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Development and characterization of multifunctional phytonanoemulgels based on licorice extract and biocompatible polymers

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ABSTRACT

Natural extract-derived cosmetics are primarily preferred by green consumers for their safety and environmental sustainability. However, many natural bioactive compounds are hydrophilic and unstable, thus having limitations in overcoming the high lipophilic barrier of stratum corneum and maintaining their beneficial properties over time. For this reason, nanostructured emulgel based on phytochemical ingredients called "phytonanoemulgel" could represent an option for a natural, safe, and effective skincare product. In this work, an extract of licorice was prepared and analyzed with a particular regard for antioxidant activity. The extract was incorporated in a phytonanoemulsion using lecithin as a vegetable, safe, and sustainable emulsifier. The optimized licorice phytonanoemulsion was physico-chemically characterized. Then, different natural polymers having additive cosmetic properties, such as sodium hyaluronate, Acacia and xanthan gums, pectin, or sodium alginate, were added to the phytonanoemulsion to form a gel. Different phytonanoemulgels were obtained and evaluated for their macroscopic appearance, droplet size, zeta potential, pH, total phenolic content and antioxidant activity. Additionally, rheological characterization was conducted. Cell viability studies verified the safety of phytonanoemulsion, and the stability of the different phytonanoemulgels was assessed over time. The licorice phytonanoemulgel based on gums demonstrated optimal physicochemical parameters, suitable rheological characteristics, and good stability over time, thus representing a promising option as a multifunctional cosmetic phytoformulation.

1. Introduction

The cosmetic market has been sensitive to sustainability for several years, with exponential growth in the organic product segments. Direct access to online information sources has led consumers to seek increasingly detailed information about cosmetic production and use chain sustainability [1]. For this reason, new cosmetic formulations should be designed with a particular focus on the naturalness and sustainability of the product, starting from the choice of raw materials and the entire composition of the formulation [2,3]. Moreover, cosmetic formulations based on a few well-known ingredients are preferred by cosmetic consumers as an indicator of sustainability and transparency. In this context, claims such as green and multifunctional cosmetics are gaining attention from environmentally conscious consumers. Cosmetic multifunctionality can be achieved by selecting cosmetic ingredients that have multiple cosmetic functions and technological properties to

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obtain formulations pleasant to apply to the skin, and stable over time [4–6]. Moreover, achieving multifunctionality by reducing the number of ingredients offers economic and environmental advantages due to shorter production and supply chains.

One of the most investigated natural extracts in the cosmetic field is licorice, the natural extract from the roots of *Glycyrrhiza glabra* L. (GG). This extract contains a variety of substances having different cosmetic actions. In particular, licorice extract contains flavonoids such as glabridin, liquiritin, liquiritigenin, isoliquiritigenin, licochalcones, and saponins such as glycyrrhizin and its aglycone, the glycyrrhetic acid. Due to these bioactive compounds, GG has demonstrated antioxidant, anti-melanogenesis, anti-inflammatory properties, and skin repair effects [7,51]. More in detail, glycyrrhizin, one of the main licorice compounds, has antioxidant and anti-inflammatory effects that can protect the skin, and act as an effective pigment-lightening agent [8]. Moreover, due to its surface-active properties, glycyrrhizin has demonstrated technological properties as a natural emulsifier for stabilizing multiphase systems such as emulsions or foams. This amphiphilic molecule is able to adsorb at oil–water or air-water interfaces, thereby reducing interfacial tension and forming stable interfacial films. These attributes make glycyrrhizin an attractive candidate for developing of clean-label or natural-origin emulsifiers [9,10]. Owing to its multifunctional bioactive compounds, GG extract is currently used in cosmetic products for skin whitening, anti-sensitizing, anti-aging, and anti-inflammatory purposes [11,52].

Formulating green cosmetics presents a challenge in utilizing the multifunctional properties of natural extracts, particularly their hydrophilicity and instability. This can be improved by using lipophilic compositions like nanoemulsions with functional natural ingredients. Additionally, including of an appropriate biopolymer can provide extra cosmetic properties while preserving the stability and effectiveness of the extract [5,12].

Nanoemulsions are heterogeneous colloidal oil and water mixtures, with one component as a dispersed phase and the other as a continuous phase [13]. These mixtures are designed to have hydrophilic and lipophilic regions, facilitating the encapsulation and delivery of diverse active ingredients [14]. Almond oil is often included in cosmetic formulations due to its emollient properties, which help enhance skin softness and hydration. It is rich in essential fatty acids, vitamins E and A, which support the skin's barrier function and provide antioxidant protection. Furthermore, almond oil acts as an effective carrier oil, aiding in the absorption of active ingredients in topical formulations [15,16]. To stabilize the system, a surfactant known as an emulsifier is adsorbed at the interface between the dispersed and continuous phases, lowering the surface tension and thus stabilizing the system. Due to the small droplet size, nanoemulsions are characterized by high kinetic stability [17,18]. However, nanoemulsions have some disadvantages, such as low viscosity, poor retention on the skin, and low spreadability [19]. These properties can be improved by transforming the nanoemulsion into a nanoemulgel by adding a suitable gelling agent. The nanoemulgel is a colloidal system with gel characteristics and improved nanoemulsion properties for dermal application [20]. The emulsion part protects the bioactive compounds from enzymatic degradation and hydrolysis, improving the permeation like other nanocarriers. The jellifying agent stabilizes the emulsion, increases the viscosity acting as a thickener, improves the spreadability, facilitates skin application, and increases product compliance [21].

Natural gelling agents are bio-polysaccharides or their derivatives that provide excellent biocompatibility and biodegradability. Moreover, these biopolymers have additional advantages such as non-toxicity, inexpensiveness, and commercial availability. However, the poor mechanical resistance represents a limitation for natural gelling agents, which require careful attention in the handling processes and vigilant monitoring of stability over time. Among the naturally derived thickeners, hyaluronic acid, pectin, alginate, and vegetable gums, such as Acacia and xanthan gums, could be considered stabilizer agents for a green nanoemulsion [22]. In particular, Acacia gum and xanthan gum are biopolymeric hydrocolloids that exhibit excellent stabilizing properties, making them suitable excipients for the formulation of green cosmetic nanoemulsions. Acacia gum, a complex mixture of arabinogalactan oligosaccharides, glycoproteins, and polysaccharides, acts as an effective emulsifying agent due to its amphiphilic nature, facilitating the formation of kinetically stable oil-in-water systems. Xanthan gum, a high molecular weight exopolysaccharide produced by *Xanthomonas campestris*, contributes to the rheological modification of the continuous phase, enhancing viscosity and inhibiting creaming and coalescence phenomena. Their synergistic effect can lead to improved droplet size distribution control and long-term physical stability, crucial for nanoscale emulsions. Furthermore, their natural origin aligns with the principles of green chemistry and sustainable cosmetic formulation [23,24].

Licorice extract is rich in bioactive compounds, making it a valuable source for various cosmetic benefits. However, the full potential of these compounds can be limited due to their hydrophilicity and instability. These limitations can be effectively addressed by incorporating licorice extract into a nanoemulsion,. Additionally, the use of biopolymers may enhance skin absorption. Adopting an integrated approach to select and combine natural ingredients in a jellified nanoemulsion is beneficial for creating minimalist, multifunctional green cosmetics.

The main objective of this study was to develop and evaluate multifunctional phytonanoemulgels based on licorice extract and natural biopolymers, focusing on their antioxidant activity, rheological properties, and stability. To achieve this goal, the following steps were undertaken, starting from the licorice extraction to the characterization of the final formulations: a) Preparation and characterization of a licorice-based nanoemulsion using natural emulsifiers. b) Transformation of the nanoemulsion into phytonanoemulgels with various biopolymers. c) Evaluation of the antioxidant activity, rheological behavior, and long-term stability.

2. Materials and methods

2.1. Materials

Licorice roots were gently provided by Naturemed s.r.l. (Calabria, Italy). Soy lecithin was purchased from Carlo Erba (Milan, Italy). Sodium alginate and Sodium hyaluronate (MW 800–1200 kDa) were sourced from Farmalabor (Canosa di Puglia, Italy). Solagum® AX was kindly provided by Seppic SA, LaGarenne-Colombes (France). Folin-Ciocalteu reagent was sourced from Titolchimica (Pontecchio Polesine, Italy). Fetal bovine serum (FBS), L-glutamine, penicillin (1000 U/mL), and streptomycin were purchased from Euroclone S.p.A. (Milan, Italy). All other chemicals were purchased from Sigma-Aldrich (Milan, Italy). Phosphate buffer solution at pH 7.4 was prepared with the following composition: 2.98 g/L Na_2HPO_4 x12H_2O, 0.19 g/L KH_2PO_4, 8 g/L NaCl. Phosphate buffer at pH 5.5 was composed of 1.5 g/L $Na_2HPO_4 \times 12H_2O$, 13.61 g/L KH_2PO_4, and 2.66 g/L NaCl. Ultrapure water (18.2 MΩ cm) was obtained with a MilliQ apparatus by Millipore (Milford, MA, USA).

2.2. Extraction of licorice roots by bio-solvent

Licorice roots (50 g) were weighed into a flask and added to a final volume of 500 mL of a mixture of ethanol/water 50:50 *v/v*. The mixture was sonicated for 1 h and kept under stirring for three days in the dark. Then, the mixture was filtered with a funnel and paper filter into a volumetric flask and dried in a rotavapor. The procedure was performed three times on the same plant material and some steps are shown in Fig. 1 .

