Title

Skin irritation potential of nanofluids containing mixtures of non-ionic/anionic surfactants and silica nanoparticles: a proof-of-concept study

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Abstract

Product formulation based on nanotechnology is booming in the cosmetics industry. The quality and performance of cosmetics can be enhanced by incorporating nanocarriers like solid or micellar nanoparticles that promote the bioactive components transport onto the skin. However, the synergy between these compounds may cause skin irritation. To mitigate skin irritability, cosmetics should incorporate nano-formulation strategies. A proof-of-concept study was therefore conducted to determine whether the addition of (i) a low-irritant cosurfactant (non-ionic) to anionic surfactants and/or (ii) low-toxicity nanoparticles (silica) could lead to safer cosmetic formulations. The skin irritation potential was evaluated *in vitro* using the Zein method.

As expected, the non-ionic surfactant has the lowest risk of skin irritation. Non-ionic/anionic combinations reduced skin irritability by 24-67% over the single anionic surfactant. In general, the addition of silica nanoparticles (NPs) to single surfactants increased the skin irritation potential, especially when the anionic surfactant molecules were arranged in a spherical core-shell structure along the NPs surface. On the other hand, the formulation stability of surfactant mixtures improves with the addition of NPs. Despite all the three-component formulations reduced skin irritability, those forming spherical core-shell structures showed the greatest reduction, possibly due to a reduction in charge density caused by the non-ionic surfactant, which may reduce protein solubilisation. Therefore, the irritation potential of anionic/non-ionic mixtures of surfactants can be further reduced by adding silica NPs.

As shown in this study, a good nano-formulation approach may have a positive impact not just on cosmetic efficacy but also on the synergy between its components, reducing skin irritation.

Keywords

Skin irritation; nonionic surfactant; anionic surfactant; silica nanoparticles; nanofluids; surfactants mixtures; zeta potential; diffusion coefficient; nanoformulation; cosmetics.

1. Introduction

A cosmetic product is defined as ”any substance or mixture intended to be placed in contact with the external parts of the human body (i.e., epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours” (Regulation (EC) No 1223, 2009). As consumers apply cosmetics on a daily basis, it has become increasingly necessary to develop milder and more suitable for sensitive skin cosmetic formulations having minimal impact on the human body.

Personal cleansing formulations are available in a wide range of formats (e.g., soap bars and liquids, body gels, shampoos, cleansing wipes, micellar water), usually containing one or more surfactants. The surfactant system must have good both performance and phase stability. Surfactants consist of hydrophilic and hydrophobic groups whose characteristics and size reduce interfacial tension upon adsorption at the liquid–gas, liquid–liquid, and solid–liquid interfaces. Surfactants are frequently marketed based on their degree of mildness to the skin; they bind to skin proteins to different extents. Surfactant formulations leaving less residual surfactant molecules on the skin after application would be the most desirable for use.

In terms of production, anionic and non-ionic surfactants represent about 90% of the total (Modor, 2022). Among non-ionic surfactants, ethoxylated fatty alcohols have high compatibility with anionic, cationic, and non-ionic surfactants, excellent cleaning efficiency, a good environmental profile, and they are cost-effective and safe for the skin (Jurado et al., 2007; Blagojević et al., 2016; Lechuga et al., 2016; Achaw & Danso-Boateng, 2021); because of weak hydrogen bonding with skin proteins, non-ionic surfactants have low skin irritation properties. On

the other hand, traditional anionic surfactants such as sodium lauryl sulphate (SLS) are highly irritating because their molecules bind to the skin proteins through strong ionic bonds; SLS concentration over 2% is considered irritating to the skin (Human patch test; 24-hour exposure) (Dahl & Trancik, 1977; CIR, 2005; Piret et al., 2008). There are other anionic surfactants like ether carboxylic acid derivatives that provide a better foaming capacity and are less irritant and toxic (Sato et al., 1996; Jurado et al. 2012); they are commonly used in cleaning products, personal care products, and cosmetics. In practice, surfactant mixtures are often preferred over single surfactants as synergy may boost performance (Muherei & Junin, 2008) and reduce skin irritation potential. For instance, Unilever (US 9655866 B2) discloses super mild surfactant systems based on a combination of specific alkanoyls or mixtures of them with a fatty acyl isethionate product which provides synergy to reduce irritation.

