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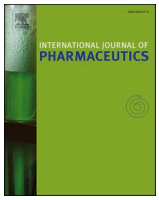

# Nanocomposite gel containing usnic acid: Characterization and evaluation of the antibacterial efficacy on Staphylococcus epidermidis

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ABSTRACT

Usnic acid (UA) is one of the most abundant secondary metabolites of lichens. Its antibacterial, anti-inflammatory, antiviral, and antitumor properties make it one of the few commercially available lichens compounds. Owing to its low solubility it has limited application, for that reason encapsulation in polymeric micelles (UA-PM) has been used to solve this aspect. Then, the obtained dispersion has been incorporated into a Sepigel-based gel for skin application (UA-PM-GEL). Polymeric micelles consisting of Soluplus, Solutol HS15 and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) were characterized in terms of size, polydispersity index, Zeta potential and morphological analysis. The nanocomposite gel (UA-PM-GEL) was characterized by determining the rheological behaviour, pH, UA recovery, physical and chemical stability and texture characteristics during one-month storage. The dialysis bag method and vertical diffusion Franz cell apparatus were used to determine the UA release from UA-PM and UA-PM-GEL. Skin-PAMPA assay allowed to evaluate the influence of micelles and nanocomposite formulation on the *in vitro* passive permeability of UA. In addition, the antibacterial activity of UA-PM and UA-PM-GEL was evaluated on the *Staphylococcus epidermidis* bacterial strain.

The micellar formulation significantly increased both the solubility and the permeability of the UA, as indicated by the Pe value obtained by Skin-PAMPA test. The release of the active ingredient, although more gradual than the colloidal dispersion, was not hindered by the viscosity of the gel system, as demonstrated by the release studies. The rheological properties of the gel outlined a good spreadability, adhesion and viscosity, suggesting easy application and removal from the application site. The pH values also proved to be suitable for skin application. The formulation was chemically and physically stable for 30 days, with a percentage of loss of the active ingredient less than 10 %. Finally, both the micelles and the nanocomposite gel revealed an effective antibacterial activity, representing a promising approach for the treatment of skin infections.

## 1. Introduction

Usnic acid (UA), isolated for the first time in 1844 from the *Usnea* genus, has been identified in several lichen genera *Cladonia*, *Lecanora*, *Ramalina*, *Alectoria*, and *Evernia* widespread in Arctic, Antarctic, tropical and subtropical areas (Douglas Andrade de Araújo et al., 2021). Its antiviral, antitumor, antioxidant, antibacterial, antifungal, anti-inflammatory and protective properties against UV rays are well documented (Croce et al., 2022; Wang et al., 2022). Unfortunately, the poor solubility together with the potential hepatotoxicity associated with the systemic application of UA have limited its therapeutic use as demonstrated by the few formulations present on the market. UA has been used both as an active component and as a preservative in personal care products such as creams, perfumes, deodorants, mouthwashes, and toothpastes due to its antibacterial and antifungal properties. A Gillette deodorant stick containing UA has been registered with US patent (Kwong & Wang, 2020). In Italy MicofootZeta, FootZeta and SterilZeta are indicated as disinfectants, antifungals and healing agents (Zugic et al., 2020). Although to a lesser extent, it has also been exploited in the agricultural industry for its antimicrobial, insecticidal, and herbicide

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activity, as an alternative characterized by a relatively limited ecological impact compared to some synthetic chemicals (Ingólfsdóttr, 2002). Furthermore, it has been used as an additive in packaging materials, in situations where natural and sustainable alternatives to the use of traditional chemical preservatives are preferred.

Following the emergence of antibiotic resistance, the antibacterial effect of UA has been evaluated with respect to various clinical isolates of bacteria resistant. A high activity was found against Gram-positive bacteria, *Bacillus cereus*, *Bacillus subtilis*, (Sultana & Afolayan, 2011), methicillin-resistant *Staphylococcus aureus* (Gupta et al., 2012; Lauterwein et al., 1995) and *Propionibacterium acnes* (Lauterwein et al., 1995; Weckesser et al., 2007). Different *in vitro* studies have highlighted the significant antibacterial activity of UA against *Staphylococcus epidermidis*, capable of both inhibiting biofilm formation and reducing cell viability at relatively low concentrations (Francolini et al., 2013). However, due to the reduced solubility, nanocarriers or hydrophilic polymers have been the protagonists of studies aimed at evaluating their possible positive influence in increasing the UA bioavailability and therefore its antibacterial activity against *S. epidermidis* (Martinelli et al., 2014; Francolini et al., 2013; Francolini et al., 2019; Pandit et al., 2021 Pagano et al., 2019).

The aim of this study was to prepare a micellar formulation capable of improving the UA aqueous solubility and to incorporate this dispersion into a gel for skin application. Polymeric micelles are thermodynamically and kinetically stable systems due to the combination of multiple amphiphilic polymers. They protect the encapsulated drug from degradation by enzymes or metabolism, but the most relevant feature is the excellent ability to promote the solubilization process of active ingredients that are insoluble in an aqueous environment, especially thanks to the high efficiency of drug loading (Shiraishi et al., 2015). The choice of polymers is important for a good performance of the micellar preparation *in vivo* (Figueiras et al., 2022). Polymeric micelles consisting of Soluplus, Solutol HS15 and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) were used (UA-PM) (Vasarri et al., 2022).

Gels are semisolid preparations for skin application easy to obtain and to apply, they can also modulate the drug solubility and release (Risaliti et al., 2018; Barrett-Catton et al., 2021; Buwalda et al., 2014). Nanocomposite gels are gel materials with nanocarrier dispersed into their structure. The resultant structure shows synergic effects useful as drug delivery systems. They are biocompatible and realize controlled release (Barrett-Catton et al., 2021; Karchoubi et al., 2024), as evidenced in previous studies (El Bejjaji et al., 2024; Mostafa et al., 2021; Moghaddam et al., 2018). Topical nanoformulations are of interest to improve the efficacy of therapeutic compounds by increasing their penetration and half-life when applied to the skin.

The UA micellar dispersion was jellified with Sepigel 305 (UA-PM-GEL) and the resulting formulation was characterized determining the rheological behaviour, pH, UA recovery, physical and chemical stability and texture behaviour during one-month storage and *in vitro* release using dialysis bag method and vertical diffusion Franz cells. Skin-PAMPA assay allowed to evaluate the influence of the micelles and the nanocomposite formulation on the passive permeability of UA. The antibacterial activity of UA-PM and UA-PM-GEL was evaluated on the *Staphylococcus epidermidis* bacterial strain using the dilution test on microplates.

## 2. Materials and methods

### 2.1. Chemicals and reagents

(+)-Usnic acid (UA), purity 98 %, Methanol HPLC grade, Acetonitrile HPLC grade, Formic acid analytical grade, Dichloromethane (CH2Cl2), dimethyl sulfoxide (DMSO), hexane HPLC grade, isopropyl myristate (IPM), Phosphate-buffered saline pH 7.4 (PBS) and D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) were purchased from Sigma Aldrich (Milan, Italy). Silicone oil was from Galeno Srl (Comeana, Prato, Italy). Soluplus and Solutol HS15 were from BASF SE, Ludwigshafen, Germany. Sepigel 305 was obtained from Farmalabor, Assago, Milan; Italy. Ultrapure water was produced using a Merck Millipore's Simplicity® UV Water Purification System (Merck KGaA, Darmstadt, Germany).

### 2.2. Analytical method

High-Performance Liquid Chromatograph, HP 1100, equipped with an autosampler and a Diode Array Detector (DAD), (Agilent Technology, Santa Clara, CA, USA) was used for UA quali-quantitative determination. Chromatographic analyses were performed at 25 ∘C, using Kinetex C18 (150 × 4.6 mm, 5 μm) column. A gradient analytical method with 0.5 mL/min flow rate was applied, using (A) formic acid/water pH 3.2 and (B) acetonitrile as mobile phases (Vasarri et al., 2024). The UV spectra were recorded at 280 nm. A stock solution of AU in $CH_3CN$ (0.616 mg/mL) was prepared and subsequently diluted with $CH_3CN$ from 10 to 250-fold and injected. The resulting straight line has a correlation coefficient $R^2$ equal to 0.9999.

### 2.3. Preparation of polymeric micelles (PM) and Usnic Acid-Loaded polymeric micelles (UA–PM)

Empty (PM) and UA-loaded micelles (UA-PM) were prepared following the thin-film hydration method (Vasarri et al., 2022). Soluplus, Solutol HS15 and TPGS weight ratio 4:1:0.5 were dissolved in a 10 mL $CH_3OH/CH_2Cl_2$ (1:4 v/v) mixture. Then, the organic solvents were evaporated and the lipid film was hydrated with 5 mL of deionized water and sonicated for 3 min to form a micellar dispersion. The same method was applied to UA-PM preparation, with the addition of UA to the organic phase. UA final concentration resulted 1 mg/mL.

### 2.4. Physical characterization

Physical characterization (average diameter and polydispersity index, PDI) were carried out by Dynamic Light Scattering technique (DLS), employing the Zsizer Nanoseries ZS90 (Malvern Molecules Instrument, Worcestershire, UK), after a 10 or 20-fold dilution with dezionized water. The zeta potential was determined by Electrophoretic Light Scattering technique (ELS), using the same instrument. The results were expressed as the average of three measurements.