2.3. Analysis of extracts

2.3.1. Total phenolic content (TPC), total flavonoid content (TFC)

The TPC was determined by using the Folin–Ciocalteu reagent

Fig. 1. Steps of extraction of licorice root.

according to Singleton et al. [25]. Briefly, 0.2 mL of a water solution of extract (1 mg/mL) was added to 1.00 mL of 1:10 diluted Folin–Ciocalteu's phenol reagent, followed by the addition of 0.8 mL of sodium carbonate solution (7.5 % *w/v*). After 30 min in the dark at 40.0 ± 1.0 °C, the absorbance at 750 nm was measured spectrophotometrically (UV–Vis 1601 spectrophotometer, Shimadzu, Milan, Italy). Distilled water was used as a blank. TPC was estimated from a standard curve of gallic acid ($R^2 = 0.999$). All measurements were performed in triplicate and results were expressed as gallic acid equivalent (GAE) in μg/mg extract (μg GAE/mg extract).

The TFC was determined using the European Pharmacopoeia method with minor modifications [26]. Briefly, 1 mL of 2 % (*w/v*) $AlCl_3$ solution was added to 1 mL of the extract solutions (1 mg/mL). After incubation at room temperature for 30 min in the dark, the absorbance was measured at 430 nm. The TFC was estimated from a standard curve of quercetin ($R^2 = 0.999$) and was expressed as quercetin equivalent (QE) in μg/mg extract (μg QE/mg of extract). The pH value of extract solutions was determined by using a digital pHmeter (Crison Instruments, S. A. Barcelona). The analysis of the pH of each solution was done in triplicate, and average values were calculated.

2.3.2. Determination of antioxidant activity (AA%)

2.3.2.1. DPPH assay. The Antioxidant Activity (AA%) of the extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) reduction assay [27]. Briefly, different concentrations of each extract, as well as ascorbic acid (used as a standard antioxidant compound), were mixed with a solution of DPPH (0.1 mM in methanol) at room temperature. The mixtures were kept in the dark for 30 min, and the absorbance was measured at 517 nm. Methanol was used as a blank solution, and DPPH was the control. Results are expressed as a percentage of inhibition of the DPPH radical according to the following equation: $AA\% = [(A_0 - A) / A_0] \times 100$, where A_0 was the absorbance of the DPPH control, and A was the absorbance of the sample with DPPH. The determination was conducted in three technical replicates, and the average values were calculated.

2.3.2.2. Reduction of in vitro oxygen species (ROS). The intracellular production of ROS within cells was assessed with a fluorimetric technique using 2′,7′-dichlorodihydrofluorescein diacetate (H_2-DCFDA, Life Technologies, UK). HaCaT (human keratinocyte cell line, CLS, Germany) sub-confluent cells grown in 96-well plates were incubated for 30 min with 20 μM of H_2-DCFDA in the dark at 37 °C. Media was removed and fresh medium was added to the cells before exposure to different sample concentrations (0.05–1 mg/mL), ascorbic acid as positive control (AA, 1 mg/mL), and culture medium as negative control for 1.5 h. Hydrogen peroxide (H_2O_2) solution (500 μM, 1 h) or exposure to UVB light (emission wavelength 312 nm) for 15 min were used for induction of ROS in cells. After exposure, ROS levels were determined at excitation 485 nm and emission 520 nm wavelengths using a fluorescence microplate reader (FLUOstar BMGLabtech, Germany). Data from 9 biological replicates are reported as the percentage of ROS reduction determined as: 100-(fluorescence of exposed cells to samples/fluorescence of negative control consistent of a culture medium without samples from the same experiment) x100. The concentration of the samples that reduces 50 % of ROS (IC_{50}) was determined by non-linear regression analysis with GraphPad PRISM® 5 software (San Diego, California USA, www.graphpad.com).

2.3.3. UHPLC-MS analysis of licorice extract

The extracts were analyzed by UHPLC-MS to quantify main bioactive compound, glycyrrhizin. UHPLC-MS analyses were performed on a Waters ACQUITY ARC UHPLC/MS system consisting of a QDa mass spectrometer equipped with an electrospray ionization interface and a 2489 UV/Vis detector. The wavelengths detected (λ) were 254 nm and 365 nm. Analysis was performed on an XBridge BEH C18 column (10 × 2.1 mm i.d., particle size 2.5 μm) with an XBridge BEH C18 VanGuard Cartridge guard column (5 mm × 2.1 mm i.d., particle size 1.8 μm). The mobile phases were H_2O (0.1 % formic acid) (A) and acetonitrile (0.1 % formic acid) (B). Electrospray ionization in positive and negative modes was applied in the mass scan range of 50–1200 Da. The following gradient was used: 0–0.78 min, 20 % B; 0.78–2.87 min, 20–95 % B; 2.87–3.54 min, 95%B; 3.54–3.65 min, 95–20 % B; 3.65–5.73, 20 % B. Flow rate: 0.7 mL/min. Glycyrrhizin and glycyrrhetinic acid standards were injected at six concentrations in a range from 0.02 to 0.12 mg/mL to build calibration curves, and the sample was injected at a concentration of 1 mg/mL prepared in MeOH.

2.4. Preparation of phytosomes (PHY), phytonanoemulsion (PNE), and phytonanoemulgel (PNEG)

Preliminary studies were performed to select the type of vegetable oil and compound percentages to form a GG extract nanoemulsion, leading to the following preparation method. The first step was the preparation of phytosomes (PHY). GG extract (20 mg) was mixed with soy lecithin (LC, 400 mg) and distilled water (20 mL) and stirred at 300 rpm for 2 h. Then, the phytosome suspension was sonicated (Transonic T310, Elma, Germany) for 10 min to reduce the dimension of PHY. After sonication, almond oil (AO, 2 mL) was added, followed by ethanol (EtOH, 2 mL) and glycerin (Gly, 0.2 g). Then, the mixture was homogenized (Ultra-Turrax

T25 basic, IKA-WERKE) at 9500 rpm for 10 min to obtain the phytonanoemulsion (PNE). After the homogenization process, various hydrophilic polymers were added to the PNE as jellifying agents to obtain the phytonanoemulgels (PNEGs). These included hyaluronic acid (HA), Solagum® AX (SG), which is a mixture of Acacia and xanthan gum, and a combination of pectin and alginate (PA). Specifically, 0.4 g of HA or SG, or PA, corresponding to 2 % of each polymer or polymer mixture, was gradually added to 20 mL of the PNE while continuously stirring at 100 rpm. The PNEGs were then stirred for 24 h and left to stand for 24 h to allow the gel to hydrate before undergoing physico-chemical and rheological characterization. In Table 1 is shown the composition of the prepared formulations.

2.5. Characterization of PHY, PNE, and PNEGs

The droplet sizes of formulation were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS ZEN3600 (Malvern Instruments, UK). The sample was diluted to 1:2000 *v/v* with deionized water. The results were reported as the mean droplet size and polydispersity index (PDI) and expressed as the average of five consecutive readings.

The zeta potential measurements were carried out at 25 °C on a Malvern Zetasizer 3000 HS instrument (Malvern Panalytical Ltd., Malvern, UK), after the same dilution.

The pH values of the formulations were determined using a digital pHmeter (Crison Instruments, S.A. Barcelona, Spain) at room temperature and the measurements were performed in triplicate.

For determination of TPC, about 1 g of each type of PNEGs was weighed in a beaker and dissolved in 10 mL of methanol. The TPC and the AA % of PNEGs were determined by the Folin-Ciocalteu method and DPPH assay, as previously reported.

2.6. Rheological characterization of PNEGs

The rheological studies were conducted on PNEGs stored at room temperature for 24 h after manufacture.

The viscosity measurements and the viscoelastic properties were performed with controlled stress Malvern Kinexus Rheometer Lab+ (Malvern Instruments, UK) using a cone and plate geometry (truncated angle 4° and radius 40 nm). Dynamic viscosity was measured on a logarithmic increment, ranging from 0.1 to 100 s^{-1}. Oscillation frequency sweep tests were performed at frequencies ranging between 0.01 and 1 Hz. All measurements were performed at 25 °C.

The tackiness and adhesiveness of gels were measured using the same equipment with a plate and plate geometry (pull-away assay or tackiness testing). Six replicates were performed for each type of PNEGs.

A parallel-plate method was adopted to measure the spreadability [28]. Gel samples (300 μL) were spread between two Sil-Tec medical-grade solid silicone sheets of rubber 304 mm × 304 mm × 0.127 mm (Technical Products Inc., Lawrenceville, GA, USA). A circle of 20 cm in diameter and 200 g in weight was applied to them for 30 s. The change in diameter was measured. Measurements were performed at room temperature (25 ± 2 °C).

Spreadability in mm^2 was calculated by the formula: $S = d^2 \bullet \pi/4$, where *S* is the spreading area (mm^2) and *d* is the spreading area diameter (mm). The test was performed in triplicate.

Table 1
Composition of phytosomes (PHY), phytonanoemulsion (PNE) and phytonanoemulgels (PNEGs).

Sample	GG (mg)	LC (mg)	H_2O (mL)	AO (mL)	EtOH (mL)	Gly (mg)	Natural polymer
PHY	20	400	20	–	–	–	–
PNE	20	400	20	2	2	–	–
PNEG HA	20	400	20	2	2	200	HA 2 %
PNEG SG	20	400	20	2	2	200	SG 2 %
PNEG PA	20	400	20	2	2	200	A1% P1%
PNEG HAu	–	400	20	2	2	200	HA 2 %
PNEG SGu	–	400	20	2	2	200	SG 2 %
PNEG PAu	–	400	20	2	2	200	A1% P1%

GG-*Glycyrrhiza glabra* L. extract, LC-lecithin, AO-almond oil; EtOH-ethanol; Gly-glycerin; HA-hyaluronic acid; SG-Solagum®AX; A-alginate; P-pectin; u-unloaded.

2.7. Stability studies

For the stability studies, 15 mL of each type of PNEG was transferred into Corning® Falcon® Centrifuge Tubes, which were then capped, sealed with parafilm, and stored at room temperature (25 ± 2 °C) in the dark.

All PNEGs were analyzed for macroscopic appearance (visual inspection) after preparation and after 4, 8, and 12 weeks. Droplet size, PDI, and pH of PNEGs were determined as described in paragraph 2.5.