The nanotechnology-based product formulation is considered one of the hottest emerging technologies available for cosmetic manufacturing. This approach enables the formation of small nanoparticles (1-100 nm) – large surface-to-volume ratio – for enhancing the characteristics and attributes of final consumer goods by giving additional properties that are generally not observed in their bulk counterpart. For instance, forming active components-readily absorbed onto the skin, enhancing UV protection, lasting, colour, finish quality, and cleansing efficiency (Singh et al., 2016). Cleansing formulations such as micellar water are one of the latest applications in cosmetics, becoming trending and widely commercialized in local and international markets. The ability of the nanoemulsion system to form small micellar nanoparticles size with high surface area allows for the effectiveness of bioactive component transport onto the skin (Yukuyama et al., 2016).

Cosmetics quality and performance have also been enhanced by incorporating bioactive components with novel nanocarriers: liposomes (Soni et al., 2016), niosomes (Yeh et al., 2013),

dendrimers (Mu & Sprando, 2010), nanocapsules (Rosset et al., 2012), and nanoparticles (Ma et al. 2008; Lu et al., 2015; Waghmode et al., 2019) within others. Nanoparticles are commonly used as abrasive materials and stabilizers (Maestro et al. 2014, Patra et al. 2016, Plomaritis et al. 2019, Jeelani et al., 2019). Silica nanoparticles has attracted significant attention in the last years (Slowing et al. 2010, Mamaeva et al. 2013, Jeelani et al., 2019; Sheng et al., 2021), being present in many formulations due to their physicochemical properties, low toxicity, good stability, and functionalization capacity with a range of polymers and molecules (Ríos et al. 2018, Lechuga et al. 2022). Silica nanoform (< 100 nm) are intentionally used in leave-on and rinse-off cosmetics to enhance effectiveness, texture, and product shelf-life. Previous studies using rabbit skin found that silica nanoparticles did not cause irritation (Park et al. 2013). However, despite potential benefits and lack of skin irritation, nanomaterials are still relatively new to the world of consumer goods, and safety concerns may still arise.

Owing to the irritation potential, cosmetics manufacturers are required to conduct testing to appropriately characterise eye and dermal toxicity of products, labelling them with the appropriate warnings and first aid information according to regional regulations; for instance, EU regulation for Chemicals (REACH) (Regulation (EC) No 1907, 2006), Consumer Product Safety Commission (CPSC) and Toxic Substances Control Act (TSCA) in the US (USEPA, 2014), or Chemical Substances Control Law (CSCL) in Japan (Japan METI). In particular, the EU regulation on cosmetics products (Regulation (EC) No 1223, 2009) pays special attention to cosmetics containing nanomaterials. The *in vivo* evaluation of the irritant potential of cosmetics has been traditionally conducted in animals, particularly in rabbits, using the Draize method (Draize et al., 1944). However, due to the increasing concern over animal use and considering its potential ban in the near future, *in vitro* alternatives should be encouraged. Zein protein has been presented and explored as a potential candidate to be used on tissue engineering applications

(Perez-Guzman & Castro-Muñoz, 2020). Among *in vitro* tests, the Zein method (Götte, 1964) is one of the most used, showing a good correlation with the Duhring chamber test – a modified patch testing method (Kästner & Frosch, 1981; Lang & Spengler, 1986). This method provides a screening tool for the evaluation of the relative mildness to the skin of surfactants (Moore et al., 2003). Zein, which resembles the keratin protein present in human skin and hair, is an insoluble corn protein in water that becomes soluble once denaturised, being the irritation potential of the surfactant formulation directly proportional to the quantity of dissolved proteins (Götte, 1964). The Zein Number (ZN) is a standard parameter used by the cosmetic industry. A major role in skin irritation is played by surfactants that interact with skin proteins, but this is only one of the mechanisms proposed in the literature that explain skin irritation. Surfactants can also interact with intercellular lipids and sebum, as well as with live skin cells.

The development of nanoformulation-based cosmetics should incorporate strategies for mitigating skin irritability. We hypothesize that adding either low-toxicity nanoparticles or low-irritant cosurfactants could lead to the formulation of safer cosmetics. This study, therefore, investigates the synergetic effects that formulating mixtures of a non-anionic and three anionic surfactants, with or without the presence of silica nanoparticles, may have on the particle size, zeta potential, particle concentration and diffusivity coefficients of the micelles/complexes formed in the bulk phase. Afterwards, the skin irritation potential is evaluated *in vitro* using the Zein method. The objectives are to determine whether the addition of either (i) a non-ionic cosurfactant to form binary mixtures with anionic surfactants or (ii) silica nanoparticles to the solutions formed by single surfactants (or their mixtures) could reduce the irritation potential of cosmetic products.