### 2.5. Preparation of nanocomposite gel (UA-PM-GEL) and UA-loaded gel (UA-GEL)

Sepigel 305 was incorporated into the micellar dispersion. The sample was manually stirred and the obtained formulation was left to stabilize until completely gelled. UA-GEL was obtained adding UA in Sepigel gel under mechanical agitation. The uniformity of the formulation was assessed by the homogeneous light yellow colour of the gel.

### 2.6. Characterization of UA-PM-GEL

#### 2.6.1. Viscosity

UA-PM-GEL viscosity (mPa·s) was measured by a Brookfield DVE-RV Digital Viscosimeter (Ametek Brookfield; Milan, Italy), using a 06 spindle in order to collect on-scale readings with a % Torque between 10 and 100. Spindle rotational speed extends on a scale from 0.3 rpm to 100 rpm and 2 min is the time required by the spindle to register a constant value of viscosity. Thus, every 2 min a new viscosity measurement was recorded. In particular, every 2 min the rotational speed was increased from 0.5 to 60 rpm to obtain an up-viscosity ramp, afterwards, it was reduced from 60 rpm to 0.5 rpm in order to get a down viscosity ramp. All the measurements were performed at 21 ± 2°C. All data were fitted on a viscosity-spindle rotational speed graph so that the

fluid could be classified from a rheological point of view.

*2.6.2. Spreadability test*

UA-PM-GEL was analysed by a Texture Analyser TA.XT.plusC (Enco, Spinea; Italy) equipped with a Heavy Duty Platform (HDP/90) and a TCC Spreadability Rig (HDP/SR) using a 5 kg load cell. An empty female cone was filled homogenously with the gel up to the surface avoiding the presence of air pockets. After that, it was locked with screws on the HDP/90. At the beginning of the test the male cone moved down with a 3 mm/s pre-test speed for 23 mm until the surface of the sample. Then, the male cone penetrates the sample to a depth of 2 mm. During penetration the force was seen to increase until the point of maximum penetration depth forcing the sample to flow outwards between the surfaces of the two cones. At the end, the male cone returned to the starting position (25 mm over the female cone) with a post-test speed of 10 mm/s. Before each measurement the instrument was recalibrated. The results are expressed as average of three measurements of three different UA-PM-GEL formulations.

*2.6.3. Scanning and Transmission Electron Microscope analysis*

UA-MP and diluted UA-MP-GEL dispersion, obtained by 20-fold dilution in ultrapure water, were analyzed by the Scanning Electron Microscope (SEM) Gaia 3 (Tescan s.r.o, Brno, Czech Republic), FIB-SEM (Focused Ion Beam-Scanning Electron Microscope), operating with electron beam voltage of 10 kV and Bright-field Transmission Electron Microscope (TEM) detector and delivered with a STEM (Scanning Transmission Electron Microscopy) detector, which provides a complementary method for image acquisition of transmitted electrons.

*2.6.4. Determination of pH*

Values of pH were determined according to the procedure reported by Dejeu et al., 2022. A Basic $20^{+}$ pH-meter (Crison Instrument, Barcelona, Spain) was calibrated with buffer solutions at pH 4.01, 7.00 and 9.21. The sample was diluted in distilled water and stirred vigorously for 5 min. After dispersion's filtration pH was determined. All of the measurements were carried out in triplicate.

*2.6.5. Extraction of UA from the gel*

The amount of UA loaded into the gel formulation was determined following the modified and optimized extraction method from a semi-solid preparation previous described (Risaliti et al., 2018). The sample was dissolved in 1 mL of $CH_3CN$ under magnetic stirring for 15 min to break down the gelled structure. 100 μL of UA-PM-GEL was diluted with 900 μL of $CH_3CN$. The sample was sonicated and centrifugated at 14000 rpm for 10 min. Finally, the amount of UA in the supernatant was determined by HPLC-DAD analysis. The experiments were performed in triplicate.

*2.6.6. Stability studies*

UA-PM-GEL was stored at 4 °C for one month. Both chemical and physical stability studies were conducted. Colour change, development of mold, rheological behaviour, spreadability and syneresis measurements were carried out on a weekly basis to predict the physical stability, together with the syneresis test. On the other hand, chemical stability was estimated by determining pH every week for one month and UA content every week up to 30 days. The recovery percentage (R%) was defined using method of paragraph 2.6.5.

*2.6.7. Syneresis test*

Syneresis refers to the expulsion process of a liquid from a gel due to the system's destabilization. The samples were stored at 25 °C and at 4 °C and the weight was monitored for 24, 48, 72 h and then weekly up to five weeks. According to Fitriani (Fitriani et al., 2019), the hydrogel was positioned on a support with a filtering base in order to collect the water released during storage. At each timepoint the gel was weighed and the syneresis value (g) was calculated by measuring the weight loss during storage then compared to the initial weight.

*2.6.8. In vitro release Study: Dialysis bag method*

The release study of UA from UA acetonitrile solution, UA-PM and UA-PM-GEL was investigated according to the dialysis bag method employing regenerated cellulose dialysis membranes (Spectrum Laboratories, Inc., Breda, The Netherlands, MWCO 12–14 kDa). The sample was inserted into the dialysis bag, put in a beaker containing 200 mL of a mixture PBS:EtOH (70:30) as release medium and kept under magnetic stirring at 37 °C. Subsequently, 1 mL of the release medium was withdrawn at predetermined intervals and replaced with the same volume of the mixture to maintain the sink conditions (Vasarri et al., 2022). The collected aliquots were analysed by HPLC-DAD. All the experiments were carried out in triplicate.

*2.6.9. In vitro release study: Vertical diffusion Franz cells*

The *in vitro* release of UA from UA-PM-GEL or UA-GEL was furthermore determined by the Franz cells apparatus. Cellulose nitrate filters (Sartotius Stedim Biotech GmbH, Germany), with 0.45 μm pore sizes and 130 μm thickness were employed as synthetic membranes and placed between the donor and the receptor chambers. The donor compartment was loaded with the gel, whereas the acceptor compartment was filled with a PBS:EtOH (70:30) mixture and maintained under magnetic stirring at 37 °C. At specified time intervals (30 min, 1, 2, 4, 6, 24 h) an aliquot was taken from the acceptor compartment and analysed by the HPLC-DAD to determine the concentration of the UA. All the experiments were performed in triplicate.

*2.6.10. Parallel artificial membrane permeability assay (PAMPA)*

UA ability to permeate the *stratum corneum* was determined using a 96-well MultiScreen-IP filter plate Millipore Corporation (Tullagreen, Carrigtwohill, Cork, Ireland). A mixture of silicone oil and IPM (70:30 v/v) in hexane (35 % v/v) was transferred to the bottom of the donor compartment for 20 min in order to completely evaporate the solvent and to simulate human skin (Casamonti et al., 2019; Markovic et al., 2012; Ottaviani et al., 2006). Then, acceptor compartment was filled with a solution of 5 % DMSO in PBS (250 μL). Moreover, the wells in the top plate (donor) were filled with 250 μL of samples. UA-PM dispersion on one hand, and a saturated solution of UA in 5 % DMSO in PBS on the other were used as donor samples; the second one was used as a term of comparison. Eventually, PAMPA sandwich was assembled. 150 μL of both compartments were picked up after 2 h and 4 h, centrifugated at 14000 rpm for 10 min and analysed by HPLC-DAD; the UA-PM acceptor and donor were analysed after a 10-fold dilution in $CH_3CN$. All the experiments were carried out in duplicate.

In addition, the Effective Permeability (Pe), expressed as $\mu g/cm^2$ was evaluated according to the following formula:

$$Pe = C \bullet \left[ -ln\left(1 - \frac{Ca}{Cequilibrium}\right) \right]$$

Where:

$$C = \frac{(Vd \bullet Va)}{[(Vd + Va)A \bullet t]}$$

$$Cequilibrium = \frac{[Cd \bullet Vd + Ca \bullet Va]}{Vd + Va}$$

A is the filter surface (0.24 $cm^2$) multiplied by the apparent porosity of the filter (f) of 20 % or 0.048 $cm^2$, $V_a$ and $V_d$ respectively represent the acceptor volume (250 μL) and the donor volume (250 μL), *t* is the time incubation (sec), finally $C_a$ represents the drug concentration in the acceptor compartment (mg/mL) and $C_d$ refers to the drug concentration in the donor compartment (mg/mL).