2.8. In vitro release studies

The *in vitro* release studies were performed using the Franz cells (15 mm jacketed cell with a flat-ground joint and clear glass with a 12 mL receptor volume; diffusion surface area = 1.77 cm^2) equipped with a V6A Stirrer (PermeGearInc., Hellertown, PA, USA). A cellulose membrane (MF-Millipore cellulose nitrate 0.45 μm, Sartorius Stedim, Biotech GmbH, Germany) was placed between the donor and receptor compartment to assess the release of polyphenols from PNEGs. 0.5 mL of each sample was poured into a donor chamber, and 12 mL of PBS/ethanol 70:30 (*v/v*) mixture was used as a receptor medium. The systems were maintained at a temperature of 32.0 ± 1.0 °C while being stirred magnetically at a speed of 100 rpm. Samples of 200 μL from the receptor medium were withdrawn at the scheduled time (1, 2, 3, 4, 5, and 6 h) and replaced with the same amount of fresh medium. The TPC released in each sample was determined by the Folin-Ciocalteu assay, as reported in the Section 2.3.1. The results were expressed as a percentage of TPC released *versus* time.

According to the observed release profiles, the zero-order (Eq. (1)) and Higuchi (Eq. (2)) models were fitted to the percentage released *vs.* time data. The models were individually fitted to each replicate tested and the data used were from 1 to 6 h.

$$F = k \cdot (t - Tlag) \quad (1)$$

and

$$F = k_H \cdot (t - Tlag)^{0.5} \quad (2)$$

where k is the zero-order release rate constant, Tlag is the lag time, and k_H is the Higuchi release constant. To select the most suitable model for each dissolution data, the determination coefficient (R^2), the Akaike Information Criterion (AIC) [29], and the predicted *vs.* observed percentage of release plots were considered. The DDSolver® software program [30] was used for drug release data analysis.

$$AIC = n \cdot \ln(WSS) + 2 \cdot p \quad (3)$$

where n is the number of data points, WSS is the weighted sum of squares, and p is the number of parameters in the model; the model with a lower AIC value can be considered the better model.

2.9. Ex-vivo skin permeation studies

The permeation/retention studies of glycyrrhizin and TPC from PNEG SG, the more stable formulation, were performed using the Franz diffusion cells. Pig skin as a membrane model was chosen due to its similarity with human skin [31,32]. It was from the flank area and was provided by a local slaughterhouse, washed, the hair on the skin

carefully clipped, and dermatomed (Nouvag, Switzerland) to a thickness of 0.72 mm. It was stored at −20 °C until use. Before the experiments, the skin was defrosted and sandwiched between the donor and receptor compartments of the diffusion cell with an effective diffusion area of 1.77 cm^2. Transepidermal water loss (TEWL) was measured (Delfin VapoMeter®, Delfin Technologies) before starting each experiment to ensure the integrity of the skin. The receptor compartment was filled with 7 mL of a mixture of PBS 7.4/ethanol 70:30 (*v/v*), thermostated at 32.0 ± 1.0 °C, and continuously stirred at 450 rpm. The PNEGs were applied (500 μL) onto the epidermis surface under non-occlusive conditions, but the donor chamber was closed with a cap to prevent any evaporation. At prefixed intervals (0, 12, 16, 20 and 24 h), 0.8 mL of the receptor phase was withdrawn and analyzed by HPLC. Successively, the skin surface was gently washed with 0.6 mL of a solution of PBS/Tween 80 0.25 % (*v/v*) twice and gently dried with a cotton swab. Then, the skin was cut into thin pieces and bottled in 3 mL of a mixture of ethanol/water 50:50 (*v/v*). The extraction of TPC and glycyrrhizin from the skin was performed by a sonication of 10 min, followed by a stirring for 24 h at 90 rpm and a second sonication of 10 min. Finally, the mixture was centrifuged at 9900 rpm for 15 min, and the supernatants were analyzed by HPLC and the colorimetric test by Folin-Ciocalteu reagent concerning glycyrrhizin and TPC, respectively. Three replicates for formulation were tested. The HPLC analysis of glycyrrhizin was conducted by a Waters equipment and UV detection (254 nm). A Synergi column 4 μm Hydro-RP 80 Ă LC column 150x3mm (Phenomenex) was used. The system was operated in isocratic mode using a mobile phase consisting of a mixture of water/formic acid 0.1 % (*v/v*) and acetonitrile/formic acid 0.1 % (*v/v*) at a phase ratio of 60:40 *v/v* pumped at a flow rate of 1.0 mL/min at 25 °C. The sample was injected at a volume of 100 μL. The glycyrrhizin retention time was approximately 2.5 min. The calibration curve was acquired in the range between 1 and 100 μg/mL and the determination coefficient R^2 value was >0.999.

2.10. Cell viability

Cellular metabolic activity was measured by an MTT assay on WS1 cells as an indicator of cell viability and cytotoxicity. Briefly, cells were seeded at a density of 7000 cells/well in a 96-well plate and left to adhere overnight. Next, WS1 cells were treated with decreasing concentration of PHY (100, 50, 25, 12.5, 6.25 μg/mL of extract) and corresponding amount of PHYu 24 h after treatment, 10 μL of a 5 mg/mL MTT solution (Merk KGaA, Darmstadt, Germany) were added to each well. The plate was incubated for 2 h at 37 °C. Then, the cell culture medium was removed, and the formazan crystals formed were solubilized by adding 150 μL/well of propan-2-ol (Merk KGaA, Darmstadt, Germany). The absorbance at 570 nm was measured with an EnSpire® multimode plate reader (PerkinElmer, Waltham, USA). The sample absorbance at 690 nm was used as a reference wavelength for correction. Each formulation was assessed in four replicates, and the results are reported as the mean ± SD.

2.11. Statistical analysis

Statistical analysis of data was performed with the GraphPad Prism software, version 5.0 or 8.0 (GraphPad Software, Boston, MA, USA). Data normality was confirmed by the Shapiro-Wilk test. Normally distributed data were analyzed using the Student's *t*-test for comparisons between two groups and ANOVA for comparisons involving more than two groups. Non-normally distributed data were analyzed using the non-parametric Mann–Whitney test. The experimental groups were compared through one-way or two-way ANOVA, followed by Dunnett's or Tukey's multiple comparisons test. For *in vitro* release studies, and due to the small sample size ($n = 3$) the medians of the Higuchi rate constants were compared using the non-parametric Kruskal-Wallis one-way ANOVA on ranks test, followed by the post-hoc Kruskal-Wallis Multiple comparison z-value test if necessary. The differences between groups were considered statistically significant for $p < 0.05$. Data were expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Extraction of GG roots and extract analysis

3.1.1. Determination of total phenolic content and total flavonoid content

Roots of GG gently provided by Naturemed srl (Italy) were extracted by a mixture ethanol/water 50:50 (*v/v*) followed by drying under vacuum. The yield of extraction of the licorice root was 28.08 %. A solution of 1 mg/mL extract was prepared and analyzed; the same concentration was used to prepare the vesicular systems for extract encapsulation. The pH value of this solution was 6.40 ± 0.02.

The TPC determined spectrophotometrically using the Folin–Ciocalteu method was 62.11 ± 0.41 μg GAE/mg extract. This value is lower than those reported by Lim [33] which indicate a range of 72.10 to 107.93 mg/g extract [33] and by Castangia et al. [34] which is 91.2 mg GAE/g extract [34]. This slight variation in the TPC could be due to the pedoclimatic conditions of plant growing and extraction methods.

The TFC was determined by a UV–vis spectrophotometric assay using aluminum chloride. The carbonyl and hydroxyl groups of flavonoids combine with the aluminum ion to form a complex, with a maximum absorbance at 430 nm. The TFC value of the GG extract was 6.39 ± 0.13 μg QE/mg extract. According to the literature, almost 300 types of flavonoids can be detected in the GG extract, especially liquiritigenin, quercetin, and glabridin [35].

3.1.2. Determination of antioxidant activity (AA%)

The phenolic compounds in GG extract determine its antioxidant activity [34]. In the present study, the AA% values determined by DPPH assay at different extract concentrations (0.1, 0.25, 0.5, 1 mg/mL) are shown in Fig. 2a. Ascorbic acid (AA) was used as a positive control due to its antioxidant properties.

Fig. 2a illustrates that the AA% increases with the GG extract concentration and, at 1 mg/mL, reaches the value of 87.38 ± 2.64 %. These increasing values of the AA% could be related to the presence of flavones and flavanones in the extract [36]. This result was confirmed by the ROS reduction test that measures the capacity to reduce the intracellular ROS generation induced by a chemical compound (H_2O_2) or by UV light *in vitro* in the HaCaT cell line. The results are shown in Fig. 2b and c. Based on these results, it could be concluded that phenolic compounds in the GG extract developed a valuable antioxidant activity for cosmetic applications.

3.1.3. UHPLC/MS analysis

The UHPLC/MS analysis was performed to determine the amount of the main saponin glycyrrhizin in the sample compared with the standard compound. Through this analysis, the concentration of glycyrrhizin in the sample resulted in 45 μg/mg of extract (Rt = 3.04 min; confirmed by molecular ion 823.72 *m/z* [M + H] in positive mode; MW = 822.94).

The presence of glycyrrhizin in the extract is important for its well-known antioxidant activity and its natural emulsifier properties that could improve the stability of cosmetic emulsions. Based on this data and according to the literature, glycyrrhizin is the more abundant bioactive compound in the GG extract [35,52].

3.2. Physico-chemical characterization of PHY and PNE based on the GG extract

PNE was developed with a focus on minimal multifunctional ingredients, as shown in Table 2 and Scheme 1.

