"Evaluation of the photoprotective potential of trans-urocanic acid incorporated in mesoporous silica"

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Abstract

The study focuses on the development and evaluation of ACUMES, a system composed of trans-urocanic acid incorporated into mesoporous silica nanoparticles, aiming to enhance photoprotection and stability against UV radiation. The incorporation process was confirmed through Fourier-transform infrared spectroscopy (FTIR), which indicated successful interaction between trans-urocanic acid (ACU) and mesoporous silica (MES). Thermogravimetric analysis revealed a significant retention of ACU within the silica matrix. Morphological analysis via transmission electron microscopy confirmed that ACUMES particles are nanometric in size with a mesoporous structure. Zeta potential measurements demonstrated the stability of the nanoparticles, with values exceeding −30 mV. The in vitro evaluation of sun protection factor (SPF) showed a remarkable increase of 273.13% in comparison to the control formulation without ACUMES. Additionally, the critical wavelength remained above 370 nm, ensuring broad-spectrum protection against both UVA and UVB radiation. The incorporation of ACU into MES also provided photo-stabilization to commonly used chemical filters such as octyl methoxycinnamate and avobenzone. However, the study highlighted the limited capacity of mesoporous silica to prevent the photoisomerization of trans-urocanic acid into its cis isomer, which is known to induce immunosuppressive effects. This finding underscores the need for further optimization of the formulation to enhance the stability of the active compound under UV exposure. The system maintained erythrocyte membrane stability, indicated nosignificant hemolytic effect of ACUMES at concentrations ranging from 5 to 500 µg/mL. suggesting its safety for topical applications. In conclusion, the ACUMES system demonstrated promising photoprotective properties, with enhanced SPF and photo-stabilizing effects. Despite the need for further improvements to prevent photoisomerization, these results support the potential of trans-urocanic acid incorporated in mesoporous silica as an innovative active ingredient in sunscreen formulations.

Key-words: urocanic acid, mesoporous silica, photoprotection

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1. Introduction

Exposure to ultraviolet radiation (UVR) is the main environmental risk factor for the development of skin cancer, including both melanoma and non-melanoma types [1]. Sunscreens containing UV filters remain a cornerstone strategy for preventing UVR-induced damage. These filters can be classified as organic (chemical) or inorganic (physical) according to their mechanism of action [2].

Reactive oxygen species (ROS) generated by UVA and UVB radiation contribute to cellular damage and promote immunosuppressive responses [3,4]. Consequently, sunscreens are crucial for minimizing oxidative stress and protecting skin health. Among the efficacy indicators for sunscreens, Sun Protection Factor (SPF) measures UVB protection, while UVA protection is assessed by parameters such as the critical wavelength (λc) [5,6].

Nevertheless, organic filters, due to their lipophilic nature, can penetrate the stratum corneum and reach vascularized dermal layers, raising concerns about systemic toxicity [7,8]. Inorganic filters like TiO_2 and ZnO, although less prone to deep penetration, can also generate ROS and impact aquatic ecosystems [4,9]. Moreover, adverse reactions such as skin irritation and allergic dermatitis have been associated with some organic filters [10].

Advances in nanotechnology offer promising alternatives by improving the stability, safety, and efficacy of UV filters [11,12]. Nanomaterials, typically ranging from 1 to 100 nm, provide unique advantages such as controlled release, increased bioavailability, reduced toxicity, and enhanced photostability [13-15]. Among innovative approaches, encapsulating UV-active

compounds into nanostructured systems represents an effective strategy to address the limitations of conventional sunscreens.
Trans-urocanic acid (ACU), a naturally occurring chromophore produced in the stratum corneum, initially showed promise as a natural UV absorber [16]. However, its application was hindered by photoisomerization from the trans to the cis form, associated with immunosuppressive effects and increased risk of photocarcinogenesis [16-18]. This led to the removal of ACU from sunscreen formulations by regulatory authorities such as the FDA.
Encapsulating trans-ACU into mesoporous silica nanoparticles could potentially protect it from photoisomerization, preserving its beneficial properties while mitigating immunosuppressive risks. In this context, the present study aimed to synthesize and characterize a novel system (ACUMES), assessing its photoprotective efficacy, photostability, and preliminary safety for topical application.

