess the alterations in peak height and pattern (Zafar *et al*., 2021). The size and shape of nanoparticles were investigated using a scanning electron microscope. Furthermore, the NLCs formulations' entrapment efficiency (EE) was determined using Equation (1.0) (Elmowafy *et al*., 2013). To test for stability, the tretinoin loaded NLCs formulation was kept in an amber glass vial sealed with paraffin tape and wrapped with aluminium foil for three months at different temperatures as per ICH guidelines (Kamath *et al*., 2023).

$$\%EE = \frac{(\text{Total amount of drug added} \;\; \text{Amount of unencapsulated drug}}{\text{Total amount of drug added}} \times 100$$

### *Addition of inorganic sunscreens to optimized NLCs formulations*

The optimized NLCs formulation was subjected to inorganic sunscreen agents i.e. ZnO and $TiO_2$. Both inorganic sunscreens were used alone ranging from (0, 2, 4, 6, 8 and 10%) to evaluate the efficacy of sunscreen agents and in combination in the ratio of 1:1 (w/w). The concentrations of inorganic sunscreen agents in NLCs formulations providing optimal sun protection factor (SPF) were noted and subjected to further analysis (Asfour *et al*., 2019).

### *Assessment of tretinoin-loaded NLCs-based sunscreen*

The transpore tape assay was adopted to calculate the sun-protection factor (SPF) (Abdel-Salam *et al*., 2017, Diffey, 1989, Kamel *et al*., 2017). Transpore tape was applied to the quartz cuvette and formulations (2 mg/cm²) were uniformly spread on a 4.5 cm² tape area, equivalent to 9 mg/4.5 cm², left to dry for 10-15 min. The quartz cuvette was then placed under a UV-spectrophotometer, conducting a wavelength scan from 400-290nm. Absorbance was measured, constructing the spectrum with a 5nm interval. The SPF was calculated using following Equation (2.0).

$$SPF = \frac{\Sigma_{290}^{400}(E\lambda B\lambda)}{\Sigma_{290}^{400}\frac{E\lambda B\lambda}{MPF\lambda}}$$

Where: $E_\lambda$ and $B_\lambda$ are constant values provided by (Diffey, 1989), $MPF_\lambda$ is a monochromatic protection factor. The UVA/UVB ratio, indicating broad-spectrum protection (Garoli *et al*., 2008), was also examined by comparing the absorbance of the UVA and UVB wavelength ranges. A higher SPF value signifies increased protection against UV rays. Photostability study was also conducted on the optimized NLCs treated with sunscreen agents. NLCs formulations were collected, dissolved in methanol and analyzed using a UV spectrophotometer within the 250-450nm wavelength spectrum. The absorbance was measured and the methanolic solution containing an equivalent amount of tretinoin as the optimized NLCs and NLCs treated with sunscreen agents underwent a similar UV spectrophotometer analysis for comparison (Ourique *et al*., 2011).

***Preparation of tretinoin-loaded NLCs-based sunscreen cream***

The tretinoin-loaded NLCs-based sunscreen was formulated into a cream dosage form. The non-lipidic phase, containing methyl paraben, propyl paraben, propylene glycol, and tween-80, was completely dissolved in hot distilled water. Simultaneously, the lipid phase, comprising cetostearyl alcohol, span-60, soft paraffin, and liquid paraffin, was heated to a temperature range of 65°C–75°C. The hot lipidic phase was then blended with the hot aqueous phase and allowed to cool. As the mixture reached room temperature, the NLCs formulation containing 0.05% tretinoin was added while consistently stirring. The components were mixed until achieving a uniform cream consistency (Apriani *et al*., 2019).

***Evaluation of tretinoin-loaded NLCs-based sunscreen cream***

*Homogeneity and appearance*

The main prerequisite to promoting a product is its uniformity and appearance. So, using a visualization approach, the cream formulation was checked for color, clarity, odor and texture after the appliance (Kamath *et al*., 2023).

***pH***

pH meter was used to check the pH of tretinoin-loaded NLCs-based sunscreen cream. By dipping an electrode into cream, the pH value was determined.

***Viscosity***

Using Brookfield viscometer DV II + Pro, the viscosity of tretinoin-loaded NLCs-based sunscreen cream was determined. The cream was held in the viscosity measuring pan and the spindle was turned at various rotational speeds (Apriani *et al*., 2019).