PNE was prepared using the emulsifying properties of lecithin and glycyrrhizin to incorporate almond oil as the hydrophobic phase. Ethanol was added to reduce the droplet sizes, and glycerin was used as

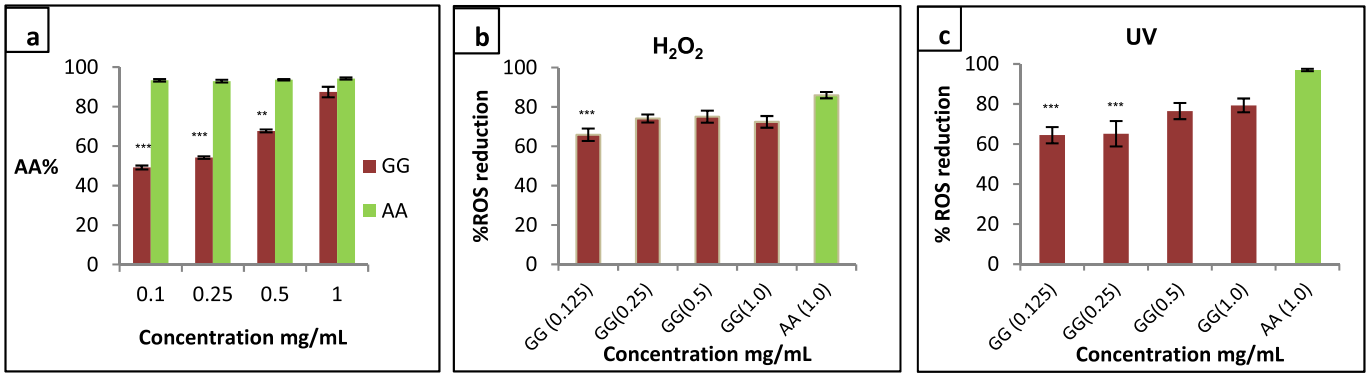


Fig. 2. a) Antioxidant activity (AA%) of licorice extract (GG) and ascorbic acid (AA). The data are expressed as the mean of three replicate experiments ± SD. (*** = $p < 0.001$; ** = $p < 0.01$; two-way ANOVA with Tukey's post-hoc test) b) Reactive Oxygen Species reduction by licorice extract solution at different concentrations after exposure to H_2O_2. c) Reactive Oxygen Species reduction by licorice extract solution at different concentrations after exposure to UVB radiation in HaCaT cells. The data are expressed as the mean of at least nine replicate experiments ± SD. (*** = $p < 0.001$; one-way ANOVA with Dunnett post-hoc test concerning GG 1.0).

Table 2
Ingredients and their cosmetic and technological properties selected for PNE preparation.

Ingredients	Cosmetic action	Technological function	References
Licorice extract	Antioxidant	Emulsifier	[37]
	Antinflammatory	Skin absorption enhancer	[9]
	Skin whitening		
Lecithin	Moisturizing	Emulsifier	[38]
	Elasticing		[39]
Almond oil	Antioxidant	Lipophilic solvent	[16]
	Moisturizing		[15]
	Elasticing		
Glycerin	Moisturizing	Skin absorption enhancer	[40]
		Co-surfactant	[41]
		Hydrophilic solvent	
Ethanol	Antiseptic	Co-surfactant	[42]
		Hydrophilic solvent	[43]

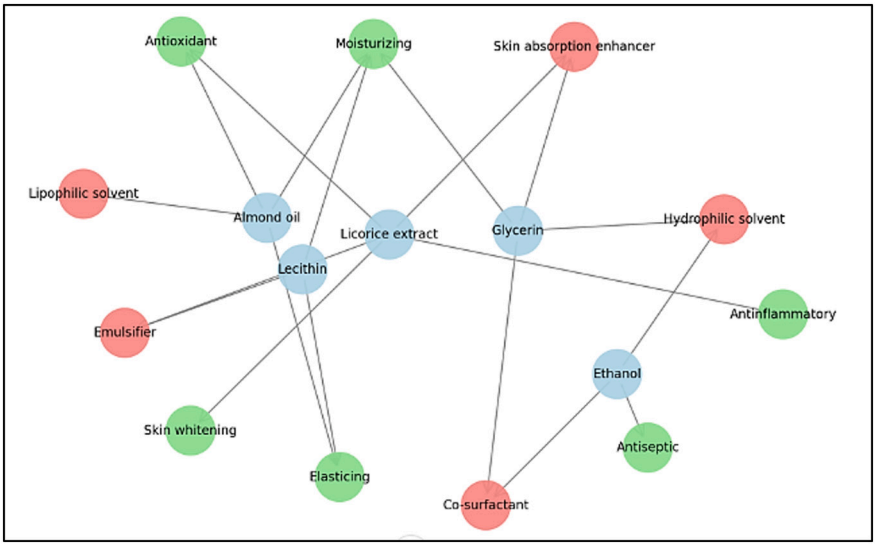
a humectant. For the preparation of PNE, lecithin and GG extract were mixed to form PHY by the auto-assembling method; then they were sonicated to favor the size reduction. Subsequently, the other components were added in the order: almond oil, ethanol, and glycerin, and homogenized. The final composition of the nanoemulsion was optimized by testing different types of hydrophobic phases and different percentages of emulsion compounds to produce a stable nanoemulsion without adding a synthetic emulsifier. The optimized formulations determined by preliminary studies were successfully prepared and *in vitro* characterized as reported in Table 3.

In Table 3, the main characteristics of PHY, PHYu, PNE, and PNEu are reported. The sizes of PHY and PHYu obtained by the autoassembling method were 684.0 ± 34.5 nm and 626.0 ± 32.4 nm for loaded and unloaded samples, respectively. The addition of almond oil, ethanol, and glycerin, followed by homogenization, produced a nanoemulsion with droplet sizes of 288.0 ± 13.6 nm and 271.8 ± 3.4 nm for loaded and unloaded samples, respectively. All the phytosome formulations

Table 3
Size (nm), PDI, ζ-potential, and pH of loaded with 1 mg/mL of GG (PHY), phytosomes unloaded (PHYu), phytonanoemulsion loaded (PNE), and phytonanoemulsion unloaded (PNEu).[a]

Sample	Size (nm)	PDI	ζ-Potential (mV)	pH
PHY	684.0 ± 34.5	0.227 ± 0.075	−51.63 ± 0.30	6.00 ± 0.50
PHYu	626.0 ± 32.4	0.290 ± 0.030	−54.53 ± 1.00	5.50 ± 0.00
PNE	288.0 ± 13.6	0.263 ± 0.013	−58.83 ± 1.27	5.33 ± 0.29
PNEu	271.8 ± 3.40	0.321 ± 0.001	−61.57 ± 0.55	5.67 ± 0.29

[a] Values are expressed as mean ± SD, (n = 3).



Scheme 1. Graphical representation of ingredient interconnections for embedding of cosmetic and technological properties in the formulation design.

present higher sizes than phytonanoemulsion droplets, probably due to the incorporation of added compounds followed by homogenization.

The PDI value increased from 0.227 ± 0.075 to 0.263 ± 0.013 for PHY and PNE, respectively. It is worth noting that the PDI of PNEu is higher than that of PNE. It could be due to the presence of glycyrrhizin in GG extract that, as a natural emulsifier, could favor a better homogenization of the nanoemulsion. In fact, a PDI <0.3 indicates a narrow distribution of droplet sizes.

The zeta potential reflects the surface charge of droplets. A value of ±30 mV is favorable for the repulsion of droplets. In Table 3, the negative zeta potential values can be attributed primarily to lecithin, as noted in the literature [44,45]. An increase in the ζ-potential value from −51.77 ± 0.25 mV to −58.83 ± 1.27 mV ($p < 0.001$; one-way ANOVA with Dunnett post-hoc test) was observed between PHY and PNE. Both values are highly negative, which suggests that the system is likely to be stable due to the electrostatic repulsion between droplets, thereby preventing their aggregation. Finally, the pH value of PNE is slightly acidic (pH = 5.33), and it is favorable to topical use.

3.3. Preparation and characterization of PNEGs

The prepared PNE was mixed with various natural polymers—hyaluronic acid, Solagum®AX, pectin, and alginate—to achieve jellification. This process increases the viscosity, making it easier to apply to the skin while enhancing the stability of the PNE. Additionally, the selected biopolymers contribute cosmetic benefits to the formulation, including moisturizing, anti-aging, soothing, and emollient properties. The PNEG formulation, which consists of adding a nanoemulsion to a gel-like matrix, makes it possible to combine the advantages of both nanoemulsion and gel. The addition of hydrogel enhances not only the stability of the nanoemulsion but also the rheological properties [46]. Different percentages of polymers or their combination were assessed before reaching the optimized formulations of PNEGs (Fig. 3). In fact, selecting the hydrocolloid type is a crucial step for obtaining stable nanoemulsion-based gels with controlled rheological properties [22]. The characterization of the optimized phytonanoemulgels is reported in Table 4.

In Table 4, the main parameters for PNEG droplets are reported. Regarding the sizes, adding polymer increases the size of droplets, particularly for PNEG PA compared to PNEG HA ($p = 0.029$; one-way ANOVA with Dunnett post-hoc test). This increase is probably due to the interaction of the polymer with the droplet surface. The value of PDI was <0.3 for all PNEGs, which confirmed that the systems were homogeneous. The ζ-potential showed a decrease in its values, becoming more negative and preventing aggregation and phase separation. These reductions may be influenced by the charges of polymers, particularly the negative charges found in hyaluronic acid, pectin, and alginate [47,48]. Concerning the TPC, the values follow the order PNEG PA ≈ PNEG HA > PNEG SG. It is expected that these values could be different for the different PNEGs and probably related to their different density values, which follow the same order. Concerning the AA%, mainly due to the polyphenol contents of GG extract, it is preserved for all PNEGs, even if there are some differences in their values following the order PNEG PA > PNEG HA >PNEG SG, showing the same order of TPC values ($p<0.05$, one-way ANOVA with Tukey's post-hoc test). Finally, the pH values of PNEG HA and PNEG SG were optimal for skin use, while the pH of PNEG PA was probably too acidic and unfitting for topical use.