2. Materials and Methods

2.1 Synthesis of Mesoporous Silica Nanoparticles (MES) and adsorption of Trans-Urocanic Acid (ACU)

Mesoporous silica nanoparticles were synthesized based on an adapted sol-gel method [19]. A solution of NH_4F (0.3 mol/L) was prepared and mixed with 2-[2-(2-hydroxyethoxy)propoxy]ethanol until the critical micellar concentration was reached. Then, tetraethyl orthosilicate (TEOS, 40 mmol) was added dropwise under magnetic stirring. The reaction was maintained under heating and then allowed to cool. The product was collected by centrifugation. Template removal was performed using an ethanolic solution containing 1% hydrochloric acid (v/v), followed by additional centrifugation and drying [19].
A stock solution of ACU (2 mg/mL) was prepared by dissolving trans-urocanic acid in purified water using an ultrasonic bath. To this solution, MES was added and kept under magnetic stirring at room temperature for 120 hours. The resulting suspension was filtered and air-dried for 24 hours to yield the ACUMES system [20].

2.2 Nanoparticle Characterization

2.2.1 Thermogravimetric Analysis (TGA)
Thermal stability and ACU content were evaluated using a Shimadzu TGA-50 thermobalance. Samples (~6 mg) were heated from 25 to 800 °C at 10 °C/min under nitrogen flow (50 mL/min).

2.2.2 Transmission Electron Microscopy (TEM)
Nanoparticle morphology and size were assessed using a JEM-2100 TEM (JEOL, Japan).

2.2.3 Poli dispersion index and Zeta Potential
Measurements were performed using a Zetasizer Nano ZS (Malvern Instruments). Samples were diluted (1:100, v/v) in purified water and analyzed at 25 °C in triplicate.

2.3 In Vitro Photoprotective Efficacy and Photostability.

In order to prepare the photoprotective formulation containing ACUMES and to evaluate the enhancement in photoprotection and its photostability, compatibility studies of these systems in emulsions were initiated. For this purpose, a semi-solid base was prepared containing Cetearyl alcohol (and) ceteth-10 phosphate (10%), caprylic/capric triglycerides (15%), Phenoxyethanol (and) caprylyl glycol (1%), and purified water (q.s.p.) for the incorporation of the active ingredients and the UV filters octyl methoxycinnamate (10%) and avobenzone (5%) [21]. The emulsions containing chemical UV filters (octyl methoxycinnamate and avobenzone) were prepared with or without ACUMES, free ACU, or MES. SPF and critical wavelength (λc) were measured using diffuse reflectance spectroscopy (Labsphere UV-2000S®), applying 1.3 mg/cm^2 of sample to PMMA plates. The formulations were irradiated under a solar simulator (Atlas Suntest CPS+), and SPF values were calculated before and after UV exposure [22, 23,24].

2.4 Photoisomerization Assessment

To evaluate whether MES protected ACU from photoisomerization, samples of ACUMES and free ACU (1 mg/mL) were irradiated for 2 hours (765 W/m^2) using the solar simulator (Atlas Suntest CPS+). Post-irradiation, samples were stirred for 48 hours to ensure ACU release and analyzed by HPLC [21].

