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Research Article

An Innovative Natural Eutectic Solvents-Based Emulsion Examined through the Lens of the Safe and Sustainable by Design Framework

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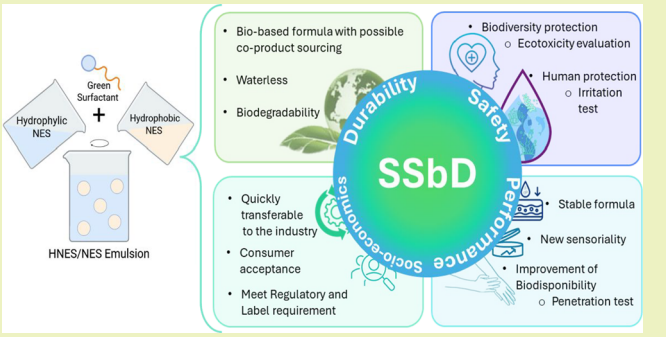
ABSTRACT: Natural eutectic solvents (NESs) are promising alternatives to conventional organic solvents, yet their use as structuring media in emulsified systems remains largely unexplored. This study reports the first fully eutectic solvents-based emulsion, in which both phases are exclusively composed of NESs. The formulation consists of a hydrophilic NES (glycerol/glucose/betaine/water, 4:1:1:11 mol/mol) as the continuous phase and a hydrophobic NES (oleic acid/1,2-octanediol, 5:4 mol/mol) as the dispersed phase, stabilized with a bio-based non-ionic surfactant (lauryl glucoside). Designed using conventional emulsion engineering principles, the system exhibits high physical stability and well-controlled physicochemical and rheological properties. As a functional demonstration, the emulsion significantly enhances the stability and dermal penetration of retinyl palmitate, a model compound highly sensitive to oxidation, photodegradation, and extreme pH. Within a Safe and Sustainable by Design (SSbD) framework, ecotoxicological assessment using *Daphnia magna* revealed no adverse effects on survival or inflammatory pathways. The formulation process is compatible with existing industrial emulsification equipment. This work establishes eutectic solvents as viable and scalable media for sustainable emulsion systems, coupling performance with reduced environmental impact.



KEYWORDS: *natural eutectic solvent, safe and sustainable by design, emulsion, ecotoxicology*

INTRODUCTION

Emulsions are a foundational class of soft matter composed of two immiscible liquid phases, one of which is divided into fine droplets in the other. The generation and the kinetic stability of these dispersed systems are generally guaranteed by the addition of emulsifying agents, mainly surfactants, which lower the interfacial tension and limit the coalescence of the internal phase droplets.[1,2] Emulsions composed of oil and water are particularly exploited in the cosmetic and pharmaceutical sectors.[2] Indeed, the biphasic architecture permits the solubilization of both hydrophilic and hydrophobic compounds of interest while providing levers to tune stability, release, and dermal delivery performance through control of droplet size distribution, interfacial composition, and continuous-phase rheology.[1,3,4] Oil-in-water (o/w) emulsions are the most frequent systems used for cutaneous application as they are easy to spread, bring freshness, and do not leave an oily film residue, allowing immediate dressing.[3]

Because of their widespread use, sustainability has recently shifted from peripheral consideration to a central design criterion for topical emulsions. Looking ahead, future directions for emulsions in cosmetics and dermatology include sustainability-aligned material selection guided by green chemistry metrics and transparent ingredient data in order to reduce persistence, ecotoxicity, and greenhouse-gas intensity while maintaining high functional performance. The EU framework "Safe and Sustainable by Design" (SSbD) offers a holistic point of view, which should be considered before innovation market launches.[5] In SSbD, human health, environmental, and societal impact were considered as early as possible in the innovation process. While nanomaterials[6,7] and energy[8,9] fields have already embraced the SSbD standard, its underutilization in the cosmetic industry persists, despite its potential as a driving force in sustainable innovation.

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Choosing eco-friendly ingredients is a key factor in reducing the environmental impact of emulsions, followed by optimizing production processes and managing water resources.[10] Over the last decade, substantial efforts have been made to develop processes[11] or propose natural solutions to replace synthetic surfactants like natural particles (Pickering emulsions),[12] biopolymers, or proteins.[13] Nevertheless, those solutions are still not ready for rapid industrial spreading. Currently, industries have tended to reduce the use of full synthetic surfactants and to replace them by bio-based surfactants like alkyl glycosides, diligently working on synthesis pathways to minimize the carbon footprint.[14−16] As far as major internal phase ingredients are concerned, petro-derived or partially petrosourced oils (*paraffinum liquidum*, dimethicone) are commonly used as the internal phase of emulsions dedicated to make up, skin care, or therapeutic treatment despite their poor sustainability profile and high carbon footprint. However, these oils are materials of constant composition and rather low cost and well-tolerated. To increase the naturalness of products and meet consumer expectations, mineral oils were partially or completely replaced by various vegetable oils. Although renewable, these oils can come from intensive crops (palm, soy), leading to deforestation, loss of biodiversity, and massive use of pesticides.[17] Their use often involves intercontinental transportation, increasing the cost and the carbon footprint of the final product. Moreover, some stability issues were reported, as some vegetable oils are highly oxidable.[18] Therefore, vegetable oils cannot be the sole substitute resource for the oily phase of emulsions, and ingredients obtained from by-products or plant-independent sources like biotechnologies are required. The ingredients of the external phase in pharmaceutical or cosmetic emulsions are also investigated. Given that the hydrophilic phase in oil-in-water emulsions is typically the largest by volume, low-water or water-minimized emulsions are increasingly gaining attention. Two main strategies are pursued in parallel: developing anhydrous self-emulsifying systems that will emulsify upon application[19] and partially or fully replacing water by polyols, already used as humectants in this type of products.[20−22] While the first strategy seems very specific and difficult to implement rapidly, replacing water by another hydrophilic solvent that meets user expectations seems achievable in the short term.

In this context, the present study proposes bio-based alternatives to traditional emulsion components. Indeed, this study explores a new type of lipophilic/hydrophilic emulsion, based on a hydrophobic eutectic solvent dispersed in a hydrophilic eutectic solvent, both bioinspired. Natural eutectic solvents (NESs) represent a class of alternative solvents that closely align with the principles of green chemistry. A eutectic solvent is defined as a mixture of two or more components that, when combined at a specific molar ratio, exhibits a melting point significantly lower than that of each individual component.[23] Natural eutectic solvents are typically composed of naturally occurring, biodegradable metabolites such as sugars, amino acids, and organic acids, which are derived from renewable resources. Unlike conventional petrochemical-based solvents, NESs exhibit negligible volatility, reducing emissions of volatile organic compounds and improving occupational safety. Their inherent low toxicity and high biodegradability could minimize environmental persistence and mitigate risks of ecological contamination.[24] Furthermore, NESs can be tailored to dissolve a broad spectrum of compounds without reliance on hazardous reagents, enabling more sustainable extraction and formulation processes. The preparation of NESs is straightforward, energy-efficient, and does not generate hazardous waste, which further supports their environmental compatibility. Collectively, these attributes position NESs as a promising solvent system for reducing the carbon footprint and advancing sustainability in chemical and pharmaceutical applications.[25,26]

As a proof of concept, two original oleic acid/1,2-octanediol (5:4, mol/mol) and glycerol/glucose/ betaine/water (4:1:1:11, mol/mol) combinations were selected for their regulatory status and physicochemical properties to form the internal and external phases, respectively. This new type of emulsion was stabilized by a bio-based surfactant and obtained with a very common emulsification process. This new HNES/NES emulsion was evaluated in terms of kinetic stability, *ex vivo* cutaneous drug delivery performance, and *in vitro* applicative properties. Considering their potential industrial deployment, the results were examined using an SSbD approach, specifically focusing on key performance, safety, and sustainability indicators.

MATERIALS AND METHODS

Chemicals

1,2-Octanediol (98%), D-(+)-glucose (99%, anhydrous, powder), glycerol (99%), and betaine (98%, anhydrous) were all obtained from Thermo Scientific Chemical (Waltham, Massachusetts, USA). Oleic acid (extra pure) and propan-2-ol (≥99.5%, HPLC grade) were purchased from Fisher Chemical (Illkirch-Graffenstaden, France). Retinyl palmitate (≥1,700,000 USP units per g, yellow powder) was purchased from Sigma-Aldrich (St. Quentin Fallavier, France). HyClone Dulbecco's Phosphate Buffered Saline (D-PBS) was purchased from Cytiva (Marlborough, MA, USA). Eumulgin VL75, a surfactant composed of lauryl glucoside, polyglyceryl-2 dipolyhydroxystearate, and glycerin, was kindly provided by AMI Ingredients (Tauxigny, France). For the ecotoxicity assays, the following reagents were purchased from Sigma-Aldrich (Germany): phosphate buffered saline (PBS, pH = 7.4), phosphate buffer (pH 8.0), Bradford reagent, sodium phosphate buffer, 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), xanthine, nitroblue tetrazolium (NBT), xanthine oxidase (XOD), hydrogen peroxide (H_2O_2), potassium phosphate buffer (pH 7.0 at 25 °C), acetylthiocholine iodide (ACTI), 5,5-dithio-bis-2-nitrobenzoïc acid (DTNB, Ellman Reagent), sodium dodecyl sulfate (SDS), tris chlorohydrate (Tris-HCl), nicotinamide adenine di-nucleotide (NADH), P-nitrophenyl phosphate (pNPP), sodium pyruvate, magnesium chloride ($MgCl_2$), and diethanolamine. Potassium periodate, methanol, trichloroacetic acid (TCA), thiobarbituric acid (TBA), and purpald were purchased from Merck (USA). Bovine albumin serum (BSA, Fraction V) was purchased from Nzytech (Portugal) and acid ethylenediaminetetraacetic (EDTA) from Riedel-Haen (Germany).