2. Materials and methods

2.1 Types of surfactants, nanoparticles, and solutions

A total of four different commercial surfactants, a non-ionic fatty-alcohol ethoxylate (FAE-$R_{12-14}E_{11}$; Kao Corporation, Spain) and three anionic ether carboxylic acid derivatives with different alkyl chain and degree of ethoxylation, laureth-4 carboxylic acid (EC-$R_{12-14}E_3$; Kao Corporation, Germany), laureth-11 carboxylic acid (EC-$R_{12–14}E_{10}$; Kao Corporation, Germany), and capryleth-6 carboxylic acid (EC-R_8E_5; Kao Corporation, Germany) were used in this work. The characteristics of the surfactants used are listed in **Table 1**. According to the specified by the supplier and depending on the carbon chain length and the ethoxylation degree, these surfactants offer a wide range of characteristics for industrial applications: FAE-$R_{12-14}E_{11}$ is a non-ionic surfactant commonly used as a high HLB (14.3) emulsifier that provides good foaming, wetting, and dispersing power, being highly compatible with anionic, cationic, or amphoteric surfactants. Among the anionic surfactants tested here, EC-R_8E_5 has a short alkyl chain (C8) and moderate degree of ethoxylation, having good hydrophilic properties (HLB 11-14). EC-$R_{12-14}E_3$ and EC-$R_{12-14}E_{10}$ have a C_{12}-C_{14} alkyl chain and their degree of ethoxylation ranges from 2.5 to 10 EO; EC-$R_{12-14}E_3$ is a lipophilic surfactant with low degree of ethoxylation and excellent wetting and lubricity ability, whilst EC-$R_{12-14}E_{10}$ is a hydrophilic surfactant with excellent mildness to skin and mucous membranes.

Hydrophilic fumed silica nanoparticles (Aerosil® 200; Evonik Industries, Germany) were also incorporated into the surfactant formulation. Nanoparticles were characterised by ultrahigh-resolution scanning transmission electron microscope (S/TEM) and a high-angle annular dark-field imaging (HAADF) system (FEI TITAN G2 60-300). The images showed amorphous structures formed by spherical-like nanoparticles (representative image shown in **Fig. 1**); Aerosil

200 particles show a sphericity value of 0.94. **Table 1** shows the values of D_m (mean diameter), surface area (S), and the tamped density (d) provided by the supplier.

The surfactant solutions were prepared at 2% wt. of active matter of the surfactant of interest. A NPs solution at 2%wt was prepared by adding the NPs A200 to milli-Q water, being both mixed by a magnetic stirrer, and later sonicated (Sonorex RK 106 S, Bandelin, Berlin, Germany) for 30 minutes at 75% amplitude intensity, 500 Watt and 20 kHz in intervals of 15-05. If used, the NPs solution was added to the surfactant formulation of interest at a ratio 1:1; the final concentration of the formulation before testing was 1% wt. of active matter of surfactant and 1% wt. of NPs. All solutions were prepared in Milli-Q water (18.2 MΩ cm^{-1}), adjusting pH to 7 ± 0.2 by adding either 1N NaOH or 1N HCl. The composition of the solutions tested is detailed in **Table 2**.

2.2 Irritation potential by the zein method

The irritant potential of the formulation of interest was determined by a modified version of the zein method described by Götte (1964), developed by Bailón et al. (2009) The method, formed by three stages, determines the amount of protein solubilized (zein) in a containing surfactant solution:

(i) Digestion of zein. 2 ± 0.01g of zein from corn (Sigma, Spain) was added to 40 mL of the surfactant solution. When the protein is exposed to the surfactant, it is denatured and dissolved into the solution. Samples were placed in a water bath at 30°C for 1h under stirring conditions. After digestion – protein far in excess – the resulting solutions were centrifuged for 20 minutes at 6000 rpm.