#### 2.6.11. In vitro test on Staphylococcus epidermidis by microplate dilution method

UA was tested on a strain of *Staphylococcus epidermidis* ATCC 12228 to evaluate its ability to inhibit its growth. For this purpose, the microdilution method was used (Sacco et al. 2020). On the bacterial line, the UA-MP formulations and gels containing UA-MP with gelling agent Sepigel 305 at concentrations of 1 % and 2 % w/v were tested. The UA-PM-GEL formulation had an active ingredient concentration of 1 mg/mL, while in the polymeric micelles the UA was present at a concentration of 1 mg/mL or 0.5 mg/mL. The bacterial strain *Staphylococcus epidermidis* was grown in Trypticase Soy Broth (TSB) (ThermoFisher Diagnostic SpA), incubated for 48 h at 37 °C and finally grown on Trypticase Soy Agar (TSA) (ThermoFisher Diagnostic SpA) at 37 °C for 48 h. Some bacterial colonies obtained were collected from the agar medium and suspended in physiological solution. The bacterial concentration contained was evaluated both by spectrophotometric reading (BioPhotometer Eppendorf srl) (OD 600) and by counting the subcultures on TSA incubated at 37 °C for 48 h. This value was found to be between 2.5–5.0 x $10^{-7}$ cfu/mL and corresponds to the concentration of the bacterial stock solution intended to be inoculated into the wells. Scaling and decreasing amounts of UA-PM and UA-PM-GEL (from 162 μL to 90 μL) were added to the wells with the complementary amount of TSB to obtain a percentage concentration between 90 % and 50 % of the antimicrobial formulation in the culture medium. Twenty μL of the bacterial stock solution were inoculated into all wells. The negative control was represented by the TSB medium without the UA-PM and UA-PM-GEL formulations, the positive control was oxacillin. Finally, the plate was sealed and incubated for 24 h at 37 °C. Subsequently, the entire content of the wells was transferred to Petri dishes, embedded in TSA and incubated at 37 °C for 48 h. The reduction capacity of the number of *S. epidermidis* was calculated by logarithmic reduction. A substance has disinfectant activity if it is able to reduce the bacterial load by 5 Log (Standard PN-EN 1040, 2006). To exclude that the observed activity was attributable to the empty formulation, polymeric micelles without active substance, Sepigel gel and Sepigel gel containing empty polymeric micelles were also tested. The test was repeated twice in triplicate. Micelles loaded with UA and incorporated into a 2 % gel were tested exclusively using the agar diffusion method because it was not possible to determine precise quantities due to the viscosity of the suspension. These tests did not indicate antibacterial activity.

## 3. Results and Discussion

### 3.1. Polymeric micelles preparation and characterization

The constituents of PM were selected among the ingredients considered safe and widely used in the food and pharmaceutical fields. Soluplus is a graft tri-block copolymer of polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol and its safety is widely reported in literature (Schmitt, 2022, Sipos et al., 2023). It forms micelles in aqueous solution due to its amphiphilic nature. Alone or in combination, it has found wide use in the pharmaceutical field for the preparation of polymeric micelles thanks to its safety and versatility characteristics (Pignatello et al., 2022). Soluplus also has a potential active role in modern antitumor strategies, since it has been demonstrated to be a potent P-gp inhibitor (Jin et al., 2015). Solutol HS15 or Kolliphor HS15 is an amphiphilic polymer consisting of polyoxyethylene esters of 12-hydroxystearic acid. It is a non-ionic surfactant characterized by high stability, solubility and low *in vivo* toxicity. It is a solubilizing agent capable of altering the MDR of drugs, modifying their binding to plasma proteins, improving their absorption and pharmacokinetics (Bergonzi et al., 2020). TPGS is a water-soluble nonionic surfactant synthesized by esterification of vitamin E succinate with the PEG chain. It has interesting characteristics as a solubilizing, gelling, dispersing and emulsifying agent. From the data reported in literature it is classified as a permeation enhancer and down-regulator of P-gp; essential functions for increasing the bioavailability of drugs (Rathod et al., 2021).

The authors used these excipients for the preparation of micelles aimed at dissolving natural substances (Bergonzi et al., 2020; Vasarri et al., 2022). In a previous publication, Soluplus, Solutol, and TPGS polymeric micelles resulted an excellent formulation for UA delivery to manifest inhibitory action on human neuroblastoma cell migration (Vasarri et al., 2022).

The procedure followed for the preparation of empty PM and UA-PM was the lipid film hydration (Vasarri et al., 2022). The selected amphiphilic polymers were able to deliver 1 mg/mL of UA, increasing its solubility by 330 times. After UA loading the system maintained good physical characteristics, as evidenced by the parameters reported in Table 1. The micellar dispersion appeared transparent and light yellow coloured, proof of the solubilization of the UA. Both empty PM and UA-PM have small dimensions with a narrow sizes distribution. UA is a weak acid with pKa 4.4 and it made the zeta potential of UA-PM more negative, as previously reported (Vasarri et al., 2022). A further small decrease of the zeta potential was also observed when UA-PM were formulated into the gel. This small variation can be attributed to the composition of the thickening agent. In fact, Sepigel consists of three components, polyacrylamide, C13-14 isoparaffins and Laureth7, that can affect the value of the potential.

UA-PM and diluted UA-PM-GEL were morphologically analysed by Electron Microscope (STEM). Microscopy observation confirmed the DLS results (Fig. 1). Image of UA-PM shows homogeneously dispersed particles, with spherical shape and dimensions comparable to those obtained by DLS. Micrographs of gel after dilution displayed spherical particles with equal or slightly larger dimensions compared to PM (80–120 nm), indicating that the micellar structure remains even after the addition of the gelling agent.

### 3.2. Preparation of the nanocomposite gel

Sepigel 305 is a product of synthetic origin, widely used in cosmetics as an excipient and obtained by polymerization in inverse emulsion. Its main composition includes for the 40–45 %: polyacrylamide, C13-14 isoparaffins and Laureth7 and water. Polyacrylamide is obtained from the polymerization of multiple acrylamide molecules and it is used for its viscosifying properties, laureth7 is an amphiphilic surfactant of synthetic origin, used for its emulsifying and cleansing action; finally, C13-14 isoparaffins are a mixture of branched saturated hydrocarbons used with an emollient and film-forming function. Due to its composition Sepigel 305 is able to incorporate both hydrophilic and lipophilic compounds, with high stability of the resulting gel. It serves not only as a thickener, but also as an exceptional stabilizer, emulsifier and texturizing agent as demonstrated by the resulting gels. They have a medium consistency and opalescence, providing a refreshing, evanescent quality with an optimal dermo-cosmetic appearance and high stability (Bergonzi et al., 2011; Risaliti et al., 2018; SEPPIC Sepigel 305. Available online: https://www.seppic.com/en/sepigel-305 (accessed on 30 September 2024); López-Arencibia et al., 2024; El Bejjaji, et al., 2024; Silva-Abreu et al., 2023; Berenguer et al., 2019).

The UA-PM-GEL formulation was obtained adding Sepigel 305 to the UA-PM liquid preparation at a concentration of 1 or 2 % w/v. As evidenced in Fig. 2, the formulation with 1 % is still liquid with a poor consistency for topical application. Increasing the concentration to 2 %

**Table 1**
Physical characterization of empty (PM), UA-loaded polymeric micelles (UA–PM) and nanocomposite gel (UA-PM-GEL). Data are reported as mean ± SD of n = 3 experiments.

| Sample | Size (nm) | PdI | Zeta Potential (mV) |
|---|---|---|---|
| **PM** | 59.30 ± 0.23 | 0.21 ± 0.00 | −6.44 ± 0.36 |
| **UA-PM** | 44.27 ± 0.17 | 0.27 ± 0.00 | −13.20 ± 1.40 |
| **UA-PM-GEL** | 52.40 ± 0.74 | 0.28 ± 0.00 | −16.19 ± 1.75 |