Reference products for NES sensorial comparison were procured from Olvea (France) for the apricot oil, Aromazone (France) for the jojoba oil, Stéarinerie Dubois (France) for the pentaerythrityl tetraethyhexanoate (Trade name: DUB PTO), and Wacker (Germany) for dimethicone (Trade Name: Belsil DM1 Plus). The commercial products used to compare the HNES/NES emulsion are detailed in Supporting Information 5 (Table S1).

Eutectic Solvent Preparation

NES glycerol/glucose/betaine/water (4:1:1:11, mol/mol) (GGBW)[27] and HNES oleic acid/1,2-octanediol (5:4, mol/mol) (OaO) were prepared by mixing the components together. The GGBW mixture was heated at 80 °C and magnetically stirred for 3 h until a colorless liquid was obtained. The OaO mixture was heated at 50 °C and magnetically stirred for 6 h until a colorless liquid was obtained. After returning to

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room temperature, eutectic solvents were stored for 24 h at 25 °C/60% humidity in an oven before being used for formulation. However, they can be stored for over a month in these conditions without noticeable changes.

Eutectic Solvent Characterization

Viscosity. Viscosity was measured using a Kinexus pro + rheometer (Netzsch, Selb, Germany) with a sandblasted Couette geometry (25 mm diameter, stainless steel). Once loaded, samples were allowed to rest for 5 min prior to measurement. Apparent viscosity values were recorded by subjecting the samples to increasing shear rates ranging from 0.1 to 1000 s^{-1} for a duration of 5 min with 25 points per decades. Results were expressed as the mean ± SD of three replicate measurements.

Density. Density values were determined with a DMA 501 instrument (Anton Paar, Les Ulis, France) at 25 °C. Results were expressed as the mean ± SD of three replicate measurements.

Surface and Interfacial Tension Measurements. Surface and interfacial tensions were measured using a KRÜSS DSA30 drop shape analyzer (KRÜSS GmbH, Germany) at room temperature (19.5 ± 0.5 °C). Droplets were formed at the tip of a stainless-steel needle (1.83 mm diameter) and analyzed using the Young–Laplace equation, accounting for liquid density at the measurement temperature. For interfacial tension, droplets were generated in an immiscible second phase. The needle and syringe were cleaned with ethanol and ultrapure water before each measurement. Water surface tension was routinely checked for verification. Reported values are the mean of at least three independent measurements, with standard deviations indicating experimental uncertainty.

HNES/NES Emulsion Preparation

The emulsions were prepared in batches of 100 g in a 150 mL glass beaker using an Ultraturrax T25 homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany) equipped with an S 25 KV-25G dispersing tool. According to Table 1, appropriate amounts of Eumulgin VL75 (INCI: lauryl glucoside (and) polyglyceryl-2 dipolyhydroxystearate (and) glycerin) and HNES were stirred at 4000 rpm for 4 min. Subsequently, hydrophilic NES was added, and emulsification was carried out at 12,000 rpm for 5 min. For formulations containing retinyl palmitate, the HNES and retinyl palmitate were stirred with a magnetic stirrer for 2 h in the dark prior to the addition of the surfactant. Each emulsion was prepared in triplicate, and each sample was distributed into separate containers for storage at 3 different temperatures: 4 °C, 25 °C/60% humidity, and 40 °C/75% humidity.

According to the 2024 amendment to the European cosmetic regulation, the maximum allowed concentration is 0.3 wt % equivalent retinol in both leave-on and rinse-off products, corresponding to approximately 1 wt % retinyl palmitate. Therefore, 1 wt % was incorporated into the formulation.

Emulsion Physicochemical Characterization

pH. The pH of a sample made of 10 wt % of emulsion diluted in distilled water was taken at 20 °C on days 1, 7, and 28 for the emulsions for each storage condition (4 °C, 25 °C/60% humidity and 40 °C/75% humidity). Results were expressed as the mean ± SD of the measurements from three replicates of each emulsion.

Rheology Measurement (Shear Test). Rheological characterization was performed at 25 °C using a Kinexus pro+ rheometer (Netzsch, Selb, Germany). To evaluate emulsion flow properties, continuous shear tests were conducted using sanded plate–plate geometry (50 mm, stainless steel). Measurements were done in triplicate with fresh samples loaded for each run.[28] An acclimation of 2 min was allowed before testing. Apparent viscosity values were recorded over 1200 s under increasing shear rates from 0.01 to 1000 s^{-1} with 25 points per decades, providing insights into product stability, user comfort, and regulatory compliance. The obtained data was fitted to the Newtonian model by rSpace software (Netzsch, Selb, Germany). Results were expressed as the mean ± SD of the measurements from three replicates of each emulsion.

DLS Measurement. Droplet size measurement was performed using a Malvern NanoZS instrument (Malvern Instruments, Malvern, UK). The formulation was analyzed without dilution. The measurements were done in triplicate on 3 independent batches of each emulsion at days 1, 7, and 28 for each storage condition.

Water Activity. Water activity (a_w) was measured using a LabSwift-a_w device (Novasina AG, Lachen, Switzerland). Each sample cup was filled to two-thirds of its volume with the sample and analyzed for 4 min, after which a_w and temperature values were recorded. Results from three emulsions without retinyl palmitate stored at 4, 25 °C/60% humidity and 40 °C/75% humidity were compared after 1, 7, and 28 days of storage.

Biological Evaluation

Skin Irritation Test. Both eutectic solvents' safety was assessed using reconstructed human epidermis (RHE, Episkin), following the in vitro skin irritation protocol[29,30] in line with the OECD Test Guideline 439. Tissues were equilibrated overnight, then exposed for 42 min to 16 μL of each eutectic solvent (triplicates), with D-PBS and 5% SLS as negative and positive controls. After rinsing, the tissues were incubated 42 h before MTT staining (1 mg/mL, 3 h). Formazan was extracted in isopropanol (70 h, 4 °C), and OD was measured at 570 nm. Tissue viability (%) was calculated as:

$$\%\ \text{Viability} = \left[\frac{(\text{Mean(OD sample for the 2well/tissue)} - \text{ODblank mean})}{\text{ODnegative control mean}}\right] \times 100 \quad (1)$$

The results are expressed as mean ± SD for the 3 tissues.

According to OECD Test Guideline 439,[31] an ingredient is considered irritant if viability is ≤ 50%. All procedures were conducted under sterile conditions.

Permeation and Retention Test. *Human Skin Permeation and Retention Experiment.* Four defrosted dermatomed human skin discs (20 mm diameter, ∼500 μm thickness, prototype III) were used for permeation experiments (Alphenyx, Marseille, France). Skin was collected during abdominal skin surgery in compliance with French law L.1445 CSP.

Skin permeation studies were performed using the Franz diffusion cell device (Permagear Inc., Hellertown, USA) with a diffusion area of 0.64 cm^2 and a receptor volume of 5 mL. Human skin biopsies were thawed overnight at 4 °C and then mounted on the cells. Receptor compartments were filled with degassed PBS (pH 7.4), maintained at 37 °C, and stirred continuously. Before application, skin samples were equilibrated with PBS for 30 min, and transepidermal water loss was measured (VapoMeter, Delfin Technologies) to assess barrier integrity.[11] A sample of 700 mg of the Ret-HNES/NES emulsion was placed

Table 1. Composition of the Emulsions

wt %	HNES/NES emulsion	ret-HNES/NES emulsion
retinyl palmitate	0	1
NES GGBW	70	70
HNES OaO	25	24
surfactant: Eumulgin VL75	5	5

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in the donor compartment (10 mg/cm^2 of retinyl palmitate, allowing a continuous flow). Samples of 200 μL of receptor solutions were collected after 4 and 8 h of incubation. The emulsions were then collected from the donor compartment. Skin samples were swabbed with cotton tips, and the *Stratum Corneum* (SC) was removed by the tape-stripping method.[11] The viable epidermis and dermis were separated by heat treatment in water at 60 °C for 1 min. Retinyl palmitate was extracted from the separated layers with 1 mL of isopropanol overnight at 4 °C then analyzed by HPLC.