2.6 Statistical Analysis

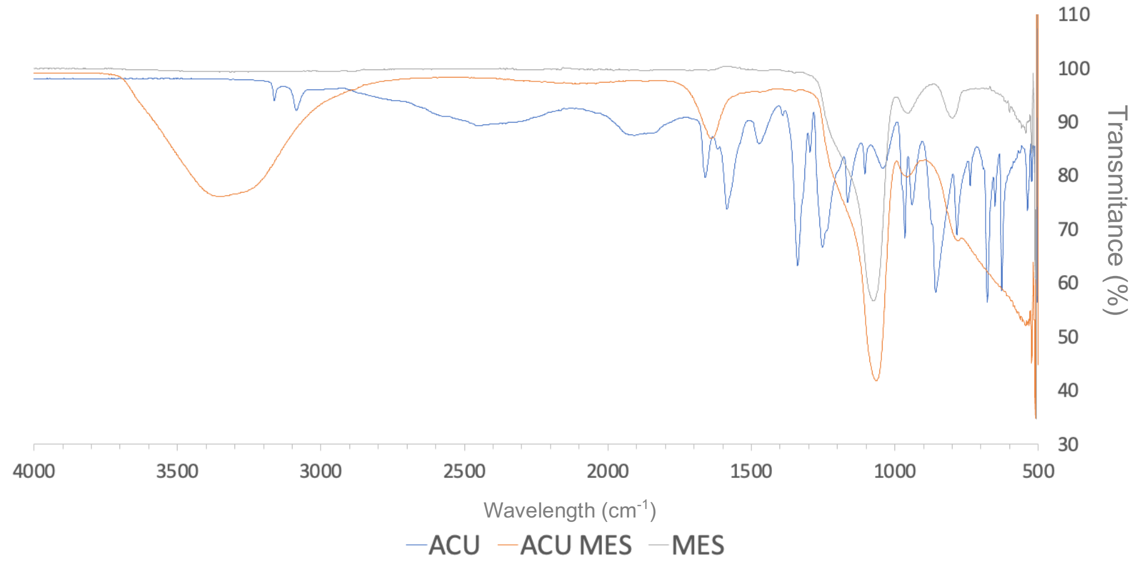
Results are presented as mean ± standard deviation. Statistical significance was evaluated at $\alpha = 0.05$ using Student's t-test or one-way ANOVA followed by Tukey's post hoc test, depending on the dataset distribution (assessed using Shapiro–Wilk test).

3. Results

3.1 Characterization of ACUMES Nanoparticles

FTIR analysis confirmed the successful adsorption of trans-urocanic acid onto mesoporous silica nanoparticles. Characteristic absorption bands corresponding to silica and urocanic acid functional groups were identified (Figure 1).

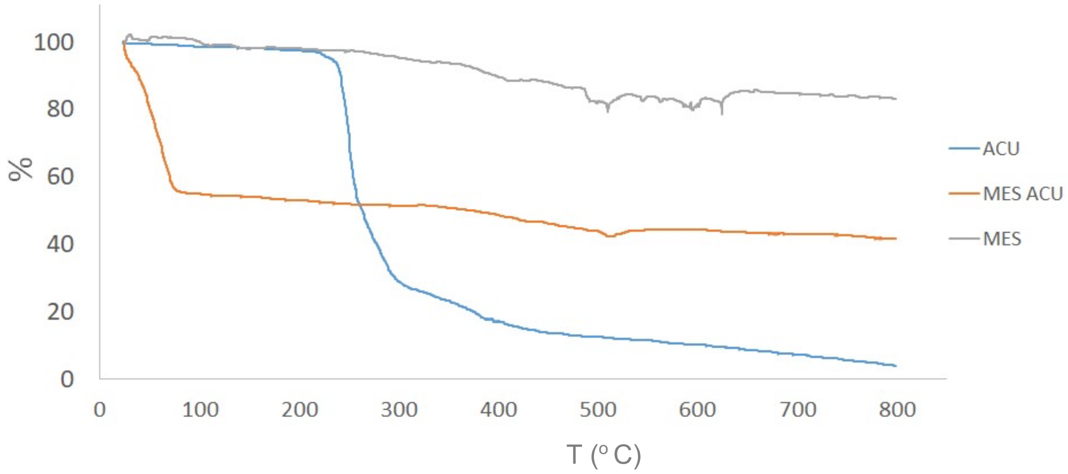
Figure 1. Mesoporous sílica, incorporated ACU and free ACU FTIR.



Legenda: ACU – trans urocanic acid; MES – mesoporous silica; ACUMES – trans urocanic acid ácido into mesoporous sílica.

TGA revealed a gradual mass loss consistent with the decomposition of organic material, confirming the incorporation of ACU into the silica matrix. The ACU content was estimated at approximately 5% (w/w) (Figure 2).