(ii) Oxidation and mineralization of organic matter. An aliquot of 0.5 mL taken from the intermediate zone (clear phase) of the centrifugated solution was added to 50 mL of Milli-Q water, adding subsequently other 7 mL of the oxidant reagent; oxidant reagent solution is

formed by 50 g $K_2S_2O_8$ (Sigma, Spain), 30 g H_3BO_3 (Panreac, Spain), 14 g NaOH (Scharlau, Spain) in 1 L of Milli-Q water. A control test was made using only water and the oxidant reagent. The samples were then autoclaved at 120°C for 45 minutes to convert all forms of nitrogen into nitrate (NO^{3-}).

(iii) Nitrate content measurement. To prepare samples for spectrophotometric analysis, the resulting samples from stage (ii) were cooled down to room temperature, before adding 0.85 mL of sulfuric acid (Scharlau, Spain). Samples were stirred and diluted 1:10, before measuring in a UV spectrophotometer at a wavelength of 220 nm. The calibration line was prepared with a series of dilutions of sodium nitrate ($NaNO_3$; Panreac, Spain) in MilliQ water (15, 10, 7.5, 5, 3.75, 2.5, 1.5, 0.75 ppm). All reagents were analytical grade.

The number of zein (ZN), mg of nitrogen (N) released per 100 grams of surfactant solution, is calculated as:

$$ZN = c / 10 \qquad \textbf{Eq. [1]}$$

Where c is the concentration of N measured spectrophotometrically in ppm (mg/1000mL). Considering that the density of the testing solution is 1 g mL^{-1}, c would be finally expressed as mg 1000 g^{-1}. Data are the average of at least two repeats. Two control cases have been implemented for validation, a negative control using deionised water and a positive control using sodium lauryl sulphate (SLS) at 1% wt.; SLS is a well-known standard surfactant with high irritant potential (ZN = 536±12).

2.3 Characterisation of particle size, concentration, and zeta potential

The size and particle concentration of surfactant micelles, nanoparticles, and particles-surfactant complexes were measured by multi-angle dynamic light scattering (MADLS) using a Zetasizer Ultra (Malvern Panalytical Ltd., Malvern, UK). The Zetasizer system is also used to analyse the

particle Zeta potential using the technique of Electrophoretic Light Scattering (ELS). The instrument settings were optimised automatically by means of the ZS XPLORER software (Malvern Panalytical Ltd., UK). All experiments were performed at room temperature. Data are the average of at least three repeats.

As for the diffusivity coefficient (D), Zetasizer obtains the hydrodynamic diameter from its diffusion in solution, using the Stokes Model:

$$d(H) = \frac{kT}{3\pi\eta D} \qquad \textbf{Eq. [2]}$$

Where: d(H) is the hydrodynamic diameter; D, the translational diffusion coefficient; k, the Boltzmann's constant; T, the absolute temperature; and η, the dynamic viscosity.

3. Results and discussion

3.1 Characterisation of the surfactant-based formulations

As highlighted in the introduction, surfactants are widely used when products are being formulated to facilitate emulsion formation by reducing interfacial tension and promoting droplet dissociation. Micellar nanoparticles are one of the latest fields applied in cosmetics, where their high surface area allows an effective bioactive component transport onto the skin. In this section, four singles surfactants and their mixtures (**Tables 1** & **2**) were characterised in absence of nanoparticles using dynamic light scattering and zeta potential measurements, determining the size, electrical potential, concentration, and diffusion coefficients of the surfactant micelles formed in aqueous solutions. The selection of the mixtures with the non-ionic fatty-alcohol ethoxylate (FAE-$R_{12\text{-}14}E_{11,}$ HLB 14.3) is based on the hydrophilic–lipophilic balance (HLB; scale 0-20) of the anionic ether carboxylic acid derivates: (i) low (EC-$R_{12\text{-}14}E_3$; HLB 5.5), (ii) medium (EC-$R_{12\text{–}14}E_{10}$; HLB 9.5, and (iii) high HLB values (EC-R_8E_5; HLB 11-14). HLB is the relationship between the size and strength of the hydrophilic and lipophilic moieties of a surfactant molecule (Zheng et al., 2015): HLB < 6.0, i.e., lipophilic, is suitable for use in water in oil (W/O) emulsions; HLB > 8, i.e., hydrophilic, is the most used in O/W emulsions (Griffin, 1949). In cosmetics, O/W nano-emulsion is playing a major role as make-up remover, facial cleanser, anti-aging lotion, and sunscreens, as well as other water-based cosmetic formulations.