3.4. Rheological characterization

Rheological characterizations were used to evaluate the resulting PNEG performance starting with measuring the relationship between the applied shear stress and the resultant rate of shear strain. As can be observed in the Fig. 4, the PNEGs show a non-newtonian behavior with a shear-thinning effect in particular for SG polymer. The loading of PNEG with licorice extract increases the viscosity of PNEG HA and PNEG PA, indicating that adding extract increases the gels' viscosity.

Viscoelastic properties were assessed within the linear viscoelastic region (LVR) to obtain more information about the PNEG structure. Fig. 5a shows that for PNEG HA and PNEG PA, the viscous modulus G″ is prevalent, and the elastic modulus G′ tends to increase at higher frequencies. In the case of PNEG SG, the elastic modulus G′ is prevalent due to this polymer's composition based on Acacia and xanthan gum (Solagum® AX).

Similarly to the loaded PNEGs, in Fig. 5b, it can be observed that the viscous modulus G″ is prevalent. The elastic modulus G′ increases at higher frequencies, particularly for HA and PA polymers. In summary, the mechanical spectra were generally similar for PNEG HA and PNEG PA, where the viscous modulus was always higher than the elastic modulus (G″ > G′) in the range of frequencies studied, evidencing a liquid-like behavior. The elastic modulus is prevalent for PNEG SG as it is composed of different types of polymers, such as Acacia and xanthan gum, leading to this different rheological characteristic. It can also be observed that the type of polymer has the primary influence on the viscoelastic behavior.

3.4.1. Adhesion test

The adhesion test gives information about the adhesiveness and tackiness of a cosmetic formulation that influences customer compliance. Fig. 6 shows the influence of different polymers on adhesivity. In particular, the Peak Normal Force-Normal force (N) is related to the tack and the Area Under Force-Time curve (N·s) to the adhesive strength. The results demonstrated that the PNEG HA has the highest value for tack and adhesive strength (−0.857 ± 0.034 N and 1.294 ± 0.065 N·s, respectively; $p < 0.001$; two-way ANOVA with Tukey's post-hoc test). This could be due to the physicochemical characteristics of hyaluronic acid, which is well-known as a jellifying agent for cosmetic products. The lower values of adhesiveness parameters are reported for PNEG SG (−0.284 ± 0.015 N and 0.693 ± 0.045 N.s), while the values are higher for PNEG PA (−0.376 ± 0.024 N and 0.765 ± 0.092 N.s).

The loaded PNEGs result in more tack than unloaded, probably due to the interactions between the GG extract and the other formulation

Fig. 3. Phytonanoemulgels of GG loaded.

Table 4
Size (nm), PDI, ζ-potential (mV), TPC (μg/g), AA%, density, and pH of phytonanoemulgel of hyaluronic acid loaded (PNEG HA), Solagum®AX loaded (PNEG SG), and pectin/alginate loaded (PNEG PA).[a]

Sample	Size (nm)	PDI	ζ-Potential (mV)	TPC (μg GAE/g of PNEG)	AA%[b]	Density (g/mL)	pH
PNEG HA	302.7 ± 4.4	0.264 ± 0.010	72.71 ± 1.14	65.37 ± 2.16	59.80 ± 0.22	1.06 ± 0.01	5.75 ± 0.27
PNEG SG	320.5 ± 6.6	0.289 ± 0.009	59.10 ± 0.27	43.80 ± 4.32	56.80 ± 1.29	1.03 ± 0.01	5.70 ± 0.27
PNEG PA	323.5 ± 10.8	0.108 ± 0.053	70.33 ± 0.30	68.75 ± 3.96	66.29 ± 0.80	1.09 ± 0.02	4.40 ± 0.42

[a] Values are expressed as mean ± SD, (n = 3).
[b] (After dilution 1:10).

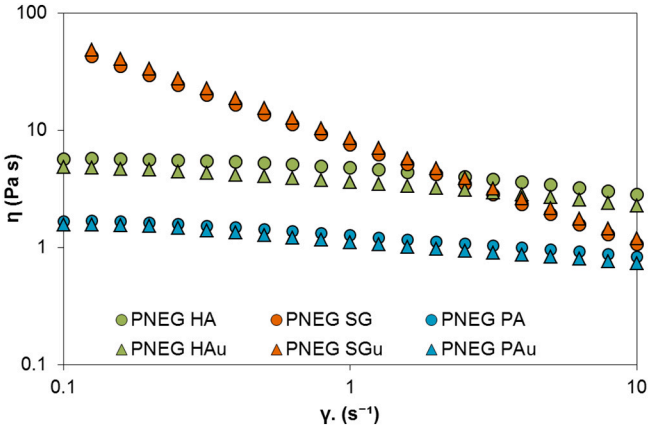


Fig. 4. Viscosity as a function of shear rate plot for PNEGs. Phytonanoemulgel of hyaluronic acid loaded (PNEG HA), Phytonanoemulgel of hyaluronic acid unloaded (PNEG HAu), Solagum® AX loaded (PNEG SG), Solagum® AX unloaded (PNEG SGu), pectin/alginate loaded (PNEG PA) and pectin/alginate unloaded (PNEG PAu).

compounds.

3.4.2. Spreadability

The spreadability test was carried out to provide more information about the distribution of PNEGs when applied to the skin. Spreadability values are reported in Fig. 7.

The measure of spreadability is a helpful parameter in cosmetic formulation as it could favor the customer compliance. As shown in Fig. 7 the PNEG spreadability follows the order PNEG PA > PNEG SG and PNEG HA ($p < 0.001$; two-way ANOVA with Tukey's post-hoc test).

3.5. Stability studies

All PNEGs were analyzed for macroscopic appearance (visual inspection) after preparation (0 week), and after 4, 8 and 12 weeks of storage in the dark at room temperature (25 ± 2 °C). Droplet size, PDI, and pH were determined as described above.

The visual inspection of formulations evidenced that PNEG SG maintained the same aspect during the 12 and 24 weeks, while a creaming phenomenon was noticed for PNEG PA and, to a minor extent, for PNEG HA (Fig. 8). However, no separation phase was observed for these formulations, and they recovered a homogenous aspect after agitation. In Table 5, it can be observed that all PNEGs maintain their vesicles in a nanoscale size, <300 nm, and in particular, a little lowering in their dimensions was observed over time for PNEG SG and PNEG PA ($p < 0.0001$, two-way ANOVA with Dunnett post-hoc). No significant increase was registered for the pH values, indicating substantial stability of chemical compounds ($p > 0.05$, two-way ANOVA with Dunnett post-hoc test). In addition, PNEG SG exhibited a homogeneous visual aspect over an extended observation period of 24 weeks, confirming that this formulation is highly suitable for creating a cosmetic product with a significantly longer shelf life.

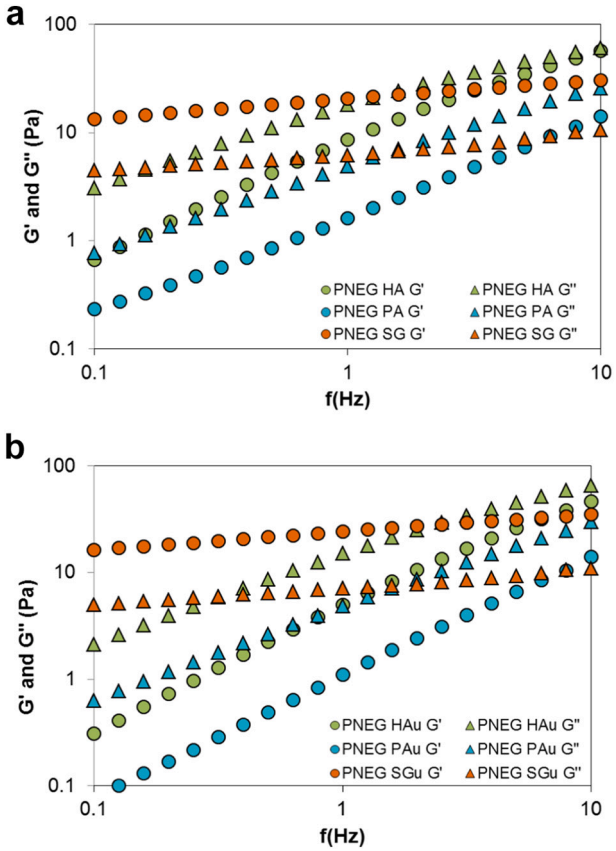


Fig. 5. G′ and G″ modulus for PNEGs a) unloaded and b) loaded PNEGs. Phytonanoemulgel of hyaluronic acid (PNEG HA), Solagum® AX (PNEG SG), and pectin/alginate (PNEG PA). G′ (Storage Modulus) and G″ (Loss Modulus).

3.6. In vitro release studies

The *in vitro* release of TPC from PNEGs is shown in Fig. 9.