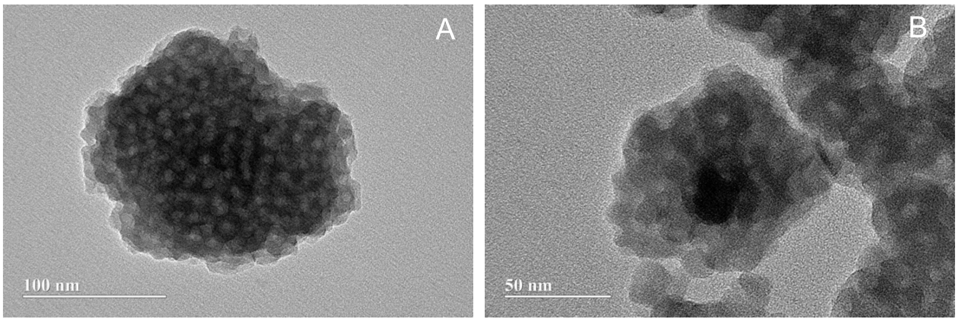
Figure 2. Thermogravimetric curve of ACU, MES ACU and MES samples



Legend: % – percentage of mass loss; ACU – trans urocanic acid; MES – mesoporous silica; ACU MES – trans urocanic acid incorporated into mesoporous silica.

The photomicrographs obtained by transmission electron microscopy for MES and ACUMES, shown in Figure 3, confirm that the silica particles are in the nanometric range and exhibit mesoporous characteristics.

Figura 3. Transmission electron microscopy for ACUMES (A) E MES (B)



Dynamic light scattering (DLS) measurements indicated a larger hydrodynamic diameter, attributed to particle agglomeration in suspension. The zeta potential of ACUMES was −111.23 ± 21.68 mV, indicating high colloidal stability. The ACUMES polydispersity index (PDI) obtained was 0.484 (± 0.053).

3.2 In Vitro Photoprotective Efficacy and Photostability

Before irradiation, the control formulation (C) exhibited an SPF of 23 ± 2.9 and a critical wavelength (λ) of 381 ± 0.35 nm. Mesoporous silica (MES) additionincreased the SPF to 33 ± 5.1, not significative, although the λ decreased slightly to 379 ± 0.64 nm. In contrast, the formulation containing ACUMES achieved a significantly higher SPF of 111.94 ± 20.6 ($p < 0.05$), while maintaining a λ of 380 ± 0.20 nm. The emulsion containing free ACU showed an SPF of 28 ± 6.2 and a λ similar to the control.
After 30 minutes of simulated UV irradiation, the SPF values decreased for all samples, but the ACUMES formulation maintained superior protection (SPF 73.57 ± 35.7), while the control presented FPS 12,95 ± 1,66.

3.3 Photoisomerization Behavior

The protection of ACU against photoisomerization was assessed through HPLC analysis. Although some degree of protection was observed, ACUMES did not completely prevent the photoisomerization of trans-ACU into the cis-isomer under UV irradiation.

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4. Discussion

The in vitro sun protection factor (SPF) evaluation demonstrated that the formulation containing ACUMES exhibited a significant SPF enhancement of 273.13% compared to the formulation containing only conventional UV filters. Moreover, the critical wavelength (λc) remained above 370 nm both before and after UV irradiation, indicating effective broad-spectrum protection. Photostability studies showed that formulations with ACUMES provided improved

protection against the photodegradation of octyl methoxycinnamate and avobenzone under simulated solar exposure when compared to formulations without the nanoparticles.
Ma *et al.* further demonstrated that sealing mesoporous silica with a dense SiO_2 shell synergistically enhanced the photostability of combined organic and inorganic UV filters and curtailed cutaneous penetration, corroborating our strategy of employing silica carriers to stabilize ACU in sunscreen matrices [25].
Despite the promising results, the limited protection against photoisomerization underscores the need for formulation optimization to improve trans-ACU stability, thereby minimizing the risks associated with cis-isomer formation, which should be the focus of future investigations. Recent ultrafast spectroscopic investigations reinforce the need to suppress the trans-to-cis conversion through molecular engineering and tailored carriers [26, 27].

5. Conclusion

ACUMES exhibited promising photoprotective and photo-stabilizing characteristics with a significant SPF increase and demonstrated safety in preliminary assays. Nevertheless, improvements are necessary to prevent trans-ACU photoisomerization effectively. Future research should explore formulation adjustments to optimize trans ACU stability, enabling its safe and effective use in advanced sunscreen products.

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