HPLC Measurement. HPLC was used to determine retinyl palmitate in the emulsions and in the skin extracts. Analyses were conducted using an Ultimate 3000 system (Thermo Fisher Scientific, Voisins-le-Bretonneux, France) controlled by Chromeleon 7.1 software. Separation was achieved on a 20 μL injection volume on a C18 column (4.6 × 150 mm, 5 μm; Interchim) maintained at 25 °C. Retinyl palmitate was detected at 325 nm using an isocratic mobile phase of isopropanol: water (90:10, v/v) at a flow rate of 1 mL·min^{-1}, with a run time of 10 min per sample. Skin extracts and receptor fluids were injected without further preparation. For each emulsion, 100 mg was dissolved in 20 mL of isopropanol. Retinyl palmitate concentrations were determined using a calibration curve. Corresponding retention time was 7.949 ± 0.015 min. Results were expressed as mean ± SD.

Ecotoxicity. *Experimental Setup.* To assess the ecotoxicity of the HNES/NES emulsions, assays were performed using the freshwater crustacea *Daphnia magna*. Three HNES/NES concentrations (10, 50, and 100 mg/L) were tested, along with a control group (no treatment). For each condition, two independent batches were prepared, each containing 20 daphnids (Figure S1 Supporting Information 1).

The exposure lasted 7 days, with an intermediate sampling at day 4. At this point, for each batch, two samples of 3 to 5 daphnids (depending on the survival at each tested concentration) were withdrawed and placed in 1.5 mL microtubes. On day 7, for each concentration the two batches were re-united and the remaining organisms were dispatched in 3 supplementary microtubes. In parallel, for each concentration, all juveniles produced during the test were counted and pooled into a single tube for later analyses.

Mortality and Reproduction. Mortality was calculated after 4 and 7 days of exposure. Reproduction rates (%) were determined on day 7. Biomarker analyses were subsequently performed on both adult and *D. magna* juveniles.

Biomarker Assays. After the exposure period, the specimens were frozen at −20 °C until analysis. Following thawing, the organisms were homogenized in 500 μL of PBS for 2 min to ensure extraction and centrifuged at 15,000 × *g* for 10 min. The resulting homogenates were used for enzymatic assays or stored at −20 °C for later analyses.

Total Protein Content (Bradford Assay). Protein content was quantified prior to biomarker analysis to normalize enzyme activities, following the Bradford assay.[32] Bovine serum albumin (BSA) was used as the standard. In a 96-well plate (Greiner Bio-One, Austria), 180 μL of the Bradford reagent was added to each well, followed by 20 μL of either standard or sample (in duplicate). Absorbance was read at 595 nm using a microplate reader (Synergy HTX, BioTek, USA), and protein concentrations were calculated from a BSA calibration curve, ranging from 0 to 4 mg/mL.

Lipid Peroxidation (TBARS Assay). Lipid peroxidation was assessed via quantification of malondialdehyde (MDA) using the thiobarbituric acid reactive substance (TBARS) method, adapted from Uchiyama and Mihara.[33]

Enzyme Activity Measurements. AChE activities were determined using Ellman's colorimetric method,[34] which measures thiol group formation. Activities (nmol/min/mL) were calculated from the slope using ε = 0.00781 μM^{-1} cm^{-1} and normalized to total protein.

Catalase activities were quantified via formaldehyde formation, using a method adapted from Johansson and Borg.[35] Catalase activities were normalized to protein content.

Super oxide dismutase (SOD) activity was determined by measuring inhibition of NBT-diformazan formation, following the method adapted from Sun et al.[36] SOD activity was expressed as the percentage inhibition relative to the negative control (presented as % of inhibition) and normalized to protein content.

GST activity, a detoxification biomarker, was determined according to the technique initially described by Habig et al.,[37] through conjugation of reduced glutathione (GSH) with CDNB. Enzyme activities were calculated from the absorbance slope, corrected for blanks, and normalized to protein content.

MDA concentration was calculated from a standard curve (0–0.1 μM) and normalized to protein content.

Acid phosphatase activity, a lysosomal integrity marker, was measured using p-nitrophenyl phosphate as the substrate, following Bergmeyer et al.'s[38] method. Activity was calculated from a p-nitrophenol standard curve and normalized to protein content.

Alkaline phosphatase (ALP) activity was assessed via hydrolysis of p-nitrophenyl phosphate (pNPP) to p-nitrophenol, following Walter et al.'s[38] method. Activity (U/mL) was calculated using ε = 8.36 mM^{-1} cm^{-1} and normalized to total protein.

Lactate dehydrogenase (LDH) activity, a general cytotoxicity marker, was determined following a method adapted from Legrand et al.,[39] by monitoring NADH oxidation at 340 nm. Activity (U/mg) was calculated using ε = 3.42 mM^{-1} cm^{-1} and normalized to protein content.

For detailed information on enzyme activity, see the Materials and Methods section in Supporting Information 1.

In Vitro Applicative Properties

Linear Spreading. Linear spreading behavior was assessed using a TA.XT Plus texture analyzer (Stable Micro Systems, UK) operated with the Texture Exponent software. The friction rig (A/FR) was fitted with an HD6 Helioplate poly(methyl methacrylate) (PMMA) plate (Helioscreen, France) and a polypropylene sheet secured to the horizontal testing platform. Polypropylene was selected as a substrate with a surface energy (39 mN/m) comparable to that of human skin.[40] A volume of 200 μL of the product was evenly applied onto the polypropylene surface, and the PMMA plate was placed on top.[41] The assay was performed in triplicate at a constant speed of 2 mm/s to record the force required to spread the product over 120 mm.[41]

Stickiness. The texture analyzer (TA.XT Plus Stable Micro Systems, UK) was configured with a P/025 stainless-steel spherical probe and a 500 g load cell, as reported by Eudier et al.[42] A 35 μL aliquot of each sample was deposited with a micropipette onto a 9.6 cm^2 circular zone of a Biody Plate #30 and distributed uniformly by performing ten controlled rotational movements at 90 rpm. The films were allowed to equilibrate for 3 min before testing. During the measurement, a force–time profile was recorded and analyzed using Texture Exponent software. Stickiness was quantified as the negative area under the withdrawal curve (g·s), corresponding to the mechanical work required to detach the probe from the emulsion-treated surface. Higher values indicate stronger adhesive interactions between the formulation and the probe and therefore greater perceived tackiness.

Glossiness After Application. A 30 μL aliquot of the product was dispensed onto a 4 cm diameter circular area of the Biody Plate #30 and spread uniformly by performing 20 circular movements at 90 rpm. Five minutes after application, a glossimeter (Courage-Khazaka electronic GmbH MPA580, Germany) was used to monitor changes in surface light reflectance over time.

Contact Angle. A 13 μL volume of the product was dispensed onto a 4.9-cm-diameter circular zone of the Biody Plate #30 and uniformly distributed through ten rotational movements at 90 rpm. After 3 min of equilibration, a droplet of distilled water was deposited onto the treated area, and the contact angle of the residual film was determined using a DSA 30 drop shape analyzer (Krüss GmbH, Germany). The angle formed at the interface between the droplet and the product film was recorded. Each experiment was performed in triplicate.

Statistical Analysis

Ecotoxicity and Physicochemical Studies. Differences in responses were evaluated using one-way ANOVA. When significant

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effects were detected (p-value < 0.05), Tukey's HSD test was applied for post-hoc pairwise comparisons. All statistical analyses were performed using 2025 JMP (Student Edition, JMP Statistical Discovery LLC, Cary, NC.).

In Vitro Applicative Properties. Statistical analyses were performed using XLSTAT software (Version 2016.02.27444, Addinsoft, France). To assess differences between products, a one-way ANOVA was applied to the dataset. When significant differences were observed ($p < 0.01$), Tukey's post-hoc test was used to identify distinct product groupings. Furthermore, principal component analysis (PCA) was conducted to explore variable correlations and to identify products exhibiting notable deviations from the rest. Linear relationships among variables were evaluated through the Pearson correlation test.

RESULTS

Eutectic Solvent Selection and Characterization

Eutectic Solvent Design and Selection. Eutectic solvents were selected and designed according to the Smart Selection Strategy described by Yagmur et al.[43] Different filters were applied to select relevant raw materials, namely : compliance with cosmetic regulations (European, Chinese), available as bio-sourced, and physicochemical properties (logP). GGBW (4:1:1:11, mol/mol) was previously described as a relevant NES with good physicochemical properties and interesting from a sensory perspective.[44]

Regarding HNESs, most mixtures described in the literature are based on short-chain fatty acids (C8 to C12) known for their irritation risk. To overcome this issue, several non-polar raw materials compliant with cosmetic regulations were selected: fatty alcohols, long-chain alkane diols, long-chain fatty acids (saturated and non-saturated), and non-polar esters. All mixtures were screened using σ-scoring and COSMOTherm solid/liquid equilibrium according to the methodology described by Yagmur et al.[43] Among all, HNES OaO (5:4, mol/mol) exhibited a typical solid liquid equilibrium (SLE) with depression of the melting point (273 K) at the eutectic ratio (Supporting Information 2 Figure S2).

Physicochemical Characterization. Both GBBW (4:1:1:11, mol/mol)[27] and OaO (5:4, mol/mol) are transparent liquids at ambient temperature. Their eutectic nature was characterized by thermogravimetry and differential scanning calorimetry (DSC) analyses (See Supporting Information 3 and 4, Figures S3 and S4).