The mean particle size and surface charge data used to predict the colloidal stability of the micelles formed in the surfactant-based formulations are shown in **Table 3**. It is worth noting that the CMC (**Table 1**) of the surfactant tested is far below the concentrations used for testing. FAE-$R_{12\text{-}14}E_{11}$ in water (pH7) forms micelles of 6.7±0.1 nm with good degree of stability (ζ -22.5 mV). Of the anionic surfactants (negatively charged headgroup), EC-$R_{12\text{-}14}E_3$ forms highly stable

medium-size micelles (57.8±2.0 nm); most of them showed a ζ of -105.3 mV, whilst a very reduced proportion had ζ values of ca. -60 mV; the different values of ζ reported may be related to impurities that commercial surfactants as the used for this work may contain. Similar happened for the other EC-$R_{12\text{-}14}E_{10}$. However, in this case, the surfactant formed the smallest micelles of the single surfactant tested (2.0±0.2 nm) yet still having two ζ: most of the micelles formed were stable (-38.5 mV) yet there was a little proportion of them with low stability (-13.0 mV). Finally, the biggest micelles were formed by the anionic surfactant EC-R_8E_5 (90.2±0.8 nm), being them highly stable (-53.1 mV).

Mixture of different type of surfactants (anionic/nonionic) is the most common base of cosmetic formulations, since usually implies synergistic effects in performance. In general, the addition of the non-ionic surfactant, FAE-$R_{12\text{-}14}E_{11}$, to the solutions of anionic surfactants significantly decreased their ζ (**Figure 2**). The size of the micelles formed also varied. The combination of FAE-$R_{12\text{-}14}E_{11}$ and EC-$R_{12\text{-}14}E_3$ led to the formation of big highly stable micelles of ca. 150 nm in diameter and two different zeta potentials, being ζ_2 of insignificant magnitude compared to the main one (ζ_1). The combination of FAE-$R_{12\text{-}14}E_{11}$ with EC-$R_{12\text{-}14}E_{10}$ led to the formation of small micelles of 2 nm and different charge that, as happened for FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_3$, ζ_1 is the governing one. Finally, the mixing of FAE-$R_{12\text{-}14}E_{11}$ and EC-R_8E_5 led to the formation of small micelles (ca. 5 nm in diameter) of low stability (-6.3 mV). Low ζ values can lead to aggregation, coagulation, or flocculation due to van der Waals interparticle attraction (Joseph & Singhvi, 2019).

Concentration data and diffusion coefficients are strongly related with the micellar size previously discussed (**Table 3**). In general, increasing the size reduced the concentration of particles measured in the solution:

(i) FAE-$R_{12\text{-}14}E_{11}$ & EC-$R_{12\text{-}14}E_3$: the formation of larger and more stable complexes reduced the number of micelles by 10^8 and 10^5 times with respect to the micelles formed only by the singles surfactants (non-ionic and anionic, respectively).

(ii) FAE-$R_{12\text{-}14}E_{11}$ & EC-$R_{12\text{-}14}E_{10}$: as the micelle size for the mixture is similar to those reported for the solutions of the single surfactants, concentration data remained practically unaltered.

(iii) FAE-$R_{12\text{-}14}E_{11}$ & EC-R_8E_5: the concentration of particles increased significantly comparing to the single anionic surfactant. However, the micelles are unstable and will tend to aggregate easily.

Regarding the diffusivity coefficient data, a decrease in micelle size generally favoured the diffusion of the particles in the bulk liquid, e.g., FAE-$R_{12\text{-}14}E_{11}$. However, in some cases, the trend is not that clear as the diffusion coefficient, apart from the size and shape of micelles, also depends on the interactions of them with bulk fluid, within others. For instance, although the mixture formed by FAE-$R_{12\text{-}14}E_{11}$ and EC-R_8E_5 is not the one forming the smallest micelles, they diffused much faster in the bulk phase that any other of the mixtures tested, likely related to their low stability in solution that may lead to aggregation and flocculation as earlier mentioned. Therefore, the most stable surfactant mixtures would be the formed by FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_3$ and FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_{10}$, having the smallest (2 nm) and the biggest (150 nm) micelle size of the surfactant mixtures tested, respectively. In practice, stable and small micelles would be preferred as it increases the active surface area available and enhances the transport of bioactive components onto the skin (Yukuyama et al., 2016). However, small surfactant micelles may also be able to penetrate the skin through different pathways more easily, affecting the skin. The interaction mechanism between surfactant micelles and skin proteins will be investigated in **section 3.3**.