***In-vitro drug release and release kinetics***

*In-vitro* drug release was conducted using the dialysis bag method. The release patterns of tretinoin from tretinoin suspension, tretinoin-loaded NLCs and tretinoin-loaded NLCs-based sunscreen cream were assessed in phosphate buffer solution (pH 5.5). These formulations were introduced into dialysis tubes with a molecular cut-off weight of 12-14 kDa, sealed and placed in the release media. The release media, maintained at 37±0.5°C, underwent stirring at 100rpm. At designated time intervals, 2ml samples were extracted from the release media, and an equivalent amount of fresh PBS media was added. Cumulative drug release was measured and plotted against time (Yanasarn *et al*., 2009). To assess the drug release mechanism, DD Solver software was utilized, applying various models, including Zero Order, First Order, Higuchi Model, Hixson-Crowell Model, and Korsmeyer-Peppas Model. This analysis aimed to identify the kinetic model that best described the drug release data for the formulations and the drug suspension.

## STATISTICAL ANALYSIS

The obtained data was statistically analyzed by SPSS version 23. *t*-test was applied for comparison of drug release from simple tretinoin suspension, tretinoin loaded NLCs formulation and tretinoin loaded NLCs-based sunscreen cream. $p<0.05$ was selected as the level of significant difference.

## RESULTS

***Preparation and optimization of tretinoin-loaded NLCs***

Box-Benkhen design model predicted an optimized tretinoin-loaded NLCs formulation based on 17 runs with variable ratios set by the model. Independent and dependent variables for the formulation were determined by the design model. Experimental results for the optimized formulation were measured and presented in table 1. Percentage error was calculated for both predicted and experimental formulations, yielding an overall desirability value of 0.780.

***Characterization of tretinoin-loaded NLCs***

The formulated Tretinoin Loaded NLCs were evaluated to determine their physical and chemical properties. The particle size, PDI, entrapment efficiency and zeta potential (table 1.0) of optimized experimental formulation were 235.3nm, 0.165, 94% and -23.6 mV respectively. Fig. 1 shows the results of various analyses conducted to evaluate the properties of the optimized formulation. Results of FTIR analysis (fig. 1A) revealed distinctive peaks in lauric acid and excipients, confirming no interactions. Also, the differential scanning calorimetry (fig. 1B) showed a lower endothermic peak for tretinoin-loaded NLCs, indicating loss of crystallinity post-integration. The outcome of the X-ray diffraction (fig. 1C) displayed an amorphous nature in optimized tretinoin-loaded NLCs, contrasting with the crystalline form of pure tretinoin. Furthermore, the scanning electron microscope (fig. 1D) depicted anisometric particles (183nm) in tretinoin-loaded NLCs, with altered lipid carriers affecting shape. Moreover, stability studies (fig. 1E) over 3 months indicated the formulation's stability, as minimal insignificant changes were observed in properties such as phase separation, redispersibility, particle size, entrapment efficiency, ZP and PDI compared to the initial samples.

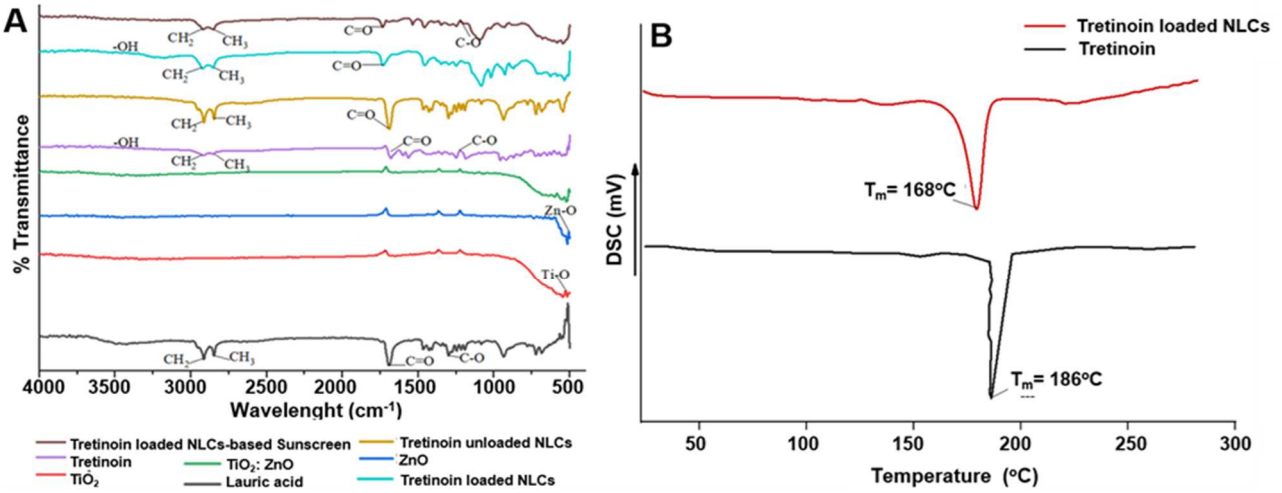
***Assessment of tretinoin-loaded NLCs-based sunscreen***