As expected, some physicochemical properties of GGBW reflect those of glycerol, its main component (43 wt %). Glycerol has a density of 1.26,[45] which is very close to that measured for GGBW, 1.24 (Table 2). However, the viscosity of GGBW is 0.082 Pa·s, which is almost ten times lower than that of pure glycerol (0.940 Pa·s at 25 °C),[46] which promotes physical stability.[47]

Regarding OaO, its density of 0.90 (Table 2) falls within the range typically reported for oils used in pharmaceutical and cosmetic formulations[48] between 0.85 and 0.91, and its viscosity (0.043 Pa·s) is comparable to vegetable oils,[49] such as walnut oil (0.0429 Pa·s). Low viscosity or medium viscosity[50] below 0.100 Pa·s usually supports emulsification by facilitating droplet disruption and dispersion. Nevertheless, the density of GGBW remains higher than that of water, resulting in a larger density difference than in conventional oil-in-water emulsions. Such a gap may, in classical systems, enhance creaming, a gravitational destabilization phenomenon.[51]

Table 2. Physicochemical Characteristics of the Both Eutectic Solvents at 25 °C

	viscosity (Pa·s)	density	surface tension ($mN \cdot m^{-1}$)
GGBW	0.082 ± 0.004	1.236 ± 0.001	62.9 ± 0.2
OaO	0.043 ± 0.003	0.903 ± 0.001	28.4 ± 0.4

The surface tension measurements of the eutectic solvents were determined by the drop shape method as well as the corresponding interfacial tension measurement between them. The surface tension of GGBW was measured at 62.9 ± 0.2 $mN \cdot m^{-1}$. This elevated surface tension is consistent with mixtures containing a significant water fraction, as water's strong hydrogen bonding markedly increases surface tension compared to most non-polar oils. In contrast, the surface tension of OaO was measured at 28.4 ± 0.4 $mN \cdot m^{-1}$, a value that lies in the range typically observed for hydrophobic oils and non-polar organic liquids with weak intermolecular cohesion at the air-liquid interface. The surface tension of OaO is comparable to that reported for medium-chain triglycerides, which typically exhibit surface tension values in the range of approximately 28–33 $mN \cdot m^{-1}$.[52] In contrast, the interfacial tension between OaO and GGBW was measured and presents a notably low value of 1.5 ± 0.2 $mN \cdot m^{-1}$. Indeed, interfacial tension between oils and water is generally of several tens of $mN \cdot m^{-1}$. Moreover, the interfacial tension between OaO and GGBW reaches equilibrium almost instantaneously, indicating a low dynamic interfacial tension as well, with rapid molecular rearrangement and strong interfacial affinity between the two phases. Low interfacial tension is generally in favor of easy emulsification and small droplet size with low coalescence risks.[53] Given the physicochemical properties of the eutectic phases, it is anticipated that small and stable droplets form if the appropriate ratio of internal and external phases is achieved when emulsifying both eutectic solvents with typical homogenizers equipped with the rotor–stator mechanism. Moreover, low coalescence is expected when surfactants are added to help stabilize the system.

Irritation Test. The skin irritation test performed on the RHE showed high cell viability for both NESs, 96.7 ± 9.7% for OaO and 96.7± 4.4% for GGBW (Figure 1), largely exceeding the 50% viability threshold defined by OECD Test Guideline 439 for non-irritant substances. The positive control shows cell viability of 0.7%, validating the experiment. These results indicate that neither the NES nor the HNES induces significant cytotoxic effects on the reconstructed human epidermis. Consequently, both formulations can be classified as non-irritant and are considered safe for topical applications.

In Vitro Application Properties of Eutectic Solvents. A principal component analysis (PCA) (Supporting Information 5 Figure S5) was carried out to explore the sensory pattern of OaO and GGBW. OaO displays the high glow typical of vegetable oils while being less sticky, whereas GGBW aligns closely with glycerin despite being ten times less viscous, supporting the physicochemical trends previously observed for NESs.[44] No spontaneous spreading was observed for the NES, indicating that particular attention should be paid to the formulation of HNES/NES emulsions. However, the spreading forces required were not significantly different between the two eutectic solvents and the different oils tested (Supporting Information 5 Table S2).

HNES/NES Emulsion Characterization

HNES/NES emulsions integrating 25 wt % of OaO and 70 wt % of GGBW were prepared with or without retinyl palmitate

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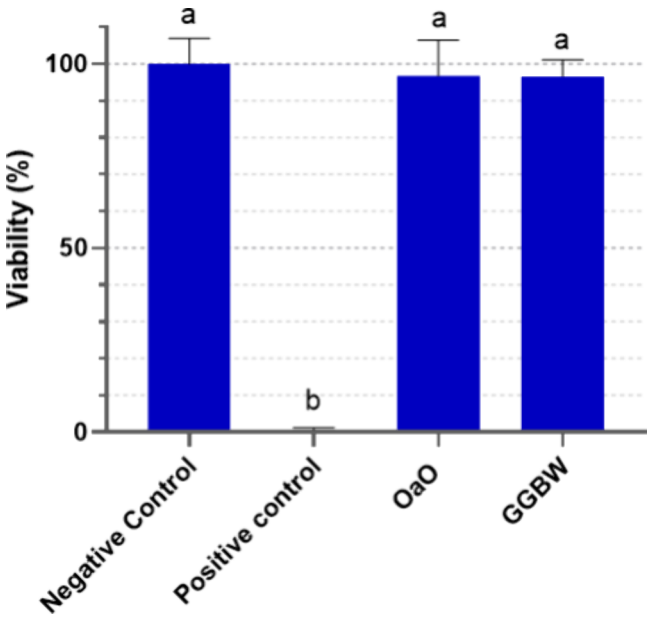

Figure 1. Result of the skin irritation test expressed in % of cell viability as mean ± SD. Different letters (a, b) indicate statistically significant differences according to Tukey's test ($p < 0.05$). (ANOVA $R^2 = 0.989$; p-value = <0.0001).

(1 wt %) using a common rotor–stator homogenizer in presence of the COSMOS-certified surfactant Eumulgin VL75.

Physical Stability. Just after preparation, HNES/NES emulsions were white and fluid, and Ret-HNES/NES emulsions were yellowish and fluid (Figure 2A,B). After 28 days, no sign of physical destabilization, creaming or phase separation, appeared for any emulsion in the tested conditions (Figure 2C,D). As expected, due to the very similar refractive indices of the two phases (GGBW = 1.449; OaO = 1.457), these emulsions gradually became translucent after the migration of the surfactant molecules from the HNES to the interface and the elimination of fine air bubbles resulting from the high shear emulsification process. Such translucent emulsions were already observed by Freitas et al. who prepared oil-in-NES emulsions.[54]

Retinyl Palmitate Stability. Emulsion containing retinyl palmitate exhibited a progressive bright yellow coloration when stored at 25 and 40 °C. This color change was reported as a sign of the degradation of retinyl palmitate when stored under non-refrigerated conditions.[55] Stability of retinyl palmitate in the emulsion was determined by HPLC. Two days after preparation, the emulsion stored at 4 °C showed a concentration of 1.04 ± 0.004%, with no detectable degradation at this temperature. Slight degradation was observed in samples stored at 25 and 40 °C, corresponding to 3 and 4% loss, respectively (Figure 3). After 28 days, the emulsion stored at 4 °C retained about 89.3% of its initial retinyl palmitate, whereas storage at 25 and 40 °C led to significant degradation, approximately 40.1 and 96.2% loss, respectively. This behavior is consistent with the known thermal and oxidative instability of retinyl palmitate, thus requiring storage at low temperature and protection from light. Retinyl palmitate is known to undergo degradation through hydrolysis, yielding retinol and palmitic acid, or through photodegradation, generating isomers and reactive oxygen species (ROS). Such degradation has been reported at moderate

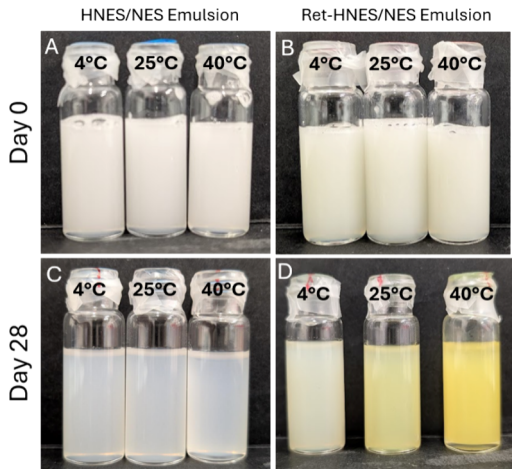

Figure 2. Picture of the HNES/NES emulsion with or without retinyl palmitate right after emulsification and after 28 days of storage at different temperatures (left to right: 4 °C; 25 and 40 °C). (A) HNES/NES emulsion at day 0. (B) Ret-HNES/NES emulsion at day 0. (C) HNES/NES emulsion after 28 days. (D) Ret-HNES/NES emulsion after 28 days.