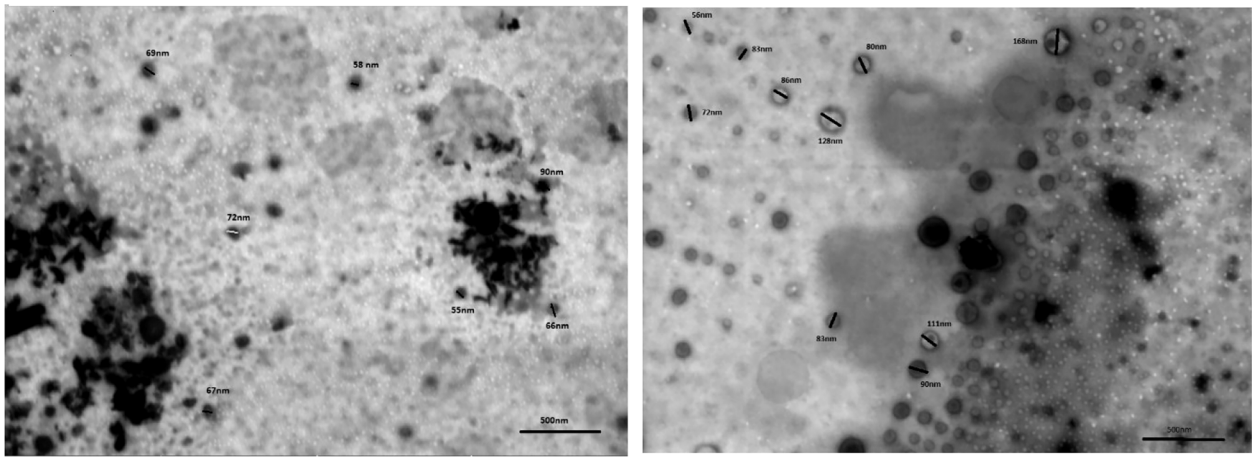


**Fig. 1.** Images of UA-MP (left) and UA-MP-GEL after 1:20 dilution (right) obtained by STEM analysis. Bar 500 nm.

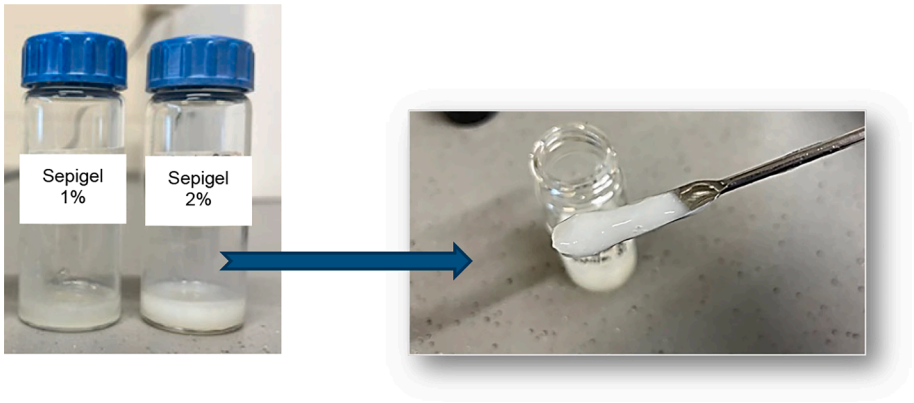


**Fig. 2.** Nanocomposite gel obtained with various percentages of gelling agent. UA-PM-GEL 1% (left, colloidal dispersion of AU-MP with 1% w/v of Sepigel), UA-PM-GEL 2% (right, colloidal dispersion of UA-MP with 2% w/v of Sepigel).

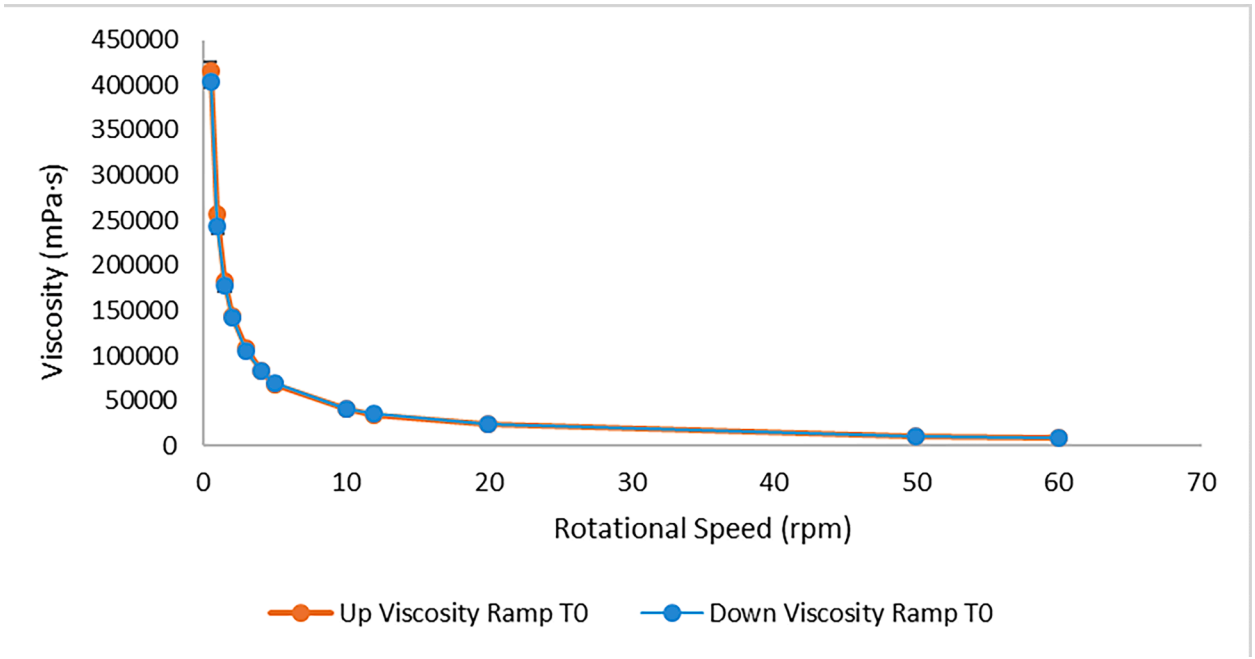


**Fig. 3.** Up and down viscosity ramps of UA-PM-GEL measured at 21 ± 2°C. Up viscosity ramp is the viscosity curve obtained from the spindle rotational speed increasing from 0.5 rpm to 60 rpm, while down viscosity ramp is the viscosity curve obtained from the spindle rotational speed reduction from 60 rpm to 0.5 rpm. Data are shown as Mean ± SD (n = 3).

results in a gel with a silky drafting, homogenous, simple application and removal from the skin. UA recovery % resulted of 90.05 ± 2.10 %. Furthermore, the gel was characterized in terms of rheological behaviour, pH, chemical and physical and texture properties.

### 3.3. Characterization of UA-PM Sepigel gel (UA-PM-GEL)

#### 3.3.1. Rheology

A rotational viscometer was used to characterize the rheological behavior of the gel. The viscosity assumed by the sample was evaluated as a function of different values of rotational speed. The data obtained were collected and reported in a graph having the viscosity (mPa·s) on the ordinate axis and the spindle rotation speed values (rpm) on the abscissa axis. Rheograms (Fig. 3) display the up and down rate ramps of viscosity, as a function of the spindle rotational speed at 21 ± 2 °C.

The gel was classified as a pseudoplastic and time-independent non-Newtonian fluid, with a viscosity (0.5 rpm) of 439000 ± 4243 mPa·s. The flow begins even under the action of modest forces, and the resistance opposed by the fluid to its flow varies as a function of the increase or decrease in the shear rate. In particular, the viscosity of the sample decreases as the applied shear rate increases. The progressive decrease in viscosity, in relation to the increase in the applied rate, reflects a continuous loss of the tendency to intertwine and pack the long-chain polymers of Sepigel 305 (Risaliti et al., 2018). The internal resistance of the system progressively decreases; this orientation produces a favorable effect on flow. It also gives the gel a shear-thinning behavior, that is a required quality for semi-solid preparations since they can be spread easily and they can show good adhesion to the site of application (Berenguer et al., 2019). The fluid is time-independent and does not exhibit thixotropic behavior because the two forward (up) and return (down) curves, are superimposable (Fig. 3). Furthermore, the viscosity values change as the applied shear force varies, but they do not change as a function of time, if measured at the same rpm value (Supplementary material, Fig. S1).

#### 3.3.2. Spreadability

Spreadability outlines a fundamental parameter in terms of texture of dermatological preparations. In particular, it refers to the creaminess, viscosity and consistency of a product. It is important as it directly affects the sensorial experience lived by consumers. The ability of a product to uniformly cover the application area is also essential to ensure uniform distribution of the active ingredient, influencing its therapeutic efficacy. As a consequence, good spreadability is useful in guaranteeing the uniformity of each applied dose. Texture Analyser evaluates and collects information about the mechanical behavior of a material when an external force is applied. It is based on the conversion of quantitative parameters into qualitative parameters, so that it is possible to make a real approximation about the sensorial description of the analysed sample (Tai, et al., 2014). In particular, for semisolid preparations for skin application, the texture analysis provides information on mechanical properties, such as ease of removal from the container, spreadability, prolonged contact time on the skin (bioadhesion) and viscosity (Lukic et al., 2012; Jones et al., 1997).

In this case, since UA-PM-GEL has to be applied on the skin, the objective of the test was to generate a real characterization of its mechanical properties (Dejeu et al., 2022). In fact, through the parameters (Fig. 4) of firmness (g), work of shear (or consistency, g s), stickness (g) and negative area (or adhesiveness, g sec) it was possible to estimate the spreadability, the tendency to be removed from the application site and the contact time of the gel on the skin. The term firmness or hardness indicates the maximum value of the force exerted by the male cone on the gel. Indeed, during the penetration, it increases proportionally up to the point of maximum penetration. A high value of firmness is related to a high value of work of shear, or the total work required by the instrument to complete the penetration; this parameter describes the consistency of the gel. Low values of firmness and work of shear correspond to a better tendency to spreadability, in practice they translate into materials that are easier to spread or more easily extractable from the container tube (Tafuro et al., 2019). The force required by the male cone to detach from the sample is recorded as stickness or cohesiveness and it is correlated to the negative area, that refers to the total work needed to separate the sample from the adhesion surface. In other words, it describes the level of cohesion or adherence in practice. Low stickiness values are related to low negative area values and they result in easier removal of the sample from the application site, as well as a shorter contact time (Wróblewska et al., 2019). In addition, the parameters of viscosity, temperature, composition and applied force can influence spreadability (Dejeu et al., 2022). Fig. 4 shows the experimental traces