All PNEGs release the polyphenols more slowly than the GG extract solution. After 6 h, the percentage of mean of TPC released is 72.88 ± 7.44 % for the extract solution, while for PNEG HA, PNEG SG, and PNEG PA, it is 34.81 ± 2.31, 50.14 ± 0.05, and 51.08 ± 0.13 %, respectively. In terms of modelling, two models were fitted to the data: the zero-order model, according to the observed release profile, and the Higuchi model, to which many release data obtained from semi-solid formulations usually fit [49] and which is also the model recommended by the European Medicines Agency for the *in vitro* evaluation of topical products [53]. Table 6 summarizes the main results obtained. According to the AIC value, the best-fitting model was the Higuchi model, except for the GG extract. The trend observed according to the Higuchi rate constant was: GG extract> PNEG SG ≈ PNEG PA > PNEG HA. The addition of polymers to the formulations to improve their stability contributed to a more sustained release of the active ingredients, probably due to the higher viscosity. However, no statistically significant differences were

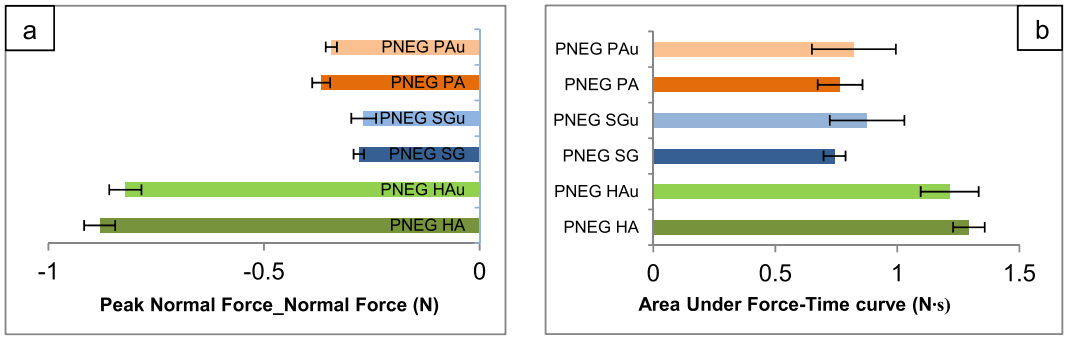


Fig. 6. a) Peak Normal Force-Normal force (N) and b) Area Under Force-Time curve (N·s) for loaded and unloaded PNEGs. Phytonanoemulgel of hyaluronic acid loaded (PNEG HA), Phytonanoemulgel of hyaluronic acid unloaded (PNEG HA), Solagum® AX loaded (PNEG SG), Solagum® AX unloaded (PNEG SG), pectin/alginate loaded (PNEG PA), and pectin/alginate unloaded (PNEG PA). Values are expressed as mean ± SD, (n = 6).

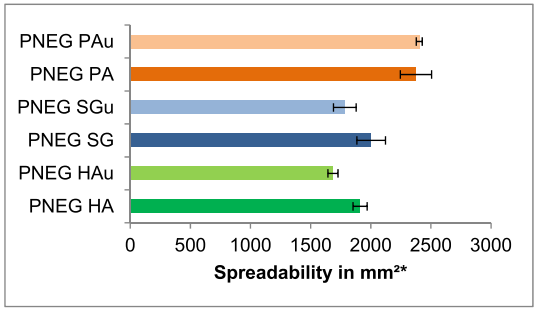


Fig. 7. Spreadability of PNEGs loaded and unloaded in mm^2. Phytonanoemulgel of hyaluronic acid loaded (PNEG HA), Phytonanoemulgel of hyaluronic acid unloaded (PNEG HAu), Solagum® AX loaded (PNEG SG), Solagum® AX unloaded (PNEG SGu), pectin/alginate loaded (PNEG PA) and pectin/alginate unloaded (PNEG PAu). Values are expressed as mean ± SD (n = 3).

found between the kH of the three PNEGs compared (p = 0.1801, Kruskal-Wallis One-Way ANOVA on Ranks test). This result is reasonable given that all polymers are water soluble, and the viscosity of the PNEGs was similar.

3.7. *Ex vivo skin permeation studies*

Considering the stability studies, PNEG SG showed the best preservation of physicochemical characteristics over time and was selected for the following study.

The permeation/retention studies of glycyrrhizin and TPC from PNEG SG were performed on dermatomed pig skin from the flank area using Franz cells. Results revealed that glycyrrhizin was indetectable in the receptor solution at 24 h post-application of the formulation on the skin, when HPLC was used as an analytical method for quantification of glycyrrhizin. Glycyrrhizin was found only in traces in the skin (*i.e.*, retained in the skin) in the range from 2 to 5 %. However, concerning the TPC, the value detected in the skin was 5.97 ± 3.24 %, while a higher percentage was found in the receptor compartment (27.68 ± 3.86 %). It can be hypothesized that, as glycyrrhizin is a highly polar compound, its penetration into the skin is limited. On the other hand, the polyphenols in the GG extract are a mixture of different compounds of various polarities, and the more hydrophobic phenolic compounds could better penetrate through the lipophilic barrier of the stratum corneum. It is worth noticing that this formulation contains ethanol and glycerin, which are considered skin penetration enhancers. In any case, and given

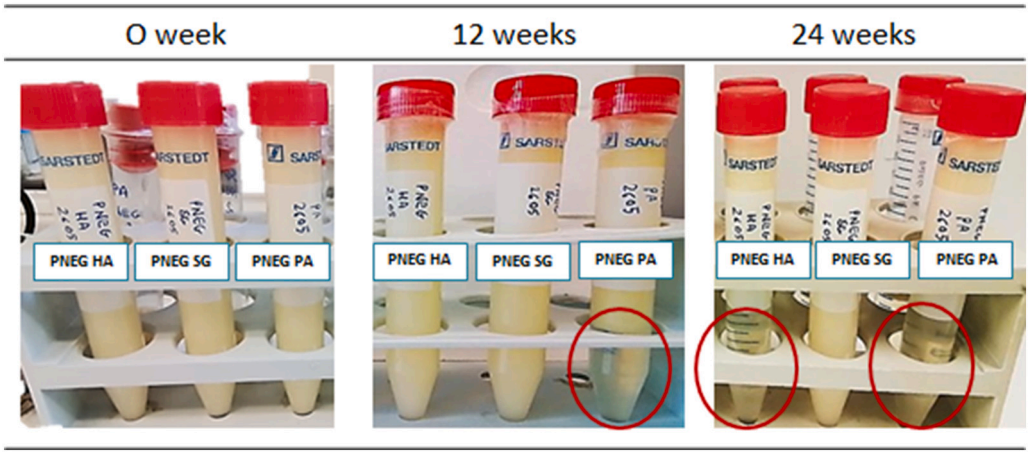


Fig. 8. Comparison of macroscopic appearance of PNEG HA, PNEG SG, PNEG PA at 0 (7a) and 12 weeks (7b) and 24 weeks (7c).

Table 5
Size (nm), PDI, pH of phytonanoemulgel of hyaluronic acid loaded (PNEG HA), Solagum®AX loaded (PNEG SG) and pectin/alginate loaded (PNEG PA) during 12 week of storage in the dark at 25 ± 2 °C.

Sample	Size (nm)	PDI	pH
Week 0			
PNEG HA	302.7 ± 4.4	0.264 ± 0.010	5.75 ± 0.27
PNEG SG	320.5 ± 6.6	0.289 ± 0.009	5.70 ± 0.27
PNEG PA	323.5 ± 10.8	0.108 ± 0.053	4.40 ± 0.42
Week 4			
PNEG HA	300.0 ± 5.3	0.278 ± 0.003	5.75 ± 0.35
PNEG SG	293.5 ± 11.4	0.225 ± 0.023	5.75 ± 0.35
PNEG PA	322.4 ± 20.0	0.145 ± 0.062	4.50 ± 0.27
Week 8			
PNEG HA	252.8 ± 12.7	0.181 ± 0.009	5.70 ± 0.29
PNEG SG	259.4 ± 39.0	0.293 ± 0.054	5.75 ± 0.50
PNEG PA	285.4 ± 26.5	0.263 ± 0.097	4.55 ± 0.50
Week 12			
PNEG HA	287.8 ± 6.1	0.265 ± 0.045	5.80 ± 0.14
PNEG SG	222.3 ± 6.2	0.268 ± 0.018	5.85 ± 0.21
PNEG PA	233.1 ± 12.0	0.194 ± 0.001	4.60 ± 0.14

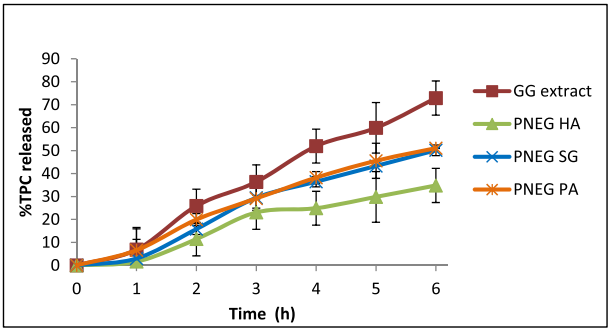


Fig. 9. *In vitro* release of TPC in PBS:ethanol (70:30 *v/v*) from GG extract solution (1 mg/mL) and PNEGs. Data expressed as mean ± SD (n = 3).

that the stratum corneum is a limiting barrier to cutaneous permeation, higher values of permeated and retained quantities of phenolic products would be expected in human skin, given the greater thickness of the stratum corneum of pig skin *vs.* human skin [32,50].

3.7.1. Cell viability studies

The biocompatibility of PHY and PHYu with GG extract was tested by MTT assay on WS1 fibroblasts. This cell type was chosen as they are the most representative cell component of skin dermis, the primary target for the topical formulations. Fibroblasts were treated for 24 h with the GG extract solution and PHY or PHYu. The data showed that none of the treatments had a detrimental effect on WS1 cells demonstrating that they are safe for cells ($p > 0.05$; one–way ANOVA with Dunnett post-hoc test; $n = 4$).

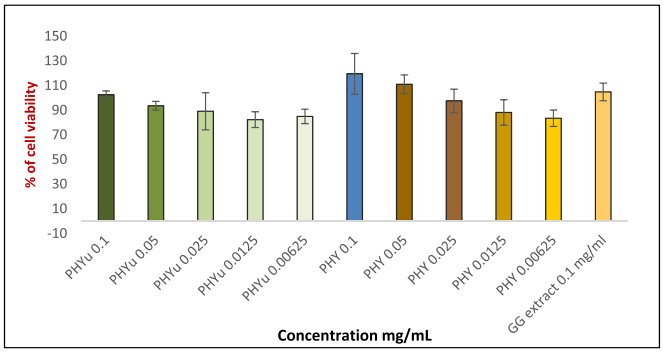


Fig. 10. MTT assays on WS1 cells treated for 24 hours with decreasing concentration of PHYu, and PHY. Data are expressed as percentage of cell viability with respect to the control (n=4).