Inorganic sunscreen agents (ZnO and $TiO_2$) were added to the optimized NLCs formulation and the resulting formulation was evaluated to determine its sun protection factor and photostability (table 2). Results revealed that a 6% concentration of either ZnO or $TiO_2$ provided optimal SPF without an undesirable whitening effect. Combining ZnO and $TiO_2$ in a 1:1 ratio exhibited the highest SPF (5.23) and effective broad-spectrum UV protection with a ratio of 0.803, against UV radiations compared with other formulations.

**Table 1**: Predicted and observed values for the optimized tretinoin loaded NLC formulation

| | Tretinoin (%) | Lipids (mg) | Tween -80 (mg) | Particle size (nm) | PDI | Zeta Potential (mV) | %EE |
|---|---|---|---|---|---|---|---|
| PF | 0.044 | 99 | 273 | 243 | 0.163 | -24.55 | 96.03 |
| EF | 0.04 | 99 | 273 | 235.3 | 0.165 | -23.6 | 94 |
| % Error | | | | 3.16% | 1.22% | 3.86% | 2.11% |

PF= Predicted Formulation, EF= Experimental Formulation, EE= Entrapment Efficiency

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Stability data of the optimized tretinoin loaded NLC formulation at 25 °C ± 2 °C/65% ± 5% RH and 40 °C ± 2 °C/75% ± 5% RH for 90 days

| Temp. | Time (days) | Phase separation | Redispersibility | Precipitate formation | PS (nm) | PDI | ZP (mV) | %EE |
|---|---|---|---|---|---|---|---|---|
| 25°C | 0 | No | Yes | No | 235.3 | 0.165 | -23.6 | 94 |
| | 30 | No | Yes | No | 238.9 | 0.172 | -23 | 92.9 |
| | 60 | No | Yes | No | 247.5 | 0.178 | -22.4 | 90.4 |
| | 90 | No | Yes | No | 261.7 | 0.182 | -22 | 89.9 |
| 40°C | 0 | No | Yes | No | 235.3 | 0.165 | -23.6 | 94 |
| | 30 | No | Yes | No | 243.8 | 0.176 | -22.9 | 90.85 |
| | 60 | No | Yes | No | 258.2 | 0.195 | -22.3 | 87.4 |
| | 90 | No | Yes | No | 271.3 | 0.254 | -21.8 | 86.8 |

**Fig. 1**: Characterization of tretinoin loaded NLCs formulation (A) FTIR-analysis, (B) DSC-analysis, (C) XRD-analysis, (D) SEM image, (E) Stability studies

**Table 2**: Sun protection factor

| Formulation | SPF values | |
|---|---|---|
| | $TiO_2$ | ZnO |
| NLCs (0% TRE + 0% sunscreening agent) | 1.29 | 1.29 |
| Tretinoin-NLCs (0.05% TRE + 0% sunscreening agent) | 1.90 | 1.90 |
| Tretinoin-IO2-NLCs (0.05% TRE + 2% sunscreening agent) | 2.88 | 3.18 |
| Tretinoin-IO4-NLCs (0.05% TRE + 4% sunscreening agent) | 3.26 | 3.41 |
| Tretinoin-IO6-NLCs (0.05% TRE + 6% sunscreening agent) | 4.08 | 4.40 |
| Tretinoin-IO8-NLCs (0.05% TRE + 8% sunscreening agents | 3.52 | 4.21 |
| Tretinoin-IO10-NLCs (0.05% TRE + 10% sunscreening agent) | 3.74 | 4.82 |
| Formulation | $TiO_2$ : ZnO | SPF values |
| Tretinoin-IO6-NLCs (0.05% TRE + 6% sunscreening agent) | 3:1 | 3.28 |
| Tretinoin-IO6-NLCs (0.05% TRE + 6% sunscreening agent) | 2:1 | 3.33 |
| Tretinoin-IO6-NLCs (0.05% TRE + 6% sunscreening agent) | 1:1 | 5.23 |
| Tretinoin-IO6-NLCs (0.05% TRE + 6% sunscreening agent) | 1:2 | 3.24 |