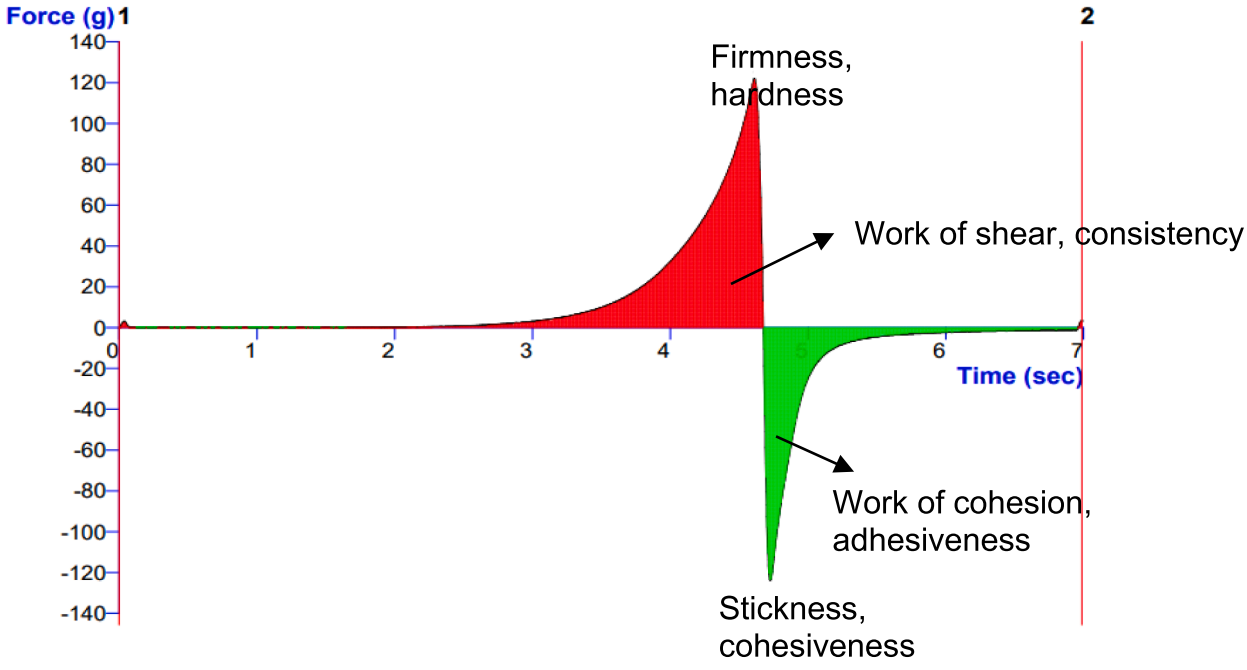


**Fig. 4.** Typical force versus time profile and textural parameters determined by the Texture analysis for UA-PM-GEL.

and the textural parameters which are calculated. Based on the data obtained (Table 2), the gel was suitable for skin application showing good consistency and adhesiveness. The data referred to UA-PM-GEL are comparable to those reported in literature (Dejeu et al., 2022; Wróblewska et al., 2019) and they were similar to those obtained with UA-GEL (Table 2).

#### 3.3.3. pH

The pH of UA-PM-GEL was 5.62 ± 0.26 resulting suitable for skin application. In fact the value not only doesn't deviate from the physiological skin values but also from those of the damaged skin that vary from 5 to 8 (Lukić et al., 2021; Risaliti el al., 2018). An acidic pH contributes to maintain the health of the skin by promoting the process of differentiation of keratinocytes and the formation of epidermal lipids, as well as to maintain a correct composition of the skin microbiome, essential for preventing disorders and pathologies caused by external agents such as bacteria and viruses (Lukić et al., 2021).

The values recorded for the UA-PM-GEL formulation are perfectly in line with those evaluated by Fitriani (Fitriani et al., 2019) for three further formulations of hydrogels containing UA in free form or as a solid dispersion which showed an average value of 5.61. The acidic character assumed by the formulations is due to the contribution of the active ingredient, in fact UA is a weak acid having a pKa of 4.4, in addition to the composition of Sepigel.

### 3.4. Stability study

#### 3.4.1. Rheology

Concerning the physical stability, lost in consistency, phase separation, colour changes and growth of mold were not evidenced during the period of the storage. Furthermore, the rheological characteristics of the formulation stored at 4 ± 2°C were analyzed weekly for four weeks. The viscosity undergoes small fluctuations over time at the same applied shear rate, despite that the system resulted stable (Fig. 5). In fact, the parameter relating to the up-viscosity ramp relative to the speed of 0.5 rpm is equal to 408500, 390500, 386000, 405110 mPa sec respectively at the first, second, third and fourth week of observation. The value recorded at time $t_0$ is 416000 mPa sec, proof of the tendency of the system to maintain the same starting viscosity profile. Furthermore, the general trend of the viscosity curves confirms the starting rheological profile of UA-PM-GEL: non-Newtonian, pseudoplastic, time-independent, non-thixotropic fluid.

#### 3.4.2. Spreadability

The spreadability profile parameters of UA-PM-GEL also were monitored over a period of four weeks (Fig. 6).

Based on the data shown in Fig. 6, a more significant reduction in the texture parameters analysed can be observed in the first week, in particular for firmness value; other parameters remain fairly constant. In the following 3 weeks, small fluctuations can be highlighted that overlap in time for all the spreadability indicators. Based on this observation, it emerges that the system undergoes a small loss of consistency after the first week of storage, while still maintaining the characteristics necessary for topical application. The high stability of the formulation is also due to the Sepigel that has an excellent physical stability profile over time. This gelling agent has also obtained positive feedback in clinical practice, as described by Risaliti (Risaliti et al., 2018). This trend can further be correlated to the viscosity profile for UA-PM-GEL which records lower values, albeit slightly, at time $t_4$ compared to time $t_0$.

**Table 2**
Parameters related to the spreadability of UA-PM-GEL and UA-GEL obtained at time t0. Data are shown as Mean ± SD (n = 3).

| Sample | *Firmness (g)* | *Consistency (g·sec)* | *Stickness (g)* | *Adhesiveness (g·sec)* |
|---|---|---|---|---|
| UA-PM-GEL | 179.87 ± 1.61 | 105.78 ± 2.65 | − 169.83 ± 1.78 | − 52.44 ± 0.88 |
| UA-GEL | 159.70 ± 1.23 | 94.59 ± 1.80 | − 159.04 ± 0.85 | − 42.44 ± 2.61 |

#### 3.4.3. Syneresis

The syneresis test was also conducted during 4 weeks to complete the definition of the physical stability of the gel. Syneresis is the process of expulsion of variable quantities of liquid previously incorporated into the three-dimensional polymer network of a gel, with its consequent reduction in volume. Several studies argue that this phenomenon occurs following the development of an osmotic pressure gradient, or a disequilibrium between the bonding forces or interactions existing at the interface between the solid dispersed phase and the liquid dispersing phase, or the level of rigidity of the gel, as well as the contraction or shrinkage of the polymer chains or particles that form the three-dimensional network of the solid dispersed phase. The space available to retain the liquid is reduced with its consequent expulsion (Guo et al., 2022). Since it exists a direct association between the reduction in weight of the sample and the occurrence of this process, based on this correlation it was possible to estimate the tendency of the gel to syneresis (Mizrahi, 2010). On a daily basis for three days and then on a weekly basis for five weeks, the weight of the sample over time was monitored.

In light of the data reported in Table 3, the gel was stable to syneresis. The weight remained constant over time, with minimal variations. Stability was maintained at both storage temperatures, although a greater, albeit slight, weight loss was recorded in the case of the formulation maintained at 25 °C. The data obtained are similar to those obtained for the hydrogel formulations studied by Risaliti (Risaliti et al., 2018).

#### 3.4.4. pH

The pH of the gel remains stable over the four weeks and always suitable for skin application (Table 4). The tendency of UA-PM-GEL to maintain the acidic character is probably due to the contribution of the encapsulated UA, that is a weak acid that has pKa 4.4. This influence was also detected in the study by Fitriani. The authors prepared hydrogels containing UA or UA solid dispersion using different gelling agents, such as sodium alginate, HPMC and Aqupec HV-505. The UA hydrogels had pH ranging from 5.50 to 5.63. and after 4 weeks at room temperature it resulted between 5.53 and 5.67 (Fitriani et al., 2019).

#### 3.4.5. Chemical stability

The content UA in the gel was defined calculating R%. The aim was evaluating the chemical stability of the formulation, considering a possible decrease in compound encapsulated inside the polymeric micelles and in the gel, and therefore its possible degradation. The gel demonstrated good stability over the 30 days, with a minimal percentage of non-encapsulated or degraded UA. In fact, at $t_0$ the R% was equal to 90.50 %, corresponding to 0.905 mg of UA per mL of formulation. After one month the value was 83.93 % corresponding to 0.839 mg of UA per mL of preparation. In light of this result, the percentage of active ingredient lost compared to $t_0$ is equal to 7.26 %, meaning that no significant quantitative alteration occurred.