4. Conclusions and future perspectives

In the present study, we prepared three different PNEGs following a natural and sustainable design using only natural origin ingredients, including plant-derived emulsifiers, biopolymers, and bioactive compounds. This green and consumer-oriented approach is highly topical in the cosmetic field. A comprehensive evaluation strategy of the pathway leading to an effective cosmetic formulation was planned: i) standardization of the extract, ii) technological and biological evaluation of the antioxidant activity, iii) rheological characterization, iv) long-term stability studies, v) *in vitro* release and *ex-vivo* permeation studies, vi) cell viability studies. Through these steps, the study offers a framework for developing eco-designed formulations. From this point of view, some steps are particularly crucial: the selection of natural extracts that have multiple cosmetic functionality and along with technological properties; the incorporation of a limited number of ingredients for a more sustainable choice; lecithin and glycyrrhizin as natural emulsifiers representing a combination compliant with the regulations in “clean-label” cosmetic products. Based on the results, PNEGs exhibited valuable antioxidant activity, suitable rheological characteristics, which improved the ease of topical application and provided a slow release of phenolic compounds, potentially enhancing the absorption of bioactive molecules. Furthermore, the ingredients used in the phytonanoemulsion were found to be safe for cells. Concerning stability, PNEG HA and PNEG PA have been observed to experience creaming phenomena, which may lead to additional stabilizers for improved stability. On a positive note, PNEG SG demonstrated good visual stability over 24 weeks, indicating a potential for an extended shelf life. Considering all the analyses performed, PNEG SG results identified this formulation as the most promising. In fact, it shows small droplet size (320.5 ± 6.6 nm) and the low polydispersion (0.289 ± 0.009), the preservation of antioxidant activity (56.80 ± 1.29 %), and a skin-friendly pH (5.70 ± 0.27). Moreover, PNEG showed an increased viscosity, shear thinning behavior, and the adhesion test showed that this formulation is nonsticky and easily spreadable. In conclusion, PNEG SG, avoiding synthetic emulsifiers or stabilizers, represents a promising natural skincare product; it offers consumers the valuable multifunctionality of licorice extract while being formulated with a limited number of natural ingredients, making

Table 6
Release constants, determination coefficients (R^2), and AIC values after fitting the zero-order and Higuchi models to the percentage of TPC released over 6 h. Values are expressed as mean ± DS.

Model		GG extract	PNEG HA	PNEG SG	PNEG PA
Zero order	K (%·h^{-1})	12.79 ± 0.003	6.36 ± 0.003	9.29 ± 0.465	8.81 ± 0.953
	R^2	0.986 ± 0.009	0.9208 ± 0.083	0.9649 ± 0.012	0.966 ± 0.002
	AIC	25.55 ± 4.43	26.25 ± 8.71	27.84 ± 2.79	27.20 ± 1.70
Higuchi	kH (%·h$^{-0.5}$)	32.67 ± 3.64	16.15 ± 0.320	23.54 ± 1.44	22.59 ± 0.072
	R^2	0.954 ± 0.039	0.959 ± 0.029	0.987 ± 0.001	0.978 ± 0.012
	AIC	32.04 ± 5.80	23.90 ± 5.23	21.84 ± 1.23	24.17 ± 2.05

it an innovative approach for both personal care and environmental sustainability. Further research could explore some limitations such as the variability in TPC due to plant material or extraction conditions, as well as the polymer interaction or the long-term stability in different storage conditions. Finally, the in vivo, clinical, and sensory evaluation could improve the translatability in dermatological applications and consumer compliance.

CRediT authorship contribution statement

Valentina Sallustio: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Joana Marto:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Elvira Escribano-Ferrer:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Lídia Maria Gonçalves:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Martina Rossi:** Methodology, Investigation. **Concettina Cappadone:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Manuela Mandrone:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Ilaria Chiocchio:** Investigation. **Simone Rossello:** Investigation. **Helena Ribeiro:** Writing – review & editing. **Teresa Cerchiara:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization.

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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.ijbiomac.2025.145241.

Data availability

The content of the present paper is object of Italian patent application n. 102023000027186 filed on 19.12.2023 and international patent application n. PCT/IB2024/062693 filed on 16.12.2024. Data will be made available on request.

References

[1] A.M. Martins, J.M. Marto, A sustainable life cycle for cosmetics: from design and development to post-use phase, Sustain. Chem. Pharm. 35 (2023) 101178, https://doi.org/10.1016/j.scp.2023.101178.
[2] C. Kolling, J.L.D. Ribeiro, J.F. De Medeiros, Performance of the cosmetics industry from the perspective of corporate social responsibility and design for sustainability, Sustain. Prod. Consum. 30 (2022) 171–185, https://doi.org/10.1016/j.spc.2021.12.002.
[3] R. Rocca, F. Acerbi, L. Fumagalli, M. Taisch, Sustainability paradigm in the cosmetics industry: state of the art, Clean. Waste Syst. 3 (2022) 100057, https://doi.org/10.1016/j.clwas.2022.100057.
[4] J.I. Kalwat, Skin minimalism - what is the new beauty trend? Biomed. J. Sci. Tech. Res. 52 (2023) https://doi.org/10.26717/BJSTR.2023.52.008303.
[5] M.-C. Kouassi, M. Grisel, E. Gore, Multifunctional active ingredient-based delivery systems for skincare formulations: a review, Colloids Surf. B: Biointerfaces 217 (2022) 112676, https://doi.org/10.1016/j.colsurfb.2022.112676.
[6] L.L. Lima, K. Bispo-dos-Santos, I.M.C. Trevisan, C. Rapóso, P.E.N.F. Velho, E. Bagatin, R.A. Rezende, J.V.L. Da Silva, G. Ricci Leonardi, Developing botanical formulations for sustainable cosmetics, Cosmetics 10 (2023) 159, https://doi.org/10.3390/cosmetics10060159.
[7] Q. Zhang, D. Qian, D.-D. Tang, J. Liu, L.-Y. Wang, W. Chen, C.-J. Wu, W. Peng, Glabridin from *Glycyrrhiza glabra* possesses a therapeutic role against keloid via attenuating PI3K/Akt and transforming growth factor-β1/SMAD signaling pathways, J. Agric. Food Chem. 70 (2022) 10782–10793, https://doi.org/10.1021/acs.jafc.2c02045.
[8] Md.K. Hasan, I. Ara, M.S.A. Mondal, Y. Kabir, Phytochemistry, pharmacological activity, and potential health benefits of Glycyrrhiza glabra, Heliyon 7 (2021) e07240, https://doi.org/10.1016/j.heliyon.2021.e07240.
[9] N. Taarji, M. Bouhoute, H. Melanie, A. Hafidi, I. Kobayashi, M. Neves, K. Tominaga, H. Isoda, M. Nakajima, Stability characteristics of o/w emulsions prepared using purified glycyrrhizin or a non-purified glycyrrhizin-rich extract from liquorice root (*Glycyrrhiza glabra*), Colloids Surf. A Physicochem. Eng. Asp. 614 (2021) 126006, https://doi.org/10.1016/j.colsurfa.2020.126006.
[10] M. Xu, Z. Wan, X. Yang, Recent advances and applications of plant-based bioactive saponins in colloidal multiphase food systems, Molecules 26 (2021) 6075, https://doi.org/10.3390/molecules26196075.
[11] M. Lianza, M. Mandrone, I. Chiocchio, P. Tomasi, L. Marincich, F. Poli, Screening of ninety herbal products of commercial interest as potential ingredients for phytocosmetics, J. Enzyme Inhib. Med. Chem. 35 (2020) 1287–1291, https://doi.org/10.1080/14756366.2020.1774571.
[12] R.K. Harwansh, R. Deshmukh, M.A. Rahman, Nanoemulsion: promising nanocarrier system for delivery of herbal bioactives, J. Drug Deliv. Sci. Technol. 51 (2019) 224–233, https://doi.org/10.1016/j.jddst.2019.03.006.
[13] M. Jaiswal, R. Dudhe, P.K. Sharma, Nanoemulsion: an advanced mode of drug delivery system, 3 Biotech 5 (2015) 123–127, https://doi.org/10.1007/s13205-014-0214-0.
[14] D.S. Shaker, R.A.H. Ishak, A. Ghoneim, M.A. Elhuoni, Nanoemulsion: a review on mechanisms for the transdermal delivery of hydrophobic and hydrophilic drugs, Sci. Pharm. 87 (2019) 17, https://doi.org/10.3390/scipharm87030017.
[15] J. Blaak, P. Staib, An updated review on efficacy and benefits of sweet almond, evening primrose and jojoba oils in skin care applications, Int. J. Cosmet. Sci. 44 (2022) 1–9, https://doi.org/10.1111/ics.12758.
[16] T.-K. Lin, L. Zhong, J. Santiago, Anti-inflammatory and skin barrier repair effects of topical application of some plant oils, Int. J. Mol. Sci. 19 (2017) 70, https://doi.org/10.3390/ijms19010070.
[17] D.J. McClements, C.E. Gumus, Natural emulsifiers — biosurfactants, phospholipids, biopolymers, and colloidal particles: molecular and physicochemical basis of functional performance, Adv. Colloid Interf. Sci. 234 (2016) 3–26, https://doi.org/10.1016/j.cis.2016.03.002.
[18] C. Solans, P. Izquierdo, J. Nolla, N. Azemar, M.J. Garcia-Celma, Nano-emulsions, Curr. Opin. Colloid Interface Sci. 10 (2005) 102–110, https://doi.org/10.1016/j.cocis.2005.06.004.
[19] B. Alhasso, M.U. Ghori, B.R. Conway, Development of a nanoemulgel for the topical application of mupirocin, Pharmaceutics 15 (2023) 2387, https://doi.org/10.3390/pharmaceutics15102387.
[20] H. Choudhury, B. Gorain, M. Pandey, L.A. Chatterjee, P. Sengupta, A. Das, N. Molugulu, P. Kesharwani, Recent update on nanoemulgel as topical drug delivery system, J. Pharm. Sci. 106 (2017) 1736–1751, https://doi.org/10.1016/j.xphs.2017.03.042.
[21] M.R. Donthi, S.R. Munnangi, K.V. Krishna, R.N. Saha, G. Singhvi, S.K. Dubey, Nanoemulgel: a novel nano carrier as a tool for topical drug delivery, Pharmaceutics 15 (2023) 164, https://doi.org/10.3390/pharmaceutics15010164.
[22] N. Riquelme, C. Savignones, A. López, R.N. Zúñiga, C. Arancibia, Effect of gelling agent type on the physical properties of nanoemulsion-based gels, Colloids Interfaces 7 (2023) 49, https://doi.org/10.3390/colloids7030049.
[23] S. Desplanques, M. Grisel, C. Malhiac, F. Renou, Stabilizing effect of acacia gum on the xanthan helical conformation in aqueous solution, Food Hydrocoll. 35 (2014) 181–188, https://doi.org/10.1016/j.foodhyd.2013.05.009.
[24] K. Kobra, S.Y. Wong, M.A.J. Mazumder, X. Li, M.T. Arafat, Xanthan and gum acacia modified olive oil based nanoemulsion as a controlled delivery vehicle for topical formulations, Int. J. Biol. Macromol. 253 (2023) 126868, https://doi.org/10.1016/j.ijbiomac.2023.126868.
[25] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: Oxidants and Antioxidants Part A, Methods in Enzymology, Academic Press, 1999, pp. 152–178, https://doi.org/10.1016/S0076-6879(99)99017-1.
[26] V. Sallustio, I. Chiocchio, M. Mandrone, M. Cirrincione, M. Protti, G. Farruggia, A. Abruzzo, B. Luppi, F. Bigucci, L. Mercolini, F. Poli, T. Cerchiara, Extraction, encapsulation into lipid vesicular systems, and biological activity of *Rosa canina* L. bioactive compounds for dermocosmetic use, Molecules 27 (2022) 3025, https://doi.org/10.3390/molecules27093025.
[27] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT Food Sci. Technol. 28 (1995) 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.
[28] V. Sallustio, G. Farruggia, M.P. Di Cagno, M.M. Tzanova, J. Marto, H. Ribeiro, L. M. Goncalves, M. Mandrone, I. Chiocchio, T. Cerchiara, A. Abruzzo, F. Bigucci,

