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Nano- and microparticle-stabilized Pickering emulsions designed for topical therapeutics and cosmetic applications

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ABSTRACT

Pickering emulsions are systems composed of two immiscible fluids, which are stabilized by solid organic or inorganic particles. These solid particles include a broad range of particles that can be used to stabilize Pickering emulsions. An improved resistance against coalescence and lower toxicity, against conventional emulsions stabilized by surfactants, make Pickering emulsions suitable candidates for numerous applications, such as catalysis, food, oil recovery, cosmetics, and pharmaceutical industries. In this article, we give an overview of Pickering emulsions focusing on topical applications. First, we reference the parameters that influence the stabilization of Pickering emulsions. Second, we discuss some of the already investigated topical applications of nano- and microparticles used to stabilize Pickering emulsions. Afterwards, we consider some of the most promising stabilizers of Pickering emulsions for topical applications. Ultimately, we carried out a brief analysis of toxicity and advances in future perspectives, highlighting the promising use of these emulsions in cosmetics and dermopharmaceutical formulations.

1. Introduction

The skin, the largest organ in the human body, prevents the loss of endogenous substances (e.g., body fluids), regulates body temperature, and acts as an organism's first line of defence against external factors (e.g., microorganisms, allergens, ultraviolet (UV) radiation, chemicals) (Prow et al., 2011; Menon, 2002; Hadgraft, 2004). The skin is composed of three primary layers: epidermis, dermis and hypodermis. Concerning to epidermis, it can be divided into four different layers: *stratum corneum* (SC), *stratum granulosum, stratum spinosum*, and *stratum basale* (Wu and Guy, 2009; Carter et al., 2019). The barrier function of the SC, the outermost layer, plays an important role in limiting cutaneous penetration and permeation of active ingredients present in cosmetics and therapeutic formulations. However, active ingredients can cross the skin by three possible routes: intercellular, transcellular or appendageal. The first route is the most used but it is slow because molecules cross through intercellular spaces (Benson, 2005). In the second route, the molecules pass through the phospholipid membranes and the cytoplasm of the dead keratinocytes. In the last route, the molecules pass through hair follicles and eccrine glands, which may be an advantage for active ingredients delivery, especially involving nanoparticles as skin delivery systems. In fact, nanoparticles-based skin formulations have been receiving great attention for cosmetics and dermopharmaceutical formulations development, since the submicron particle size and high surface area of nanoparticles allow a enhanced cutaneous permeation, improved active ingredient retention in the skin, sustained release of active ingredients, optimal target delivery, improved stability and protection of active ingredients (Santos et al., 2020; Santos et al., 2019; Paiva-Santos et al., 2021; Paiva-Santos et al., 2021; Santos et al., 2019; Santos et al., 2019). Topical active ingredients delivery can be challenging, it is often necessary to combine multiple strategies to achieve a suitable topical active ingredients delivery and the desirable effects. Additionally, other factors need to be considered to, such as the vehicle used, active ingredient's physical properties and the active ingredient-vehicle interactions. The vehicle employed in topical formulations is very important since it remains in the area of application and can

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increase drug release. Non-toxic, non-irritant and non-allergenic chemical enhancers may also be included into topical formulations to in order to improve flux of active ingredients through skin (Williams and Barry, 2012; Lane, 2013).

Emulsions are technological systems broadly used in cosmetic and pharmaceutical formulations, including creams, lotions and gels (Costa et al., 2019). Emulsions can be defined as heterogeneous and thermodynamically instable systems constituted by a emulsifying agent is and one phase intimately dispersed in another phase in the form of droplets (Marto et al., 2016). The topical application of emulsions presents several advantages, such as enhanced drug release and improved permanence on the skin (Marto et al., 2016). Emulsions are promising formulations for the treatment of skin disorders such as atopic dermatitis or eczema (Lémery et al., 2015). However, environmental and toxicity concerns (Albert et al., 2019), namely skin reactions like contact dermatitis or inflammation (Lémery et al., 2015), cell damage (Low et al., 2017) and carcinogenicity (Yang et al., 2017). The irritating potential is generally higher for cationic surfactants in contrast to non-ionic or anionic surfactants, and decreases when the surfactant concentration decreases as well. Thus, developing new approaches aiming to reduce toxicity and stabilize emulsions is a major area of interest within the field of cosmetic and pharmaceutic industries. Recent studies have been focusing on "surfactant-free" emulsions, where surfactants are replaced by other emulsifying agents, particularly polymers and solid particles (Costa et al., 2019; Albert et al., 2019).

A Pickering emulsion is a surfactant-free emulsion stabilized by colloidal solid particles at the interface of the two immiscible fluids. The emulsion stabilization is based on an irreversible and effective adsorption of solid particles at the surface of emulsion droplets (Winuprasith and Suphantharika, 2015).

Solid nanoparticles have been explored in a wide range of applications, including catalysis, cosmetic, food, oil recovery and therapeutic. (Albert et al., 2019; Yang et al., 2017) Several types of solid particles, either organic or inorganic, can be applied as cost-effective stabilisers of Pickering emulsions (Low et al., 2017). The enormous potential of the solid particles in effectively stabilizing emulsions depends on particle size, morphology, concentration, dual wettability, and particle–particle, particle–water phase and particle–oil phase interactions (Marku et al., 2012; Chevalier and Bolzinger, 2013; Matos et al., 2013). Concerning to particle size, solid nano- and microparticles have been gaining attention as efficient stabilizers of Pickering emulsions due to their unique properties, including small particle size, large surface area, unique composition and architecture (Marto et al., 2016).