### 3.5. In vitro release study: Dialysis bag method

The *in vitro* release profile reveals important information on behavior of the formulation, both PM and gel, and the influence of the carrier on the mechanism of drug release. The release study of UA from UA-PM-GEL was investigated according to the dialysis bag method (Hua, 2014) and compared with UA release from a acetonitrile solution and from UA-PM (Vasarri et al., 2022). Usually, the dialysis release method is a useful test to evaluate *in vitro* release from micro- and nano-particulate delivery systems. Recently, it was also proposed for topical

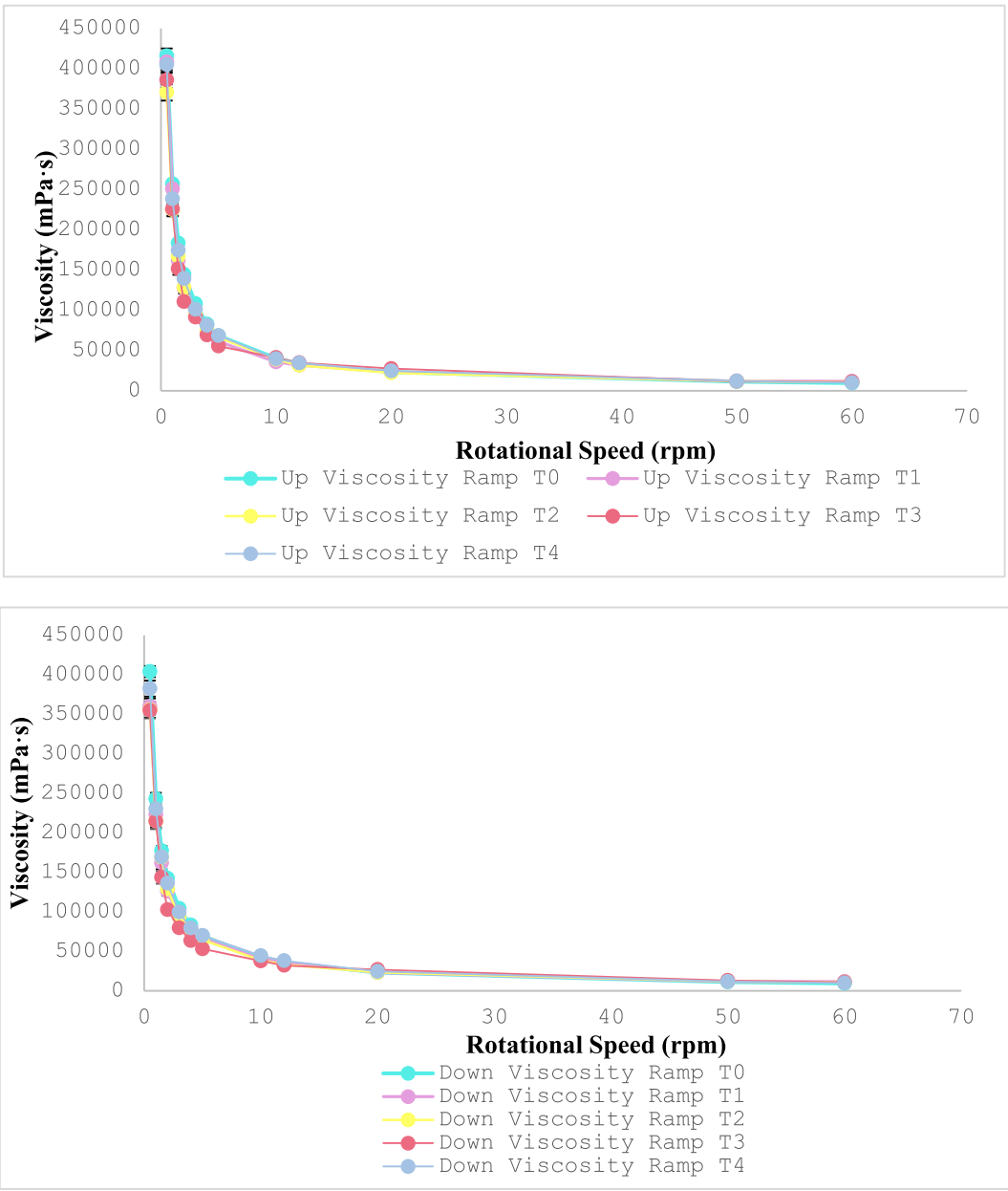


**Fig. 5.** Viscosity stability of UA-PM-GEL during 30 days of storage at 4 ± 2 ∘C. Data are shown as Mean ± SD (n = 3).

formulations (Hua, 2014).

Fig. 7 shows the UA release profile from the UA acetonitrile solution, UA-PM and UA-PM-GEL over 24 h. A slow and gradual release is highlighted from the gel, with any burst effect, instead observed for the release from the solution. The release of UA from the solution reached 100 % in 24 h, of which 60 % already released in the first two hours. The release of UA from the non-gelled PM was slower, with a maximum of 69.19 % in the same time interval (Vasarri et al., 2022). However, the release from the gel was more gradual, starting from 3.5 % in the first hour up to 36.66 % at 24 h. The results confirmed that UA-PM-GEL does not prevent the release of the active ingredient even if it was more gradual than the liquid colloidal dispersion, due to the semi-solid property of the system.

### 3.6. *In vitro release study: Vertical diffusion Franz cells*

The *in vitro* test with vertical diffusion Franz cells was used to predict the passive diffusion of a compound from a topical preparation such as gels or creams to the skin. Skin simulation is usually achieved through the use of human skin, synthetic skin or artificial membranes. In this test a cellulose nitrate filter was used as membrane and the behaviour of UA-PM-GEL and the UA-GEL was compared (Fig. 8).

A superimposable trend of the UA release from both the formulations can be noted until 6 h, with a release percentage of 9.20 % for UA-PM-GEL and 8.83 % from UA-GEL. The release from the UA-PM-GEL remains linear and constant up to the value of 30.87 % at 24 h. The release was superimposable to that obtained with the dialysis bag method. The release of UA from the UA-GEL was slower and more gradual and reached the 13.68 % at the end of the assay. The results evidenced that the release was low for both gels, due to a slow diffusion of UA through the viscous matrix of the gel. However, the polymeric micelles improved the solubility of UA in water and the amount of drug release during the time.

Considering the quantity (μg/cm$^2$) of UA permeated through the membrane and recovered in the acceptor compartment, the permeation extends from a minimum of approximately 2.57 μg/cm$^2$ at the first hour

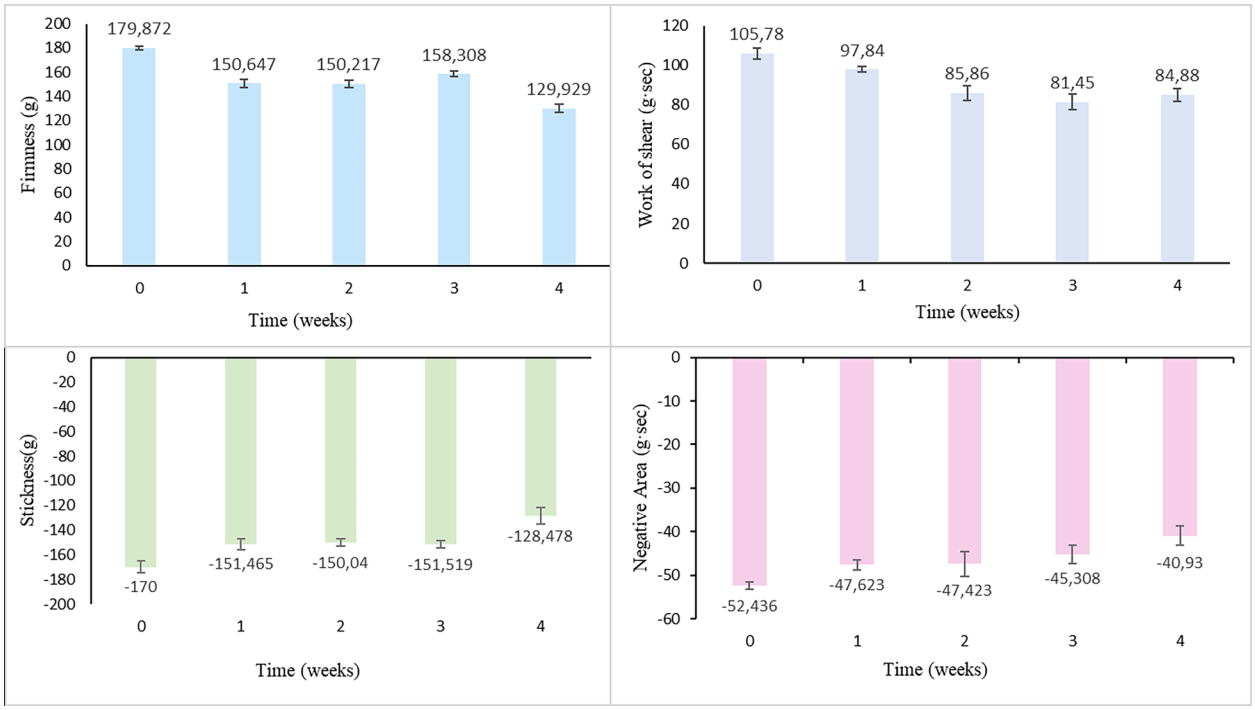


**Fig. 6.** Firmness (g), work of shear (g·sec), stickness (g) and negative area values (g·sec) values for UA-PM-GEL recorded over four weeks. Each data point corresponds to the mean ± SD of n = 3 measurements of two formulation samples.