3.2 Characterisation of the containing silica nanoparticles formulations

In this section, hydrophilic Aerosil® 200 (A200) silica nanoparticles (NPs) of 12 nm size (Guha, 2010, Ríos et al., 2018) were added to the surfactant-based formulations characterised in **section 3.1**. The mean particle size, electrical potential, concentration, and diffusion coefficients data are reported in **Table 3**.

The silica NPs dispersed in water pH 7 are negatively charged, forming agglomerates of 147.6±3.1 nm in diameter (**Table 3**). In general, the mixing of single surfactants with NPs led to both a decrease of ζ (suggesting surface adsorption of surfactants onto the NPs) and differing surfactant self-assembly structures of distinctive characteristics (**Figure 3**). The less stable system, yet stable, is the one formed by the non-ionic surfactant and the NPs solution (ζ -17.9±0.3 mV). The hydrodynamic size increased slightly for the non-ionic surfactant/NPs mixture (ca. +18 nm) regarding the NPs solution, suggesting that a micelle-decorated silica model may occur (**Figure 3A**). This mechanism of interaction between non-ionic surfactants (e.g., decaoxyethylene n-dodecylether) and silica nanoparticles has been also reported in previous works (Kumar et al., 2012) using small-angle neutron scattering (SANS). It is believed that non-ionic surfactants tend to adsorb onto the nanoparticle surface (Lugo et al., 2010; Kumar & Aswal, 2011; Kumar et al., 2012) involving hydrogen bonding (Zhang & Somasundaran, 2006), forming a shell around the nanoparticle which increases the size of the complex formed. The hydrodynamic size of the complexes formed for EC-$R_{12\text{-}14}E_3$ and EC-$R_{12\text{-}14}E_{10}$ did not vary significantly regarding the NPs alone, suggesting that a spherical core-shell structure as detailed in **Figure 3B** may occur. However, other works have reported no physical interactions between anionic nanoparticles and anionic surfactants (e.g., SDS) as two components having similar charges may show strong electrostatic repulsion that prevent them from directly adsorbing

(Kumar et al., 2012). A much more marked size increase was observed in the dynamic light scattering data for the surfactant EC-R_8E_5, where three size distribution peaks were found: the main size distribution (65%) was at a particle size of 225 nm, and the other two at ca. 480 nm and 790 nm (11.1 and 23.9% respectively). The main size distribution suggests the formation of surfactant micelle-decorated structures, whilst the other two secondary size distributions suggest macromolecule-mediated aggregation, leading to the formation of big complexes. Although it is believed that anionic surfactants tend to no interact directly with negatively charged nanoparticles (Kumar et al., 2012), the high hydrophilicity of the EC-R_8E_5 surfactant molecule may favour interactions with the NPs. Overall, the most hydrophilic surfactants (i.e., FAE-$R_{12\text{-}14}E_{11}$ and EC-R_8E_5) seem to lead to the formation micelle-decorated silica particles, where especially the charged one (EC-R_8E_5) favoured particle aggregation. The configuration micelle decoration on a nanoparticle surface is often preferred for an effective packing of hydrophobic tails for micelles as compared to that formed by bilayers (Lugo et al., 2010).

When surfactant mixtures were added to the suspension of NPs, both ζ and the complexes size varied. For the first formulation, FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_3$, its mixing with NPs decreased ζ yet little variation in the complex size was found compared to the surfactant mixture or NPs alone, suggesting that a spherical core-shell may occur. Despite the ζ reduction, these complexes are still the most stable of the three formulations tested. On the other hand, the combination of NPs with the FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_{10}$ mixture increased ζ. Although the slight increase on the complex size may suggest the formation of surfactant micelle-decorated structure, the zeta potential of the formulation converged from the two values of the surfactant mixture to only one, suggesting that surfactant molecules would be uniformly distributed on the NPs surface, and consequently, would increase the stability of the system compared to the behaviour of the surfactants mixture alone. The addition of NPs to the most unstable surfactant mixture, FAE-$R_{12\text{-}}$

$_{14}E_{11}$/EC-R_8E_5, led to an increase of the stability of the suspension, forming complexes of bigger size; the main size distribution was found at ca. 220 nm, like the formed between EC-R_8E_5/NPs in absence of FAE-$R_{12\text{-}14}E_{11}$. However, in this case, the surfactant mixture favoured the formation of even bigger and less stable agglomerates than in the previous case, increasing the risk of flocculation; the low ζ of this formulation of surfactants in deionised water (pH 7) might also favour electrostatic interactions with the surface of the negatively charged NPs.