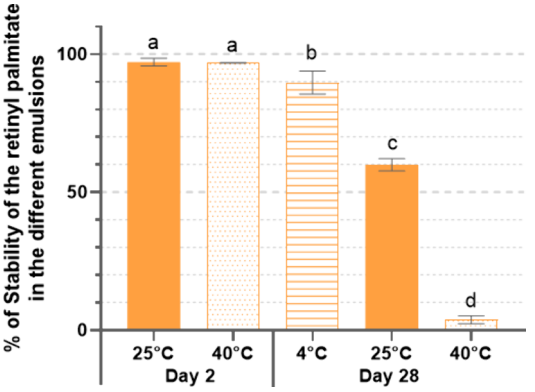

Figure 3. Percentage of stability of retinyl palmitate in the emulsions at different storage conditions compared to the quantity of retinyl palmitate in the cream stored at 4 °C, 2 days after preparation (mean ± SD). Different letters (a–d) indicate statistically significant differences according to Tukey's test ($p < 0.05$). (ANOVA $R^2 = 0.996$; p-value = <0.0001).

ambient temperatures, as described by Pignitter et al.,[56] and is further accelerated at higher temperatures, as shown by Malau et al.[57] However, Carlotti et al.[58] demonstrated that in oil-in-water emulsions, retinyl palmitate is intrinsically more stable than retinol, even though oxidative pathways still occur, highlighting the need for antioxidants. Notably, no antioxidant was included in the HNES/NES emulsion presented here, which makes the observed stability profile particularly noteworthy.

pH Monitoring. The pH values ranged from 5.1 to 5.5 on Day 1 and remained stable over time, staying between 5.0 and 5.7 (Supporting Information 6 Figure S7), at all storage temperatures and time points (Day 7 and 28). No significant variations were observed over time, indicating good pH stability. The physiological pH of the skin ranges broadly from 4.0 to 7.0, with most guidelines recommending that topical formulations maintain a pH between 5.0 and 5.5 to preserve the skin barrier and

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avoid irritation.[59] The emulsions therefore fall within the acceptable dermatological range, supporting their suitability for topical application.

Droplet Size. Dynamic light scattering (DLS) measurements showed that emulsion droplets size ranged between 400 and 620 nm for both types of emulsions (Figure 4). For HNES/NES blank emulsion, droplet size appears slightly larger, even if not significant, for the HNES/NES emulsion stored at 25 °C and moreover 40 °C. However, droplets remain within the submicrometric range. For Ret-HNES/NES, mean droplet size at day 1 is similar to the emulsion without retinyl palmitate but shows larger standard deviations. Corresponding droplet polydispersity index (PDI) values were relatively high (>0.3). However, since the emulsions are not nanoemulsions and the refractive indices of the two phases are close, PDI values should be interpreted with caution. These results mainly indicate a heterogeneous droplet size distribution, with mean droplet sizes presented in Figure 4. Particular attention should therefore be paid to stability monitoring. Nevertheless, based on the results obtained after 28 days of storage, no visible destabilization was observed. Overall, droplet size remains small considering the preparation method with a rotor–stator homogenizer. For comparison Egner et al. observed droplets more than two times larger with a similar protocol applied to oil-in-water emulsions containing only 5% of the internal phase.[60] Such low droplet size, already observed for NES-based emulsions,[61] is probably due to the low interfacial tension between the phases major components. Such a fine dispersion likely contributes to the high physical stability observed throughout the storage period. Indeed, it is well established that emulsions with smaller droplet diameters tend to be more resistant to destabilization phenomena such as coalescence or creaming.[47,62]

Rheology. Rheological analyses showed that emulsions mainly behave as Newtonian fluids, with viscosity remaining constant within the applied shear rate range. Measured viscosities ranged from 0.15 to 0.25 Pa·s (Figure 5), values consistent with oil-in-water emulsions formulated without any thickening agent using this type of the surfactant.[63] The rheological behavior of the HNES/NES emulsion remained stable over 28 days, regardless of storage temperature.

At Day 1, no significant difference was observed between the HNES/NES emulsion and the Ret-HNES/NES emulsion, with a viscosity of, respectively, 0.196 and 0.178 Pa·s (Table 3). However, after 28 days, the retinyl palmitate emulsion exhibited a slight decrease in viscosity at 25 and 40 °C (only significant at 40 °C), with a viscosity, respectively, of 0.160 and 0.116 Pa·s, likely associated with the well-known thermal instability of retinyl palmitate when not stored under refrigerated conditions.

Water Activity. Water activity was measured at day 1 at 25 °C and again after 7 and 28 days under the different storage conditions (4, 25, 40 °C). All values ranged between 0.52 and 0.56 (Figure 6). According to European Pharmacopoeia challenge test criteria,[64] the lowest water activity value that still allows growth of one of the reference microorganism (*A. brasiliensis*) is 0.77. Furthermore, the lowest a_w reported in the literature to allow microbial development corresponds to the xerophilic fungus *Xeromyces bisporus*, which can grow at a minimum a_w of 0.61.[65]

Given that the emulsions maintained a water activity below 0.60 after 28 days under all storage conditions, microbial

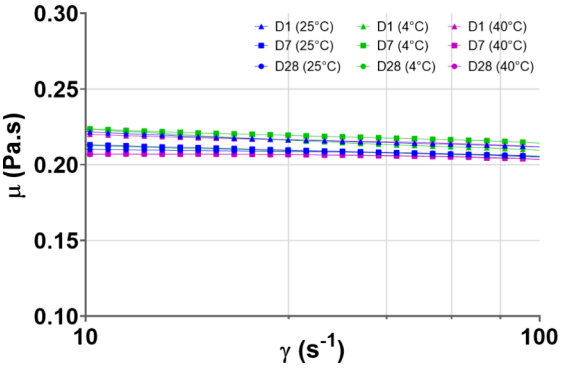

Figure 5. Shear flow of the HNES/NES emulsion at days 1, 7, and 28 under different storage conditions. Blue: emulsion stored at 25 °C, green: emulsion stored at 4 °C, purple: emulsion stored at 40 °C; triangle for the emulsion at day 1; square for the emulsion at day 7; and circle for the emulsion at day 28.

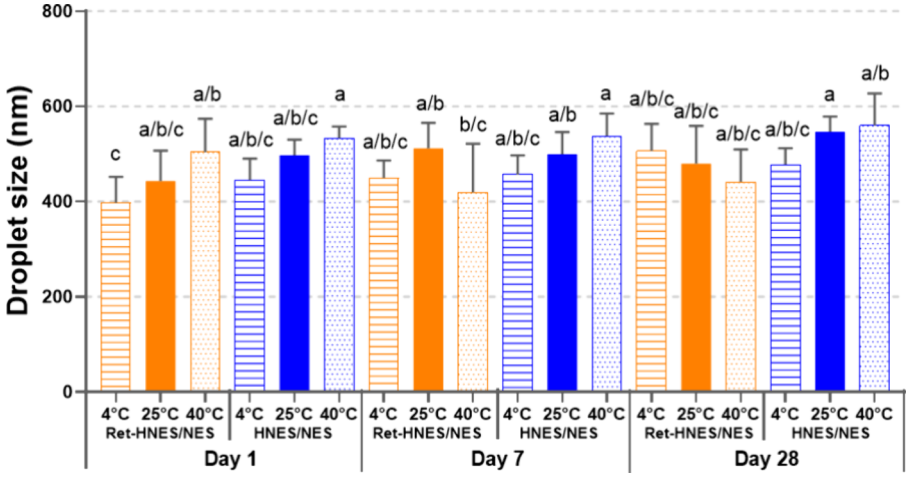

Figure 4. Droplet size measurement of the HNES/NES emulsion (in blue) and Ret-HNES/NES emulsion (in orange) on day 1, 7, and 28 under the different storage conditions expressed as mean ± SD. Non-shared letters indicate statistically significant differences according to Tukey's test ($p < 0.05$). (ANOVA $R^2 = 0.398$; p-value = 0.260).

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Table 3. Comparison of Viscosity Values (in Pa·s) Obtained Using the Newtonian Model Fit, along with the Corresponding Correlation Coefficients, for the HNES/NES and Ret-HNES/NES Emulsions (Mean ± SD[a])

		HNES/NES emulsion		ret-HNES/NES emulsion	
storage temperature (°C)	days	μ (Pa·s)	correlation coefficient	μ (Pa·s)	correlation coefficient
4	1	0.201[a] ± 0.017	0.9992	0.184[a] ± 0.014	0.9992
	7	0.205[a] ± 0.019	0.9991	0.189[a] ± 0.003	0.9989
	28	0.189[a] ± 0.004	0.9991	0.178[a] ± 0.002	0.9990
25	1	0.196[a] ± 0.007	0.9993	0.178[a] ± 0.019	0.9991
	7	0.192[a] ± 0.013	0.9993	0.177[a] ± 0.013	0.9990
	28	0.188[a] ± 0.002	0.9990	0.160[a] ± 0.010	0.9987
40	1	0.191[a] ± 0.007	0.9987	0.176[a] ± 0.004	0.9991
	7	0.191[a] ± 0.011	0.9993	0.175[a] ± 0.002	0.9986
	28	0.187[a] ± 0.013	0.9991	0.116[b] ± 0.025	0.9976

[a]Different letters (a, b) indicate statistically significant differences according to Tukey's test ($p < 0.05$). (ANOVA $R^2 = 0.83$; $p < 0.0001$).