**Table 3**
Syneresis test results (g) at 4 ± 2°C and 25 ± 2°C for 4 weeks.

| T | t0 | 24 h | 72 h | 1 week | 2 week | 3 week | 4 week |
|---|---|---|---|---|---|---|---|
| **4 ± 2°C** | 5.44 | 5.44 | 5.44 | 5.42 | 5.40 | 5.37 | 5.36 |
| **25 ± 2°C** | 5.65 | 5.64 | 5.65 | 5.49 | 5.38 | 5.25 | 5.10 |

**Table 4**
Ph stability of au-mp-gel stored at 4 ± 2°C for 4 weeks. Data are reported as mean ± SD of n = 3 experiments.

| week | UA-MP-GEL |
|---|---|
| 0 | 5.63 ± 0.26 |
| 1 | 6.06 ± 0.06 |
| 2 | 6.16 ± 0.17 |
| 3 | 6.06 ± 0.11 |
| 4 | 6.18 ± 0.04 |

up to a maximum of 21.61 μg/cm$^2$ at 24 h for UA-GEL. Differently, the UA-PM-GEL formulation released approximately 9.20 μg/cm$^2$ in the first 2 h up to 80.45 μg/cm$^2$ at 24 h. The dose of UA released after 24 h from the UA-GEL (21.61 μg/cm$^2$) is almost equivalent to that released in 6 h from UA-PM-GEL where the micelles are incorporated in the gel (24.26 μg/cm$^2$). The micellar system takes four times less time to achieve the same result obtained with UA-GEL in 24 h.

### 3.7. Skin-PAMPA

The *in vitro* permeation test PAMPA has gained particular interest in the pharmaceutical field. It is a rapid, economical and reproducible method, which allows to predict the passive transcellular absorption not only of formulations containing single molecules, but also of complex matrices such as plant extracts (Casamonti et al., 2019). It is widely used for the permeability screening of new molecules and formulations before further *in vivo* tests; a notable advantage lies in the possibility of varying the composition of the medium used for the functionalization of the membranes in order to mimic the different biological barriers.

UA is an extremely hydrophobic molecule, with a solubility in water of 3 μg/mL (Lukáč et al., 2012). The medium in which UA was solubilized was a solution consisting of DMSO 5 % v/v in PBS. An UA saturated solution was obtained with a final concentration of 0.20 mg/mL. The permeation of UA from its saturated solution was evaluated and compared to that of the UA-PM. The solution for Skin-PAMPA consists of a mixture of silicone oil and isopropyl myristate in hexane (Markovic et al., 2012; Casamonti et al., 2019).

From the analysis of the results referred to Pe value, PM offer an important contribution in increasing the permeation of UA (Table 5). At 2 h, the Pe relative to the saturated solution was equal to 2.27·10$^{-6}$ cm/s versus 1.42·10$^{-5}$ cm/sec for UA-PM. The trend remained constant over time, since at 4 h the Pe recorded for UA-solution is 2.47 10$^{-6}$ cm/s and 1.45 10$^{-5}$ cm/s for UA-PM. In both cases, a very slight increase in permeation was recorded between 2 and 4 h. The values referred to the Pe of UA from the solution were in the same order of magnitude as those obtained by Galanty (Galanty et al., 2021). Although UA is a fairly permeable molecule, the carrier allows to increase Pe by an order of magnitude. The reliability of the results obtained was confirmed by the R% which is greater than 80 % in both cases.

The percentage of UA from UA-solution permeated across the artificial membrane (Table 6) was 1.22 % and 1.53 % respectively at 2 and 4 h. Otherwise the percentage of permeation for UA-PM was 8.51 % and 16.10 % at the same timepoints. As a result, the carrier has an active role and represents a valid strategy to increase not only the solubility, but also the permeability of UA.

UA-PM-GEL was also evaluated in PAMPA test. The test time was prolonged up to 24 h since no permeation occurred at 4 h. The obtained results were 4.09 ·10$^{-7}$ cm/s for Pe and 6.71 ± 0.35 % for the percentage of UA permeated. This evidence confirms the effectiveness of the formulation in ensuring the release of the UA, as well as ensuring prolonged contact with the absorption site. The Pe was lower than the values of UA-PM because of the influence of the longer time on the calculation of the Pe factor. This is due to the different viscosity of the

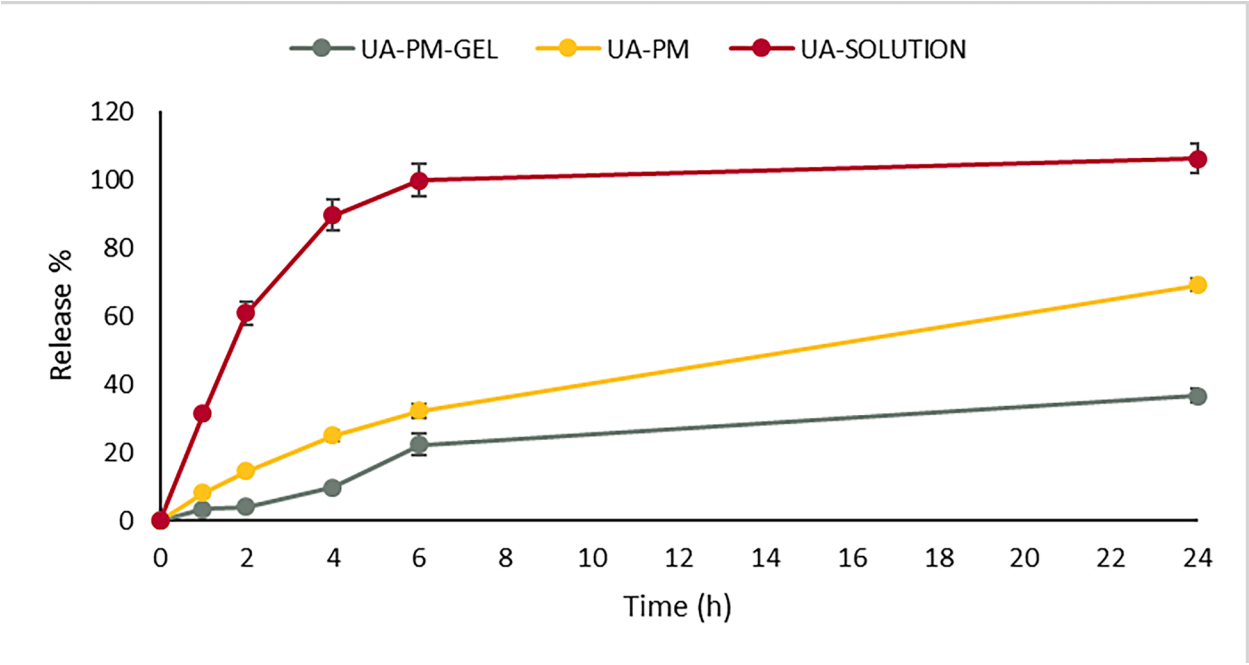


**Fig. 7.** UA release profile from a UA acetonitrile solution, UA-PM and UA-PM-GEL over 24 h. Data are reported as mean ± SD of n = 3 experiments.

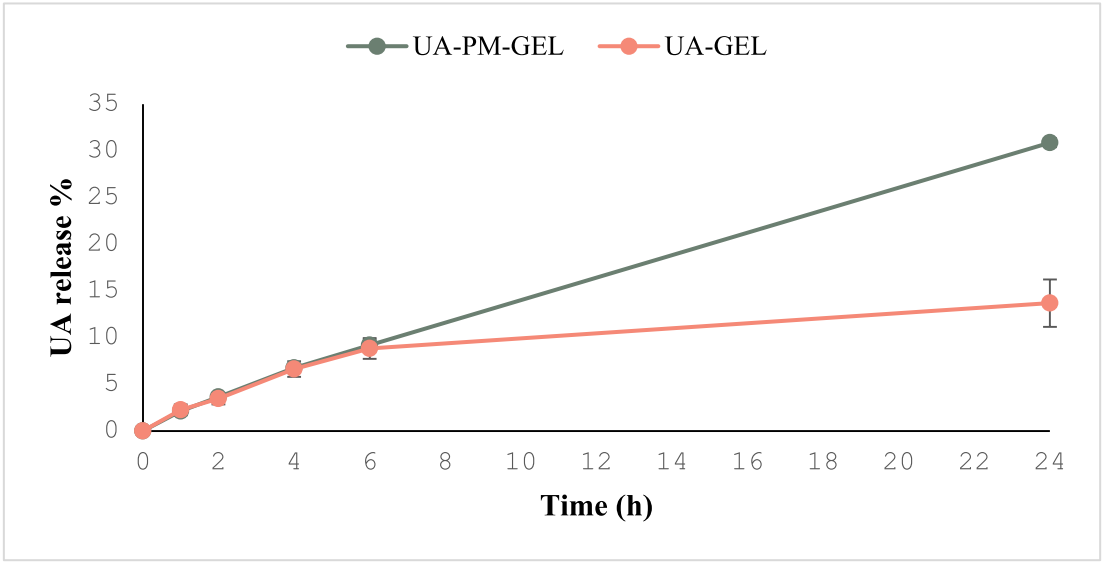


**Fig. 8.** Release profile of UA from UA-GEL and UA-PM-GEL by vertical diffusion Franz cell. Data are expressed as mean ± SD of n = 3 experiments.