Concentration and diffusion coefficient data are also shown in **Table 3**. In general, the formation of large complexes tends to decrease both concentration and diffusion coefficient compared to those of the surfactant mixtures in the absence of nanoparticles; only the mixture formed by FAE-$R_{12\text{-}14}E_{11}$+ EC-$R_{12\text{-}14}E_3$+A200 showed a higher diffusivity than in the absence of NPs, being this fact possibly related to the high variability reported for the particle size of the mixture.

Further studies are being carried out to fully understand the self-assembly interaction mechanisms of the surfactant structures on aggregates of silica nanoparticles and will be published in future works. The scope of the present work is to understand the underlying mechanisms that product formulation (e.g., charge and size) may have on the potential irritation of human tissues such as skin.

3.2 Skin irritation potential

Due to the direct application of personal care products on the skin, the cosmetic industry is willing to develop milder and more suitable for sensitive skin products. This implies a conscientious selection of the ingredients included into the formulation before testing. The Zein number (ZN) (**section 2**) gives an indicative of the potential skin irritation by surfactants. According to Goëtte´s correlation shown in **Table 4** (Goëtte, 1964), they can be classified as non-

irritant, moderate irritant, irritant, or strong irritant. **Figure 4** shows the values of the ZN obtained in this study for all the formulations tested (**sections 3.1** & **3.2**).

As highlighted in the introduction, the skin irritation potential of the three anionic surfactants is greater than the measured for the non-ionic surfactant, since anionic surfactants can solubilize zein protein to a much more extent than non-ionic surfactants could do (Moore et al., 2003). The irritation potential of the formulations tested in decreasing order is: EC-$R_{12\text{-}14}E_{10}$ >EC-$R_{12\text{-}14}E_3$ > EC-R_8E_5> FAE-$R_{12\text{-}14}E_{11}$, being the ZN number higher for those anionic surfactants with longer alkyl chain and mid/low HLB. It stands out the anionic surfactant EC-$R_{12\text{-}14}E_{10}$, classified as moderate-irritant, is less irritant than other commonly used anionic surfactants such as sodium laureth sulphate (SLS), sodium lauryl ether sulphate (SLES), or linear alkylbenzene sulfonate (LAS) (Goëtte, 1964; Pezron et al.,1996; Cohen et al., 2016), being possibly related to the physicochemical properties of the surfactant micelles formed in solution. Looking at the ZN value of the three anionic surfactants, the smaller the micelle size, the higher the irritation potential. This could be likely related to that smaller surfactant micelles would increase the surface area available for interaction with the zein protein, favouring solubilisation.

In case of the mixtures of surfactants, a remarkable decrease on the irritation potential was observed once the non-ionic surfactant, FAE-$R_{12\text{-}14}E_{11}$, was mixed with the anionic surfactants. The reduction of the ZN, ranging from 67% for the EC-$R_{12\text{-}14}E_3$ to 24% for EC-$R_{12\text{-}14}E_{10,}$ is significantly high (> 60%) for those anionic surfactants having low and high HLB values (EC-$R_{12\text{-}14}E_3$ and EC-$R_8E_{5,}$ respectively). Therefore, under the tested conditions, the mixing of anionic with a non-ionic surfactant reduces the skin irritation capacity of the surfactant system formulated; despite the differences in particle size, a lower surface charge density may reduce the electrostatic interactions between micelles and the zein protein (Moore et al, 2003).