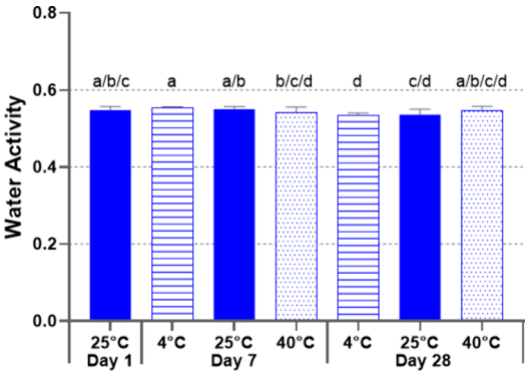

Figure 6. Water activity measured at day 1 at 25 °C in comparison with days 7 and 28 at the different storage conditions expressed as mean ± SD. Non-shared letters indicate statistically significant differences according to Tukey's test ($p < 0.05$). (ANOVA $R^2 = 0.348$; ip-value = 0.0149).

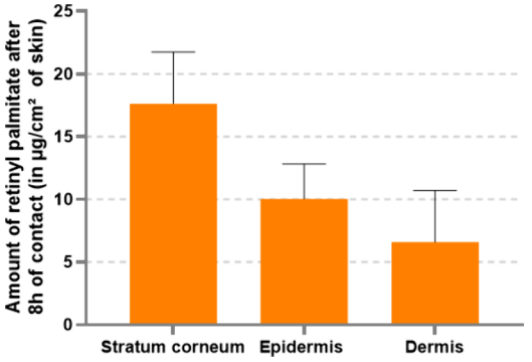

Figure 7. Amount of retinyl palmitate in the different layers of the skin after 8 h of contact, expressed as mean ± SD.

proliferation is highly unlikely. The stability of water activity over time in samples stored at 40 °C and 75% relative humidity suggests that the formulation does not exhibit significant hygroscopic behavior.

Permeation Results. Results of the permeation test after 8 h of exposure are presented in Figure 7. Reported values indicate that 17.6 μg/cm^2 of retinyl palmitate accumulated in the *stratum corneum*, 10.0 μg/cm^2 in the viable epidermis, and about 6.6 μg/cm^2 in the dermis. Overall, this corresponds to a total accumulation of approximately 34.2 μg of retinyl palmitate/cm^2 across the entire skin. Retinyl palmitate, a lipophilic derivative of retinol, tends to preferentially accumulate in the *stratum corneum*, which is composed mainly of corneocytes that are non-viable keratinized cells that favor the retention of lipophilic compounds. Ret-HNES/NES demonstrates a remarkable enhancement in retinyl palmitate delivery compared with previously reported systems. Duell et al. used a 95% ethanol solution loaded at 0.6%, which yielded less than 25 ng/mg of skin, corresponding to roughly 5 μg/cm^2 of human skin after 72 h of exposure.[66] Bjerke et al. reported a penetration of only 0.11 μg/cm^2 after 24 h of exposure to an oil-in-water emulsion containing 0.55% retinyl palmitate.[67] Frelichowska et al. reported that 0.3 μg/cm^2 of retinol was detected in pig skin after 24 h, following application of an o/w emulsion containing 0.1% retinol (0.4 mg/cm^2 applied). To enable comparison with the NES penetration test (10 mg/cm^2 applied), this value was extrapolated to a theoretical maximum of 7.5 μg/cm^2.[68] Given its lower molecular weight, retinol is expected to penetrate the skin more easily than retinyl palmitate. Moreover, unlike most published studies, which report little to no diffusion of retinyl palmitate into deeper skin layers,[69] the NES-based emulsion demonstrates penetration extending down to the dermis, highlighting the strong dermal bioavailability of our system.

***In Vitro* Applicative Properties of the HNES/NES Emulsion.** HNES/NES emulsion properties were investigated *in vitro* on artificial skin and compared with those of two pharmaceutical emulsions (Bepanthen Sensicalm and Dexeryl) and two cosmetic serums (Mixa "Anti-imperfection" and Avène "Hyaluron Activ B3").

Analysis of the spreading behavior was performed with a texturometer on a non-biological skin model (Biody) (Supporting Information 5 Figure S6). The PCA revealed that the HNES/NES emulsion was positioned in the same region of the score plot as the commercial references Avène Serum and Mixa Serum, indicating comparable spreading-related characteristics (Supporting Information 5 Figure S6 and Table S3). In contrast, Bepanthen Sensicalm was located in a distinct area of the map and was more strongly associated with viscosity. These spatial relationships suggest that the HNES/NES emulsion shares sensory attributes linked to ease of spreading and lubrication with several commercial products, while maintaining its own specific profile through a stronger association with residual gloss after application (Supporting Information 5 Figure S6 and Table S3). Indeed, the glossmeter values obtained with the commercial products range from 11 ± 1 to

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20 ± 3 AU, whereas the HNES/NES emulsion generated a significantly glossier residue, reaching 34 ± 2 AU.

As shown in Supporting Information 5 Figure S6 and Table S3, all commercial products generated a measurable contact angle. In contrast, no stable droplet could be formed on the film produced by the HNES/NES emulsion. This behavior indicates that the HNES/NES residual film exhibits substantially higher surface energy and hydrophilicity than the commercial references.

In Figure 8, The PCA biplot (F1 = 48.35%, F2 = 27.55%, total = 75.90%) shows that the HNES/NES emulsion is positioned on the right side of the F1 axis, separated from Bepanthen Sensicalm and Dexeryl formulations but on the same side of Mixa and Avène serums. This indicates that its sensory and physicochemical profile differs substantially from the other tested products.

Along F1, the HNES/NES emulsion is positioned opposite to attributes such as viscosity, friction coefficient, contact angle, as well as to the emulsions Bepanthen Sensicalm and Dexeryl. This means it is characterized by higher spreading ability, more fluidity, lower viscosity, and a thicker initial feel compared to these pharmaceutical products. Its position closer to the negative side of F2 places it relatively far from stickiness, suggesting that the HNES/NES emulsion is perceived as less sticky than some products like Dexeryl or cosmetic serums, which load positively on stickiness.

Overall, PCA outcome is fully consistent with instrumental measurements, clearly isolating the HNES/NES emulsion due to its higher lubrication, markedly higher gloss, and strong hydrophilic behavior. The emulsion HNES/NES forms its own sensory-functional identity, distinct from both the fluid serums (located on the right) and the richer, stickier creams positioned above the origin. The HNES/NES emulsion therefore provides a new sensorial experience that can be classified as a hybrid product between a serum and a cream.

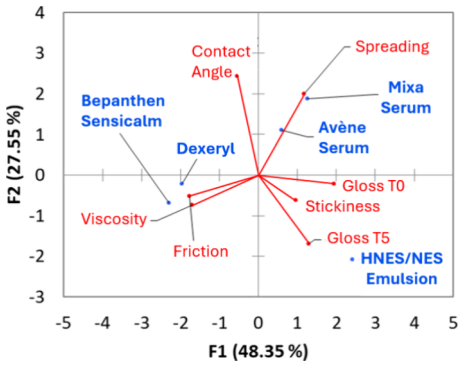

Figure 8. Principal component analysis (PCA) projection of product groupings.

Ecotoxicity Results

Cumulative mortality of daphnids was assessed after 4 days (96h) of exposure, following the OECD guidelines 202,[70] and 7 days. After 7 days, the cumulative mortality (Figure 9A) was slightly higher at 10 mg/L compared to the control (7.5 vs 2.5%). At 50 and 100 mg/L, cumulative mortality reached a similar level of about 50%, indicating a clear plateau. In addition, reproduction (Figure 9B) decreased as the emulsion concentration increased.

After 4 days of exposure, daphnids showed signs of oxidative stress, with a marked induction of antioxidant enzymes. SOD activity increased by at least a factor of two compared with controls (Figure 11B), and CAT activity (Figure 10A) showed a comparable trend. At 100 mg/L, however, CAT activity was not significantly different from the control, suggesting a possible early acclimation at this concentration. After 7 days, no significant differences were observed for SOD or CAT in either adults or juveniles.

For GST (Figure 10C), involved in detoxification, no significant differences were detected after 7 days in adults or juveniles. At day 4, however, GST activity in exposed daphnids was lower than in controls. Combined with the SOD and CAT profiles, this suggests that GST did not respond at the early stages of exposure.

Regarding lipid peroxidation (LPO), the (Figure 10D) levels were higher in exposed daphnids than in controls at day 4, indicating oxidative damage. At day 7, no significant differences were detected in adults. In juveniles, LPO levels were lower in exposed organisms than in controls, which may indicate physiological adjustments limiting oxidative damage over time.

Concerning neurotoxicity (AChE activity Figure 10F), no significant differences were observed at either time point or life stage, compared to the controls. As expected, AChE levels were higher in juveniles than in adults.

For digestive enzymes, ACP activity (Figure 10H) did not differ between treatments. ALP activity (Figure 10G) showed a slight increase in exposed adults (day 4 and 7) and juveniles compared to controls, suggesting a mild stimulation of digestive processes under moderate stress or enhanced assimilation of nutrients present in the emulsion.