**Table 5**
Pe values (cm/s) referred to a saturated solution of UA in DMSO 5 % v/v in PBS and of UA-PM. Data are expressed as mean ± SD of n = 3 experiments.

| Time | Pe (cm/s) | | | |
|---|---|---|---|---|
| | UA-sol | SD | UA-PM | SD |
| 2 h | 2.27E-06 | 1.55E-06 | 1.42E-05 | 1.15E-05 |
| 4 h | 2.47E-06 | 5.05E-07 | 1.45E-05 | 1.24E-06 |

**Table 6**
Percentage of AU permeated by a saturated solution of UA in DMSO 5 % v/v in PBS and by UA-PM in the PAMPA assay. Data are expressed as mean ± SD of n = 3 experiments.

| Time | UA-solution | UA-PM |
|---|---|---|
| 2 h | 1.22 ± 0.51 | 8.51 ± 1.76 |
| 4 h | 1.53 ± 0.41 | 16.09 ± 0.52 |

formulation that delayed the release of the drug, compared to the micellar colloidal dispersion and the solution. However, the semi-solid formulation allowed skin application, prolonging the contact time with the site of absorption and action of the UA.

The evaluation of the absorbed dose of active ingredient after 4 h (A4) or 24 h (A24) of incubation (Table 7) further confirmed the ability of the micellar dispersion and the gel to enhance the permeation of UA. The values were significantly higher than those of saturated solution: 147.31 ± 2.82 μg/cm$^2$ for UA-PM and 29.11 ± 1.54 μg/cm$^2$ for UA-PM-GEL, compared to 1.93 ± 0.51 μg/cm$^2$ of the solution.

**Table 7**
Absorbed dose (A4 after 4 h; A24 after 24 h) of incubation, for UA-solution, UA-MP and UA-MP-GEL. Mean ± SD (n = 4).

| Sample | A μg/cm$^2$ |
|---|---|
| UA-SOL | 1.93 ± 0.51 ($A_4$) |
| UA-PM | 147.31 ± 2.82 ($A_4$) |
| UA-PM-GEL | 29.11 ± 1.54 ($A_{24}$) |

### 3.8. Microbiological analysis

The literature reports the antibacterial activity of UA (Francolini et al., 2013; Kumar et al., 2019). Therefore, *in vitro* tests were conducted to evaluate whether micellar and gel formulations also retained antibacterial activity against the bacterial strain *Staphylococcus epidermidis*. The antimicrobial effectiveness of UA is mainly linked to its ability to bind to bacterial DNA, interfering with replication and transcription processes (Maciag-Dorszyńska et al., 2014). Moreover, damage to the cell membrane has been highlighted, primarily caused by the inhibition of ATP synthesis, leading to the leakage of intracellular components (Gupta et al., 2012).

Due to reduced solubility, nanocarriers or hydrophilic polymers have been the focus of studies aimed at evaluating their potential positive influence in increasing the bioavailability of UA and thus its antibacterial activity against *S. epidermidis* (Francolini et al., 2013). The authors complexed UA with the hydrophilic polymer pAcDED, finding that the most advantageous ratio with UA was 70/30. These studies showed a MIC of 5 ± 1 μg/mL and an inhibition zone of 15 ± 2 mm. The role of the polymer is to facilitate the interaction between the bacterium and UA, which can interact with its metabolic functions. In the study by Martinelli et al. (2014), the antibacterial effectiveness against *S. epidermidis* was evaluated using carboxylated poly(L-lactide) (CPLLA16-UA) as a carrier to increase the solubility of UA and free UA. The tests showed a reduction in bacterial load of 2.5 logarithmic orders, while in the second case, it was 1.5 logarithmic orders; these values indicated better activity of CPLLA16-UA in inhibiting bacterial growth.

Francolini and colleagues (2019) set up a UA-liposomal formulation, assessing its activity against *S. epidermidis* and comparing it to that of free UA. The results demonstrated that the formulation had better activity. Pandit and collaborators (2021) developed graphene flakes loaded with UA, which proved capable of releasing the active ingredient in a prolonged manner over time, reducing the growth of *S. epidermidis*.

Finally, in the study by Pagano and collaborators (2019), UA was incorporated into three hydrogels based on NaCMC. The agar diffusion method confirmed the antibacterial activity of UA on *S. epidermidis*.

In this study, the antibacterial activity against *Staphylococcus epidermidis* ATCC 12228 was evaluated *in vitro* for both free UA and UA formulated in PM and gel at 1 % w/v of Sepigel. The UA-PM-GEL formulation at 2 % of gelling agent could not be tested due to excessive viscosity. The UA-PM and UA-PM-GEL formulations were loaded with UA at a concentration of 1 mg/mL.

Antibacterial activity was assessed using the microplate dilution method; in fact, serial concentrations of UA and UA formulations were tested against the bacterial strain. Each formulation underwent five increasing dilutions, starting from a 10 % dilution up to a 50 % dilution.

From the analysis of the results reported in Table 8, it is possible to confirm the antibacterial activity of UA described in the literature (Francolini et al., 2013; Kumar et al., 2019). UA at a concentration of 1 mg/mL was active at all tested dilutions, with a reduction of 6 Log. A reduction of the bacterial load by 5 Log was observed only at the highest concentration of 0.9 mg/mL of the active ingredient. This reduction demonstrates their antibacterial activity against *S. epidermidis*. The same formulations UA-PM and UA-PM-GEL loaded with 1 mg/mL of UA showed a reduction in bacterial growth of 4 Log at a concentration of 0.8 mg/mL of UA. Empty PM, empty gel, and empty gel loaded with empty PM showed no antibacterial activity against the tested strain.

**Table 8**
Antibacterial activity of UA, UA-PM, UA-MP-GEL 1 %, on *Staphylococcus epidermidis* ATCC 12228. The bacterial count value expressed in CFU/mL found following the application of UA or formulated UA. The concentration of *S. epidermidis* was 2.7x10$^7$ CFU/mL. The concentration tested was 5.4x10$^5$ CFU/20 μL.

| *Sample Dilution* | **AU 90%** | **AU 80%** | **AU 70%** | **AU 60%** | **AU 50%** |
|---|---|---|---|---|---|
| CFU/mL | 0 | 0 | 0 | 0 | 9 |
| *Sample Dilution* | **AU-MP 90%** | **AU-MP 80%** | **AU-MP 70%** | **AU-MP 60%** | **AU-MP 50%** |
| CFU/mL | 4 | 86 | >300 | >300 | >300 |
| *Sample Dilution* | **AU-MP-GEL 90%** | **AU-MP-GEL 80%** | **AU-MP-GEL 70%** | **AU-MP-GEL 60%** | **AU-MP-GEL 50%** |
| CFU/mL | 1 | 91 | >300 | >300 | >300 |

The results demonstrated that the formulations retained the antibacterial properties against *S. epidermidis* as UA. The results provide a solid basis for future investigations and the development of targeted pharmaceutical formulations, highlighting the potential of UA as an alternative or complement to existing therapies.

## 4. Conclusions

The UA delivery in polymeric micelles increased more than 300 times the solubility. The nanocomposite gel obtained with 2 % of Sepigel as gelling agent is a good formulation for the topical application and the UA release. The gel is a pseudoplastic and time-independent non-Newtonian fluid and its rheological properties outlined a good spreadability, adhesion and viscosity profile, suggesting easy application and removal from the application site. The pH value also proved to be suitable for skin application. The nanocomposite gel produced a UA gradual release with a percentage of 37 % and 31 % after 24 h, as resulted by dialysis bag method and Franz diffusion cell, respectively.

The formulation was chemically and physically stable for 30 days, with a percentage of loss of the active ingredient lower than 10 %. The texture parameters remained fairly constant, with a more significant reduction in the first week in particular for firmness value. Furthermore, the stability is highlighted by the minimal loss of water recorded in the syneresis test.

UA passive permeability was increased of an order of magnitude when the molecule is formulated in polymeric micelles, as proved by Skin-PAMPA. The absorbed dose after 4 h or 24 h of incubation further confirmed the ability of the micellar dispersion and the nanocomposite gel to enhance the permeation of UA with values significantly higher than those of saturated solution: 147.31 ± 2.82 μg/cm2 for UA-PM and 29.11 ± 1.54 μg/cm2 for UA-PM-GEL. Finally, both the micelles and the gel revealed an effective antibacterial activity; both formulations retained the antibacterial properties against *S. epidermidis* as UA suggesting promising approach in the field of treatment of skin infections.

## CRediT authorship contribution statement

**Rebecca Castellacci:** Investigation, Formal analysis, Data curation. **Cristiana Sacco:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Rosa Donato:** Investigation, Formal analysis, Data curation, Conceptualization. **Maria Cristina Salvatici:** Writing – original draft, Formal analysis, Data curation. **Anna Rita Bilia:** Writing – review & editing. **Maria Camilla Bergonzi:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2025.125232.

## Data availability

Data will be made available on request.

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