**Table 3**: Evaluation of tretinoin loaded NLCs-based inorganic sunscreen cream

| Parameters | Results |
|---|---|
| pH | 5.4 |
| Viscosity | 29,000cP |
| Organoleptic characteristics | |
| Appearance | Clear homogeneous |
| Texture after application | Smooth |
| Color | Milky White/ Slightly yellowish |
| Odor | Odorless |
| Phase separation | No |

**A** UVA/UVB ratio of best fitted Optimized tretinoin NLCs based 6% sun screening agents.

| Formulation | UVA/UVB ratio |
|---|---|
| Tretinoin-IO6-NLCs (0.05% TRE + 6% $TiO_2$) | 0.609 |
| Tretinoin-IO6-NLCs (0.05% TRE + 6% ZnO) | 0.593 |
| Tretinoin-IO6-NLCs (0.05% TRE + 6% $TiO_2$ +ZnO) 1:1 | 0.803 |

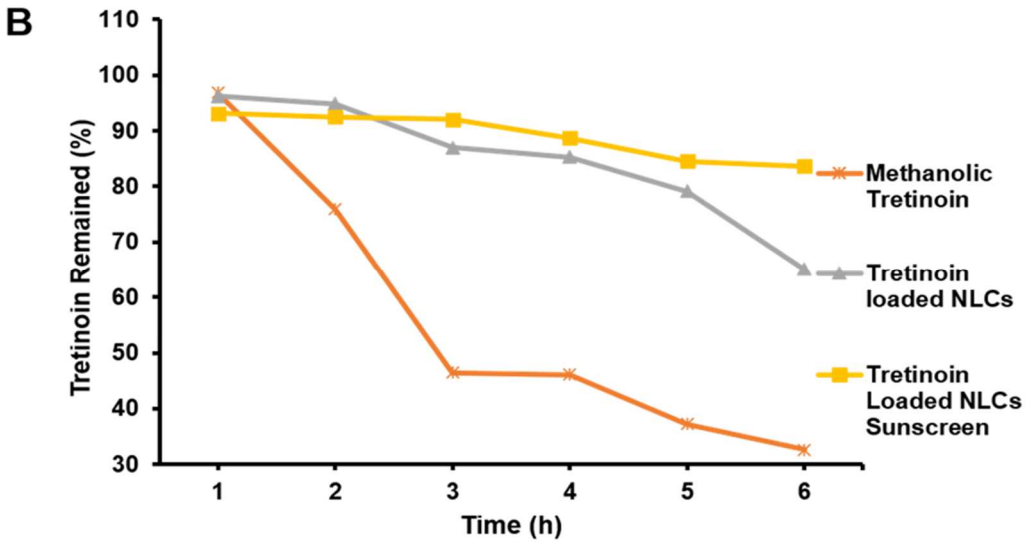


**Fig. 2**: Evaluation of tretinoin loaded NLCs-based sunscreen cream (A) UVA/UVB ratio of best fitted optimized tretinoin NLCs based 6% sun screening agents, (B) Photodegradation profile of methanolic tretinoin solution, tretinoin loaded NLCs and tretinoin loaded NLCs-based sunscreens