Regarding cell integrity (LDH activity Figure 10E), no significant differences were observed between exposed and control organisms. LDH activity remained low in adults after 7 days and in juveniles, indicating minimal cellular lysis.

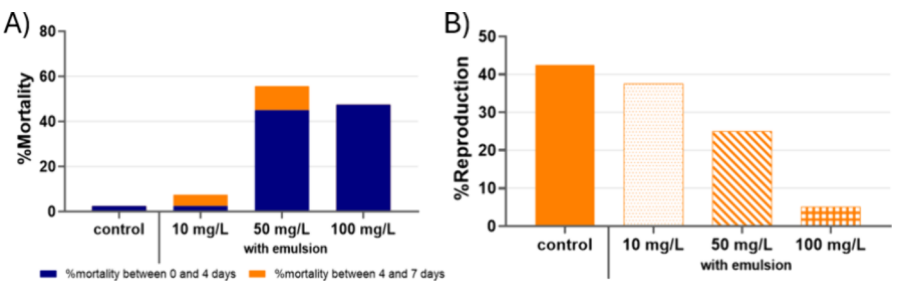

Figure 9. Results of the ecotoxicity test on daphnids. (A) %Mortality after 4 and 7 days. (B) %Reproduction after 7 days.

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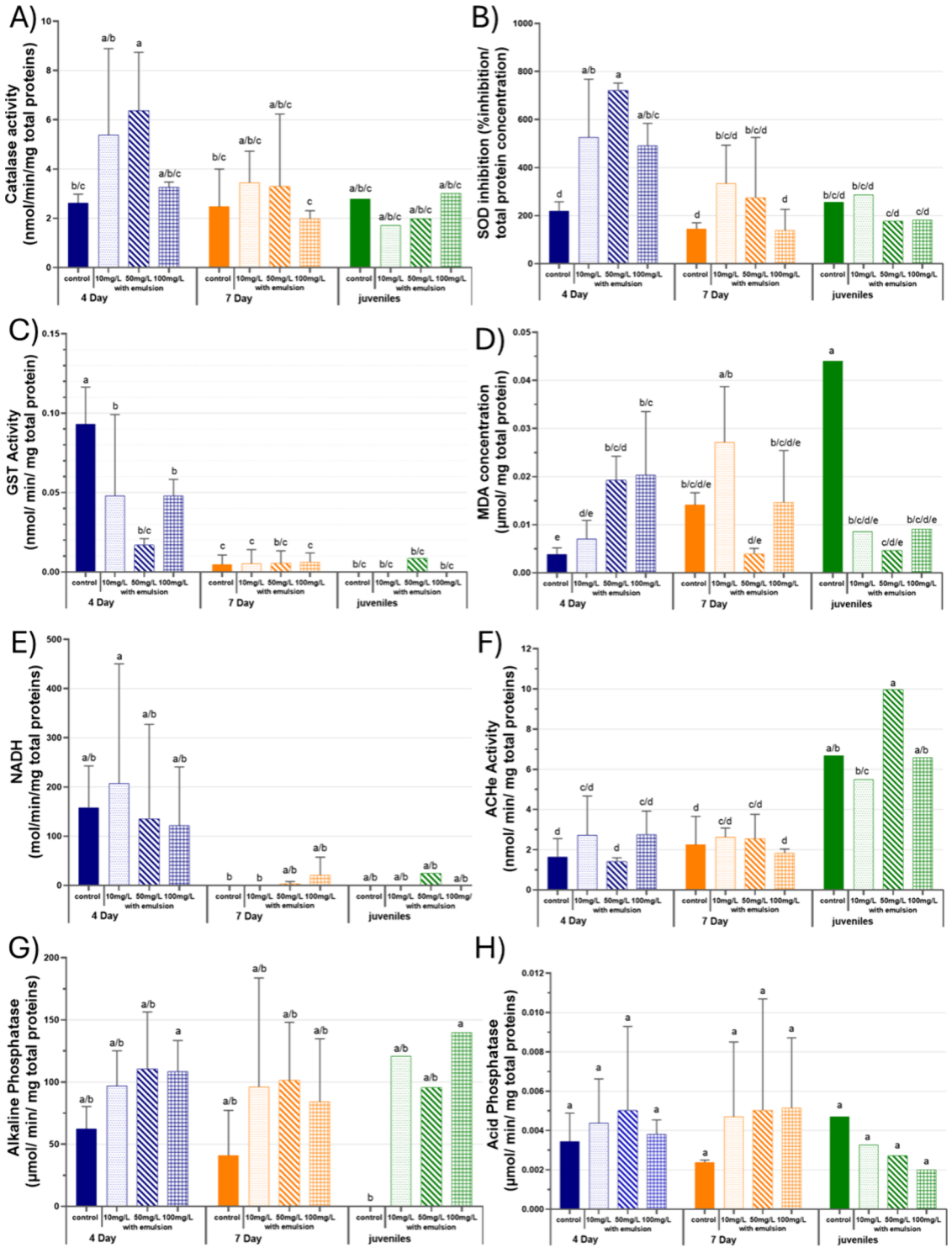

Figure 10. Results of the biomarker assays on daphnids (A) CAT activity (ANOVA $R^2 = 0.46$; p-value = 0.307); (B) SOD inhibition (ANOVA $R^2 = 0.72$; p-value = 0.005); (C) GST activity (ANOVA $R^2 = 0.74$; p-value = 0.0034); (D) lipoperoxydation (MDA concentration) (ANOVA $R^2 = 0.70$; p-value = 0.0091); (E) lactate dehydrogenase (NADH content) (ANOVA $R^2 = 0.39$; p-value = 0.491); (F) ACHE activity (ANOVA $R^2 = 0.81$; p-value = 0.0004); (G) alkaline phosphatase (ANOVA $R^2 = 0.43$; p-value = 0.383); and (H) acid phosphatase (ANOVA $R^2 = 0.17$; p-value = 0.97).

Overall, the emulsion induced a clear but transient oxidative response after 4 days of exposure, as evidenced by increased SOD and CAT activities together with elevated LPO levels. These effects were no longer observed at Day 7, suggesting that surviving daphnids may have partially adapted to the oxidative challenge. Few studies have investigated the ecotoxicity of emulsions or eutectic solvents. However, Ferreira et al.[71] reported similar alterations in catalase and superoxide dismutase

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Figure 11. Summary of the SSbD pillars applied to the HNES/NES emulsion.

activities when testing a citric acid–trehalose NES; thus, oxidative stress increased with NES concentration, reflecting an imbalance between ROS production and endogenous antioxidant defenses. Comparable mechanisms may be involved here, potentially linked to the glucose component, as previously suggested by Abnosi et al.,[72] while betaine is generally described as protective against oxidative stress. Glycerol and 1,2-octanediol have not been reported as ROS inducers, although Martins et al.[73] observed increased SOD activity in European sea bass fed an oleic acid-rich diet. Taken together, these findings suggest that the surfactant may be a major contributor to the observed oxidative response. While no ecotoxicity data is available for Eumulgin VL75, other types of surfactants were tested by Tian et al.[74] They reported similar increases in SOD, CAT, and LPO. No effects were detected on neurotoxicity (AChE), digestive enzymes (ACP, ALP), or cell integrity (LDH), and GST activity was not mobilized despite the early oxidative response. Mortality reached a plateau between 50 and 100 mg/L, suggesting the activation of antioxidant mechanisms limiting further lethality.

■ DISCUSSION

Sustainability is a central challenge that should be integrated at the early stage of innovation using newly developed tools and frameworks like SSbD (Safe and Sustainable by Design). SSbD assessment is a qualitative and semi-quantitative index designed as a decision tool, which aims to align technical and economical relevance with sustainability and regulations, combining four main pillars: Performance, safety, durability, and socio-economic considerations. In this discussion, the results will be analyzed according to these four pillars (Figure 11).

Performance

The performance pillar of SSbD aims to demonstrate that a sustainable alternative exhibits better or similar performances than a gold standard reference. Regarding cosmetic emulsions, performance evaluation is based on physical stability measurement, stabilization and vectorization of active ingredients, and sensory properties.

The emulsion developed in the present study is the first emulsified system composed exclusively of eutectic solvents and a non-ionic bio-based surfactant. This formulation remained physically stable for one month at 40 °C, with no observable instability, like creaming or phase shift, nor any physicochemical signs of destabilization. No evolution was detected in terms of rheological behavior or droplet size. It is reasonable to assume that stability will be satisfactory in the long term and may even be increased by adding thickeners. Another noticeable performance criterion is the enhanced chemical stability of retinyl palmitate within the emulsion. The ability of eutectic solvents to stabilize fragile active ingredients is well-documented.[75−77] This ability remains consistent when they are formulated in an emulsion.

The Ret-HNES/NES emulsion demonstrates significant improvements in retinyl palmitate delivery, outperforming conventional emulsions and ethanolic systems.[66,67,69] The HNES/NES emulsion vectorizes a significant amount of retinyl palmitate to the dermis, underscoring the strong dermal bioavailability enabled by this innovative system.