B. Luppi, Design and characterization of an ethosomal gel encapsulating rosehip extract, Gels 9 (2023) 362, https://doi.org/10.3390/gels9050362.
[29] H. Akaike, A new look at the statistical model identification, IEEE Trans Automat Contr 19 (1974) 716–723, https://doi.org/10.1109/TAC.1974.1100705.
[30] Y. Zhang, M. Huo, J. Zhou, A. Zou, W. Li, C. Yao, S. Xie, DDSolver: an add-in program for modeling and comparison of drug dissolution profiles, AAPS J. 12 (2010) 263–271, https://doi.org/10.1208/s12248-010-9185-1.
[31] E.C. Jung, H. Maibach, Animal models for percutaneous absorption, J. Appl. Toxicol. 35 (2015), https://doi.org/10.1002/jat.3004.
[32] E.C. Jung, H.I. Maibach, Animal models for percutaneous absorption, J. Appl. Toxicol. 35 (2015) 1–10, https://doi.org/10.1002/jat.3004.
[33] T.K. Lim, Glycyrrhiza glabra, in: Edible Medicinal and Non-Medicinal Plants, Springer Netherlands, Dordrecht, 2016, pp. 354–457, https://doi.org/10.1007/978-94-017-7276-1_18.
[34] I. Castangia, C. Caddeo, M.L. Manca, L. Casu, A.C. Latorre, O. Díez-Sales, A. Ruiz-Saurí, G. Bacchetta, A.M. Fadda, M. Manconi, Delivery of liquorice extract by liposomes and hyalurosomes to protect the skin against oxidative stress injuries, Carbohydr. Polym. 134 (2015) 657–663, https://doi.org/10.1016/j.carbpol.2015.08.037.
[35] S. Wahab, S. Annadurai, S.S. Abullais, G. Das, W. Ahmad, M.F. Ahmad, G. Kandasamy, R. Vasudevan, M.S. Ali, M. Amir, *Glycyrrhiza glabra* (licorice): a comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology, Plants 10 (2021) 2751, https://doi.org/10.3390/plants10122751.
[36] N. Martins, L. Barros, M. Dueñas, C. Santos-Buelga, I.C.F.R. Ferreira, Characterization of phenolic compounds and antioxidant properties of Glycyrrhiza glabra L. rhizomes and roots, RSC Adv. 5 (2015) 26991–26997, https://doi.org/10.1039/C5RA03963K.
[37] T.H. Fatoki, B.O. Ajiboye, A.O. Aremu, In silico evaluation of the antioxidant, anti-inflammatory, and dermatocosmetic activities of phytoconstituents in licorice (Glycyrrhiza glabra L.), Cosmetics 10 (2023) 69, https://doi.org/10.3390/cosmetics10030069.
[38] C. Vater, V. Hlawaty, P. Werdenits, M.A. Cichoń, V. Klang, A. Elbe-Bürger, M. Wirth, C. Valenta, Effects of lecithin-based nanoemulsions on skin: short-time cytotoxicity MTT and BrdU studies, skin penetration of surfactants and additives and the delivery of curcumin, Int. J. Pharm. 580 (2020) 119209, https://doi.org/10.1016/j.ijpharm.2020.119209.
[39] W. Johnson, W.F. Bergfeld, D.V. Belsito, R.A. Hill, C.D. Klaassen, D.C. Liebler, J. G. Marks, R.C. Shank, T.J. Slaga, P.W. Snyder, L.J. Gill, B. Heldreth, Safety assessment of lecithin and other phosphoglycerides as used in cosmetics, Int. J. Toxicol. 39 (2020) 5S–25S, https://doi.org/10.1177/1091581820953123.
[40] A. Amiri-Rigi, S. Abbasi, Microemulsion-based lycopene extraction: effect of surfactants, co-surfactants and pretreatments, Food Chem. 197 (2016) 1002–1007, https://doi.org/10.1016/j.foodchem.2015.11.077.
[41] N. Parisi, M. Paz-Alvarez, P.J. Matts, R. Lever, J. Hadgraft, M.E. Lane, Topical delivery of hexamidine, Int. J. Pharm. 506 (2016) 332–339, https://doi.org/10.1016/j.ijpharm.2016.04.069.
[42] Z.-E. Li, Y.-G. Jin, S.-Z. Hu, Y. Liu, M.-H. Duan, S.-H. Li, L.-J. Sun, Fan Yang, Fang Yang, Design, optimization, manufacture and characterization of Milbemycin oxime nanoemulsions, Pharmaceutics 17 (2025) 289, https://doi.org/10.3390/pharmaceutics17030289.
[43] Y. Zhang, Y. Cao, X. Meng, C. Li, H. Wang, S. Zhang, Enhancement of transdermal delivery of artemisinin using microemulsion vehicle based on ionic liquid and lidocaine ibuprofen, Colloids Surf. B: Biointerfaces 189 (2020) 110886, https://doi.org/10.1016/j.colsurfb.2020.110886.
[44] A.M.N. De Toledo, N.C.C. Silva, A.C.K. Sato, C.S.F. Picone, A comprehensive study of physical, antimicrobial and emulsifying properties of self-assembled chitosan/lecithin complexes produced in aqueous media, Future Foods 4 (2021) 100083, https://doi.org/10.1016/j.fufo.2021.100083.
[45] K.V. Jardim, J.L.N. Siqueira, S.N. Báo, M.H. Sousa, A.L. Parize, The role of the lecithin addition in the properties and cytotoxic activity of chitosan and chondroitin sulfate nanoparticles containing curcumin, Carbohydr. Polym. 227 (2020) 115351, https://doi.org/10.1016/j.carbpol.2019.115351.
[46] M. Miastkowska, A. Kulawik-Pióro, M. Szczurek, Nanoemulsion gel formulation optimization for burn wounds: analysis of rheological and sensory properties, Processes 8 (2020) 1416, https://doi.org/10.3390/pr8111416.
[47] I. Benalaya, G. Alves, J. Lopes, L.R. Silva, A review of natural polysaccharides: sources, characteristics, properties, food, and pharmaceutical applications, Int. J. Mol. Sci. 25 (2024) 1322, https://doi.org/10.3390/ijms25021322.
[48] B. Van Rooyen, M. De Wit, J. Van Niekerk, Pectin and alginate functional biopolymers: factors influencing structural composition, functional characteristics and biofilm development, Coatings 14 (2024) 987, https://doi.org/10.3390/coatings14080987.
[49] P. Costa, J.M. Sousa Lobo, Modeling and comparison of dissolution profiles, Eur. J. Pharm. Sci. 13 (2001) 123–133, https://doi.org/10.1016/S0928-0987(01)00095-1.
[50] D. Gerstel, C. Jacques-Jamin, A. Schepky, R. Cubberley, J. Eilstein, S. Grégoire, N. Hewitt, M. Klaric, H. Rothe, H. Duplan, Comparison of protocols for measuring cosmetic ingredient distribution in human and pig skin, Toxicol. in Vitro 34 (2016) 153–160, https://doi.org/10.1016/j.tiv.2016.03.012.
[51] M. Ferreira, M. Magalhães, R. Oliveira, J. Sousa-Lobo, I. Almeida, Trends in the use of botanicals in anti-aging cosmetics, Molecules 26 (2021) 3584, https://doi.org/10.3390/molecules26123584.
[52] P. Ciganović, K. Jakimiuk, M. Tomczyk, M. Zovko Končić, Glycerolic licorice extracts as active cosmeceutical ingredients: extraction optimization, chemical characterization, and biological activity, Antioxidants 8 (10) (2019) 445, https://doi.org/10.3390/antiox8100445.
[53] European Medicines Agency. Draft guideline on quality and equivalence of topical products (EMA/CHMP/QWP/770828/2018), 2018. https://www.ema.europa.eu.