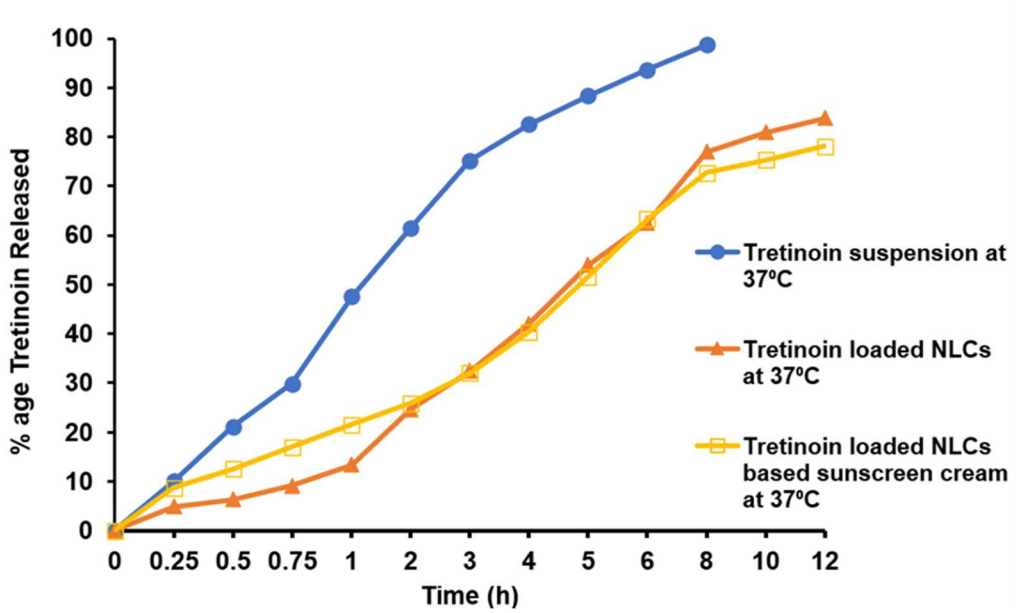


**Fig. 3**: *In-vitro* drug release studies of tretinoin suspension, tretinoin loaded NLCs formulation and tretinoin loaded NLCs-based sunscreen cream

Formulation with combined $TiO_2$ + ZnO (6%) in 1:1 demonstrated a superior UVA/UVB ratio and was selected for further in-vitro studies (fig. 2A). Furthermore, photostability studies (fig. 2B) revealed that the optimized sunscreen formulation significantly reduced tretinoin degradation under UV exposure. After 6 hours, only 32% of methanolic tretinoin remained compared to 65% for tretinoin-loaded NLCs and 83% for the optimized sunscreen formulation (6% $TiO_2$ + ZnO 1:1). This suggests that the NLCs and the sunscreen agents together contribute to the photostability of tretinoin, reducing its photo degradation rate.

***Preparation and assessment of tretinoin-loaded NLCs-based Sunscreen Cream***

The successful incorporation of tretinoin-loaded NLCs-based sunscreen into a cream dosage form was assessed and evaluated for a variety of attributes as shown in table 3.

***Homogeneity and appearance***

Tretinoin-loaded NLCs-based sunscreen cream was found to be light yellow in color, milky, creamy and smooth in texture. The formulated tretinoin-loaded NLCs-based sunscreen cream was uniform and homogenous.

***pH***

The pH of tretinoin-loaded NLCs-based sunscreen cream /was found to be 5.4, which is non-irritating and acceptable for the skin (table 3).

***Viscosity***

The viscosity of the tretinoin-loaded NLCs-based sunscreen cream was found to be 29,000cP at 20rpm spindle speed (table 3). The cream was found to have the desired viscosity, indicating the ease of application with minimal shear or stress.

***Drug release studies and kinetics***

Results of the drug release studies, shown in fig. 3, revealed the release behavior of tretinoin from suspension, tretinoin-loaded NLCs, and tretinoin-loaded NLCs-based sunscreens cream. At the 4th hour, drug release percentages were 82.59%, 42.07%, and 40.42%, respectively. By the 8th hour, the percentages increased to 98.66%, 76.98% and 72.70%, demonstrating sustained release. After 12 hours, tretinoin-loaded NLCs and tretinoin-loaded NLCs-based sunscreen cream exhibited 83.76% and 78.05% drug release, indicating delayed release for prolonged skin retention. The data was also evaluated for statistical analysis which showed that drug release from simple tretinoin suspension was significantly different from tretinoin loaded NLCs formulation and tretinoin loaded NLCs-based sunscreen cream ($p$<0.05). Moreover, various *in-vitro* release models were applied, with tretinoin suspension following the first-order model, while tretinoin loaded NLCs and tretinoin loaded NLCs-based sunscreen cream followed the Korsmeyer-Peppas model. The calculated values of "n" for both formulations (0.700 and 0.587, respectively) suggested a combination of surface erosion and diffusion in the release mechanism. High $R^2$-values (0.9933 and 0.9739) confirmed the Korsmeyer-Peppas model as the best fit.

## DISCUSSION

The current study was designed to fabricate and optimize topically applied tretinoin-loaded NLCs from natural fatty acids to potentiate the photo-stability of tretinoin and to

enhance its photoprotective efficacy by blending optimized formulation with inorganic sunscreens.