From a sensory perspective, the HNES/NES emulsion introduces an innovative hybrid texture positioned between a serum and a cream despite not containing any texturing agents. Its spreading behavior reflects this duality, as the formulation distributes easily over the skin, ensuring large surface coverage with a minimal amount of the product. These spreading properties, combined with the stabilization and penetration enhancer activity, could lead to a reduction in emulsion consumption both in the pharmaceutical or cosmetic sectors, where efficiency and resource optimization are increasingly valued in a sustainability context.

This study highlights that this simple ternary formulation achieves satisfactory performances on four different indicators (stability, preservation of active ingredient, penetration, and sensory), thereby underscoring the evident potential for multifunctionality in these ingredients. This finding is in alignment with SSbD requirements.

Safety

Safety is a critical pillar of the SSbD framework, as it considers the entire ecosystem, not only human health, but also the potential impacts of a product on wildlife and the broader environment, during the entire life cycle of the product.

The initial stage of the assessment process involves conducting a thorough analysis of the safety and risks associated with raw materials (ECHA and OECD). The only potential safety questions raised by the HNES/NES emulsion relate to high concentrations of 1,2-octanediol, which could present an irritation risk, and to lauryl glucoside present in the chosen commercial surfactant, which is also irritating but at doses higher than those used in cosmetic applications. According to the irritation test performed on both eutectic solvents (cell viability up to 95%), it is reasonable to conclude that both HNES and NES are safe for both professional and consumer use, despite the high concentration of 1,2-octanediol in the HNES.

Another key consideration when evaluating the safety pillar is the potential elimination of controversial molecules due to their negative impact on the environment and human health. Disputed substances often include antimicrobial preservatives. The use and permitted concentrations of these preservatives are becoming more regulated. According to the low-water activity ($a_w < 0.6$) observed with the HNES/NES emulsion, antimicrobial preservatives are not required, which is a major achievement from the safety perspective.

From an end-of-life perspective, ecotoxicity evaluations are not yet systematically carried out at the product development stage. Integrating ecotoxicity screening early in formulation design is becoming essential in the context of biodiversity loss,

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particularly in aquatic environments. Safety assessment is based here on the OECD ecotoxicity evaluation protocol on daphnids (OECD 202). These results indicate low acute aquatic toxicity and align with commonly accepted benchmarks for topical cosmetic and pharmaceutical products.[78] Therefore, eutectic solvents by themselves did not seem to significantly contribute to ecotoxicity, which appears to be mainly driven by the surfactant.

Socio-Economy

The socio-economic pillar raises two key considerations: realistic industrial development and consumer acceptability.

In the cosmetic industry, it is vital to consider the compatibility of innovations entering the market with the existing laboratory and industrial tools used by formulators. Indeed, the need for significant changes to production tools represents a major entry barrier for innovations, markedly eco-innovations.[79,80] It is particularly relevant given the high production volumes and the use of the same equipment to produce multiple end products. The major strengths of the new emulsified system described here are its remarkably minimalist formula and its production under conditions routinely used in research and development laboratories across the industry.

A key challenge for new products entering the market is meeting the regulatory compliance requirements and accessing toxicological data.[80] One of the major advantages of the HNES/NES emulsion is that it is composed of ingredients identified by an INCI name (international nomenclature of cosmetic ingredients), already authorized without restrictions in major cosmetic markets, including China. This formulation approach allows for innovation while circumventing a critical barrier to industrial adoption.[43] This point illustrates the need to integrate SSbD decision-making tools from the design stages onward, to shorten regulatory procedures and avoid expensive safety studies.

Consumer acceptability is a central socio-economic concern for cosmetic products, as it directly influences market viability and real-world uptake of sustainable formulations.[81,82] Product designers must incorporate consumer-centric criteria, such as transparent sustainability communication and sensory qualities early in the formulation process.[83,84] The HNES/NES emulsion presented in this work is designed to meet consumer expectations in terms of environmental and sensory aspects. All raw materials used are available as bio-sourced and compatible with COSMOS certification, a globally recognized mark of reliability and safety. Eutectic solvents proposed in this study are based on raw materials that can be obtained from the biorefinery of by-products. For example, previous studies have shown the possibility of glucose extraction from the peanut shell with a yield of 80.7%[85] or the extraction of betaine using sugar beet molasses by-products with a wider yield variation of 60–90%.[86] In terms of sensory properties, the unique profile of the HNES/NES emulsion suggests that it will be widely accepted and embraced by consumers. Indeed, its non-sticky finish and its light-glossy effect are highly sought after in cosmetic formulations, especially in face creams or primers. This enhancement of user comfort and improved product acceptability make it an asset in a range of product development scenarios.

Durability

The durability pillar is often understood to relate solely to environmental impact and long-term sustainability, which obscures the importance of technical sustainability for industrial processes. As stated in the socio-economic section above, the compatibility of the industrial plans with the production of the innovative HNES/NES emulsion is also a major asset in the durability perspective of potential industrial deployment.

As far as the environmental dimension is concerned, it is based on several criteria, including the bio-based origin of ingredients, the carbon footprint, water consumption, and ingredient biodegradability. This emulsion combines two eutectic solvents, one hydrophilic and one hydrophobic, both derived from natural metabolites. In addition, the selected surfactant is formulated entirely from ingredients of natural origin and complies with the supplier's technical and safety specifications.

A fundamental aspect of the recent "waterless life cycle" idea focuses on formulation design, encouraging the development of anhydrous or highly concentrated products intended for leave-on or rinse-off with low volumes of water.[20] The HNES/NES emulsion described in this study integrates the emerging concept of low-water skin-dedicated products. It is reasonable to expect that the approach could be adapted for use with two anhydrous NESs, resulting in waterless finished products.

Replacing conventional oils with HNESs offers several strategic advantages from both a formulation and sustainability perspective. As demonstrated by Martin et al.,[44] the sensorial properties of HNESs depend strongly on the metabolites used, enabling fine control over texture and skin feel. This tunability makes it possible to reproduce the softness of vegetable oils or, alternatively, achieve a drier, ester-like finish simply by adjusting the HNES composition, providing formulators with a versatile and customizable bio-based alternative.

Eutectic solvents are usually considered as biodegradable when their constituents are themselves biodegradable. Earlier studies have shown that hydrophilic NESs are generally classified as "readily biodegradable", particularly those composed of glycerol and glucose,[87–89] and that oleic acid and non-tertiary alcohols, such as 1,2-octanediol, also fall into the "readily biodegradable" category.[90,91] Regarding the surfactant system, both lauryl glucoside and polyglyceryl-2 dipolyhydroxystearate[15] are likewise classified as "readily biodegradable." Consequently, the final HNES/NES emulsion can be considered as biodegradable.

■ CONCLUSIONS

The present study is the first to address the development of a submicrometric emulsion composed of two natural eutectic solvents, a hydrophilic one used as the external phase and a hydrophobic one used as the internal phase. This emulsion was prepared following the current practices for formulating such systems, in terms of phase proportions and the use of a surfactant to stabilize the interface. Its water activity indicates low needs in preservatives to ensure its microbial stability. The emulsion proved to be physically stable for one month at 40 °C, 75% humidity and exhibited physicochemical, rheological, and sensory characteristics compatible with topical use. In addition, it promoted the penetration of a lipophilic active molecule placed in the internal phase all the way to the dermis, which is rarely achieved with this type of formulation. This first product was prepared at room temperature, using common tools and processes, which suggests an efficient potential scale-up of this new type of emulsion.

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Viewed through the lens of the Safe and Sustainable by Design framework proposed by the European Union, the emulsion is not only performant, but can be considered sustainable and durable in many respects, due to the nature of its ingredients, its low ecotoxicity, and the potentially minimized carbon footprint of its projected industrial production, especially if the eutectic solvents are obtained from industrial by-products. This first study opens the way to the development of a whole range of new emulsions based on rationally selected natural eutectic solvents, minimalist in their composition and potentially formulated without substances of concern.

■ ASSOCIATED CONTENT

Data Availability Statement

The data supporting this article have been included as part of the Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.6c04904.

Experimental details and specific results about ecotoxicity evaluation (SI 1), NES design (SI 2), NES characterization (SI 3, 4, and 5), HNES/NES emulsion characterization (SI 6), safety indicator estimation (SI 7) (DOCX)

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Author Contributions

L.D. conceived the study, designed and performed the experiments, analyzed the data, and wrote the original draft. M.D. contributed to conceptualization, data analysis, funding acquisition, and manuscript revision. E.G. contributed to conceptualization and manuscript revision. E.V. performed experiments, analyzed the data, and contributed to the original draft. A.V. contributed to manuscript revision. F.-X.L. contributed to data analysis and the original draft. D.C. contributed to the investigation. L.R. provided resources. M.G. contributed to supervision and manuscript revision. A.R.C.D. contributed to supervision and manuscript revision. L.B.-D. contributed to conceptualization, methodology, investigation, validation, funding acquisition, supervision, and manuscript revision. E.M. contributed to conceptualization, methodology, validation, funding acquisition, project administration, supervision, and manuscript revision. All authors reviewed and approved the final manuscript.

Notes

The authors have no conflict of interest to declare.

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