When nanoparticles are applied into the cosmetic formulation, especial attention should be paid to the synergetic effects. Although silica NPs do not show potential skin irritation according to *in vivo* and *in vitro* analyses (Park et al. 2013), they can affect the formation and stability of the surfactant micelles, and therefore, impact on the skin irritation potential of cosmetics. In general, the mixture of single surfactants (anionic or non-ionic) and NPs increased markedly the skin irritation potential, especially when the anionic surfactant molecules were located along the nanoparticle surface in a spherical core-shell structure; e.g., the mixture EC-$R_{12\text{-}14}E_3$/A200 increased its irritability degree from non-irritant to moderate irritant. Only one of them, NPs/EC-R_8E_5, reduced 2.3 times its skin irritation potential regarding the single surfactant, likely due to the formation of large aggregates (ca. 200-800 nm) (**section 3.2**) that may reduce the surface area available for surfactant-protein interactions, limiting protein solubilisation. On the other hand, when surfactant mixtures were added to the suspension of NPs, the stability of the formulation tends to increase, forming surfactant/NPs complexes of ~ 160 nm, or even bigger size depending on the characteristics of nano-formulation tested. Despite all the three-component formulations reduced skin irritability, the most marked decrease was shown by the ones forming spherical core-shell structures, FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_3$/A200 and FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_{10}$/A200, likely related to a reduction in the charge density caused by the addition of the non-ionic surfactant that may reduce protein solubilisation. The low irritability of the mixture EC-$R_{12\text{-}14}E_3$/FAE-$R_{12\text{-}14}E_{11}$ and A200 is especially remarkable as ZN decreased from 183.5 for the single anionic surfactant to 8.0 for the tertiary mixture, despite of the binary mixture of EC-$R_{12\text{-}14}E_3$ +A200 was moderate irritant (ZN = 341.9).

4. **Conclusions**

The aim of this proof-of-concept study was to examine whether the addition of either low-irritant cosurfactants or low-toxicity nanoparticles would contribute to the formation of safer cosmetic formulations. We therefore investigated whether adding either a non-ionic cosurfactant or silica nanoparticles to the solutions formed by single surfactants (or mixtures) would reduce their skin irritation potential.

As expected, the lowest skin irritation potential is shown by the non-ionic surfactant (FAE-$R_{12\text{-}14}E_{11}$), having stable micelles of size 6.7±0.1 nm and high diffusivity in water (65.6±0.5 $\mu m^2 s^{-1}$). Among the ionic surfactants, and because of its reduced micelle size (2.0±0.2 nm), the most convenient formulation for this type of nano-formulated cleansers would be the formed by EC-$R_{12\text{-}14}E_{10}$, having the smallest stable micelles (-38.5 mV) of the single surfactant tested and low skin irritation. However, any of the other two anionic surfactants would also be suitable as their micelle size is below 100 nm, having both excellent stability and low irritability.

The mixing of the anionic surfactants with the non-ionic reduced the skin irritation potential (24-67%), of which FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_{10}$ and FAE-$R_{12\text{-}14}E_{11}$/EC-R_8E_5 led to the formation of very small micelles (ca. 2 nm and 5 nm, respectively); only the micelles formed by FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_{10}$ were stable. Although in practice, stable and small micelles would be preferred, they may also be able to penetrate more easily into the skin through different pathways, affecting other skin layers.

When hydrophilic Aerosil® 200 silica nanoparticles (NPs) were added to the single surfactants, the skin irritation potential of the nanofluids increased in most of the cases, especially when the anionic surfactant molecules were arranged along the NPs surface in a spherical core-shell structure; only the formation of large aggregates by NPs/EC-R_8E_5 (ca. 200-800 nm) seems to reduce skin irritability, likely related to the reduction of the surface area available for

surfactant-protein interactions. In contrast, when surfactant mixtures were added to the NPs suspension, the stability of the formulation tends to increase, forming surfactant/NPs complexes of ~ 160 nm, or even bigger size depending on the characteristics of the nano-formulation tested. Despite all the three-component formulations reduced skin irritation, the most marked decrease was shown by the ones forming spherical core-shell structures, FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_{3}$/A200 and FAE-$R_{12\text{-}14}E_{11}$/EC-$R_{12\text{-}14}E_{10}$/A200, likely related to a reduction in the charge density caused by the addition of the non-ionic surfactant that may reduce protein solubilisation.

According to the results, the addition of silica NPs to solution of single surfactants increases their skin irritation potential, unless the surfactant molecule favours the formation of large NPs aggregates, reducing the surface area available for interaction with proteins. On the other hand, the addition of a non-ionic cosurfactant to anionic surfactant solutions reduces skin irritability, decreasing it even further as NPs were incorporated into the surfactant mixture.

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Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due the dataset on which this paper is based is too large to be retained or publicly archived with available resources but are available from the corresponding author on reasonable request.

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Authors' information (optional)

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