Tretinoin-loaded NLCs were prepared using modified microemulsion technique (Khan *et al*., 2021) and then optimized by a three-factor Box-Behnken design. The design model predicted 17 runs. All 17 experimental formulations were fabricated, and responses were measured. Relating all the formulation's factors and responses the design expert model predicts an optimized formulation against available documented data, having a desirability of 0.780. To check the compatibility between the tretinoin and excipients, FTIR was performed. The FTIR spectra revealed that there was no discernible alteration in the peaks of tretinoin and excipients. All of them were compatible with each other and the results are in accordance with the previous study with the same ingredients (Alotaibi *et al*., 2021).

The stability and agglomeration tendency of NLCs can be evaluated by their zeta potential and PDI which are the manifestations of their electro-kinetic properties. The minimum ±20 mV of zeta potential and 0-1 of PDI is considered good for the stability of NLCs (Czajkowska-Kośnik *et al*., 2021). The pharmacokinetic parameters, like absorption, distribution, and endocytosis, of the nanoparticles are influenced by their size. A size close to 300nm is considered good for prolonged delivery as in our study (Khosa *et al*., 2018, Shah *et al*., 2009).

The prepared NLCs showed very good entrapment efficiency. However, when the concentration of tween-80 was increased, entrapment efficiency was decreased. Increased concentration of surfactant might enhance the drug partitioning from oily to aqueous phase leaving behind a minute concentration of drug entrapped (Soni *et al*., 2018).

Differential scanning calorimetry results depicted that tretinoin lost its crystallinity after being integrated into NLCs. When the materials were structured as NLCs, the endothermic temperature was substantially lower. The decline of the endothermic peak may have been driven by the contribution of added surfactant (Khalil *et al*., 2024).

A standard method for evaluating the crystallinity and conformation of solid materials is X-ray diffraction. X-ray diffractogram showed intense sharp peaks of tretinoin indicating its crystalline nature. However, the optimized tretinoin-loaded NLCs presented broader peaks with minimal sharpness than pure tretinoin. These findings demonstrated that the NLCs completely enclosed tretinoin, which then changed from its crystalline form to an amorphous one (Abdelbary and Fahmy, 2009).

Nanoparticle's shape and size were investigated via scanning electron microscope which showed an anisometric morphology. When drying is carried out for lipid carriers, the alteration of lipids occurs in the sample. This might be a reason for the particle shape divergence from sphericity. These non-spherical particles, however have much higher drug-loading capability as compared to the spherical ones (Veerawat Teeranachaideekul, 2007).

All the sunscreen-based tretinoin-loaded NLCs formulations were subjected to transpore tape assay to check the *in-vitro* photo-stability. The results of SPF analyses showed that tretinoin-loaded NLCs-based 6% of both $TiO_2$ and ZnO have the highest SPF. Moreover, the UVA/UVB ratio showed that the tretinoin-loaded NLCs-based 6% inorganic sunscreens (1:1) had broad spectrum protection. Furthermore, the photo-stability study of methanolic tretinoin solution, tretinoin-loaded NLCs, and optimized tretinoin-loaded NLCs-based 6% $TiO_2$ + ZnO 1:1 sunscreen formulation was performed under UV light within the UV chamber. Results revealed that the photo-degradation rate was methanolic tretinoin > tretinoin loaded NLCs > optimized tretinoin loaded NLCs-based 6% $TiO_2$ + ZnO 1:1 sunscreen formulation. Hence it was observed that with the loading of tretinoin into NLCs, the photo degradation rate was reduced. It was further reduced when 6% TiO2 +ZnO 1:1 sunscreen was incorporated into tretinoin-loaded NLCs (Chu *et al*., 2022, de Araújo *et al*., 2024).

Stability studies for the tretinoin-loaded NLCs-based cream were taken out for 3 months and showed that formulations were stable for a longer period. These results followed the previous studies and the present formulation could therefore be regarded as a stable formulation (Ijaz *et al*., 2019). The release studies showed that drug release from tretinoin-loaded NLCs and tretinoin-loaded NLCs-based cream exhibited sustained release profile behaviour. It indicates that both tretinoin-loaded NLCs and tretinoin-loaded NLCs-based cream have delayed release and might be useful for prolonging drug retention time on the skin (Mohamad *et al*., 2022).

## CONCLUSION

In conclusion, the present study affirms the NLCs formulation as a viable carrier for topically administering tretinoin, reducing its photosensitivity, and suggesting that incorporated sunscreens may provide prolonged protective effects against harmful UV radiations. Future studies may be conducted by incorporating other sunscreen i.e., natural phytochemicals in such NLCs formulations holding promise for solutions to many of the problems of existing sunscreens.

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