Currently, Pickering emulsions have been received considerable attention from cosmetic and pharmaceutical industries in the development of innovative topical formulations or multifunctional delivery systems (Low et al., 2017; Chevalier et al., 2015). The growing interest in Pickering emulsions arises from their significant advantages in opposition to the conventional surfactant-based emulsions, namely superior stabilization against coalescence, minimization of Ostwald ripening phenomena, higher biocompatibility, and low cytotoxicity (Chevalier et al., 2015; Kang et al., 2018; Wang and Wang, 2016).

The aim of this review is to summarize the recent literature describing the potential of several types of organic and organic solid particles in the production of Pickering emulsions with potential dermopharmaceutical and cosmetic applications. Moreover, new insights and concerns related to safety concerns of Pickering emulsions will be discussed.

2. Stabilization parameters for Pickering emulsions

The effectiveness of Pickering emulsion stabilization by solid particles depends on several parameters, namely wettability (Chevalier and Bolzinger, 2013; Binks and Clint, 2002), size (Binks and Lumsdon, 2001; Dickinson, 2012), morphology (Kalashnikova et al., 2012; Luu and Striolo, 2014; Madivala Gurappa et al., 2009; Fujii et al., 2007; de Folter et al., 2014; He et al., 2007), charge (Ridel et al., 2016; Pushpam et al., 2015), surface roughness and coverage (Levine et al., 1989), adsorption at the oil/water interface (Levine et al., 1989; Binks and Kirkland, 2002), and concentration of solid particles (Frelichowska et al., 2010; Binks et al., 2005; Juárez and Whitby, 2012). Additionally, other parameters such as inherent properties of emulsion phases (Thickett and Zetterlund, 2015), the oil phase /aqueous phase ratio (He et al., 2013), salt concentration (Yang et al., 2006), and pH (He et al., 2013; Tang et al., 2014; Zhu et al., 2016), present an important role in Pickering emulsion stabilization.

Wettability of solid particles is point out in several studies as a key parameter for Pickering emulsion stabilization. (Wu and Ma, 2016) Solid particles typically present a dual wettability, which is responsible for their spontaneous accumulation at the interface of oil and water phases. The wettability of solid particles is characterized by the angle formed between the interface and the particle, known as contact angle (θ), which is directly related to the type of the stabilized emulsion. More specifically, when θ is under 90°, solid particles are predominantly hydrophilic and an oil-in-water (O/W) emulsion is obtained is. Contrarily, when θ is above 90°, solid particles are predominantly hydrophobic and a water-in-oil (W/O) emulsion is obtained. In order to achieve a durable emulsion stabilization, the particle wettability should be close to 90°. (Fig. 1) Solid particles can also be employed in the stabilization of water-in-water (W/W) or oil-in-oil (O/O) Pickering emulsions (Binks and Tyowua, 2015; Nicolai and Murray, 2016). Multiple W/O/W, O/W/O (oil-in-water-in-oil) and O/O/O (oil-in-oil-in-oil) Pickering emulsions can be obtained using one or two types of solid particles (Albert et al., 2019; Albert et al., 2018; Tyowua et al., 2017; Binks and Rodrigues, 2003).

The polarity and viscosity of the oil phase can induce alterations in the contact angle, compromising the the type, stabilization, or droplet size of the emulsion obtained. (Albert et al., 2019) For instance, a study conducted by Binks and Lumsdon (Binks and Lumsdon, 2000) showed that solid particles (i.e. silica nanoparticles) characterized by an intermediate wettability enable the stabilization of O/W emulsion composed of non-polar oils (e.g. hydrocarbons) and W/O emulsions composed of polar oils (e.g. esters and alcohols). In this study, the solid nanoparticles presented a hydrophilic character in contact with non-polar oils and a hydrophobic character in in contact with polar oils (Binks and Lumsdon, 2000).

The the oil phase /aqueous phase ratio can disturb the size of the droplets and change the type of emulsions obtained. Some studies have suggested that an increase of the dispersed phase ratio can be followed by a phase inversion (e.g. from W/O to O/W) or a shift in the emulsion type (e.g. from simple to multiple) (He et al., 2013; Tang et al., 2014; Binks and Lumsdon, 2000). When the dispersed phase increases, the interfacial area also increases. The same amount of particles is not able to stabilize a greater interfacial area thus the droplets become larger (Albert et al., 2019). Additionally, the phase where solid particles are dispersed prior to emulsification can determine the type of emulsion obtained. From interaction established between the solid particles and the liquids can result a variation of particles' hydrophobic character. Thus, solid particles with initially similar wetabbility can present a different wettability depending on the liquid where they were dispersed. For example, particles initially dispersed in an oil phase often give rise to W/O (Albert et al., 2019; Binks and Lumsdon, 2000; Yan et al., 2001).

Pickering emulsion stabilization depend on the particle adsorption at the oil/water interface, as mentioned before. The adsorption free energy is the energy necessary to remove a solid particle with a spherical shape from the interface of the emulsion (Albert et al., 2019; Levine et al., 1989). Therefore, Pickering emulsions are more stable than conventional emulsions, since the adsorption of the particles at the oil/water interface is irreversible due to the high energy required to remove the particles, which can be up to 10^8-fold higher than the thermal energy (Wu and Ma, 2016; Benhamou and El-Moudny, 2017). A specific radius, contact angle and interfacial tension are determinant to the adsorption

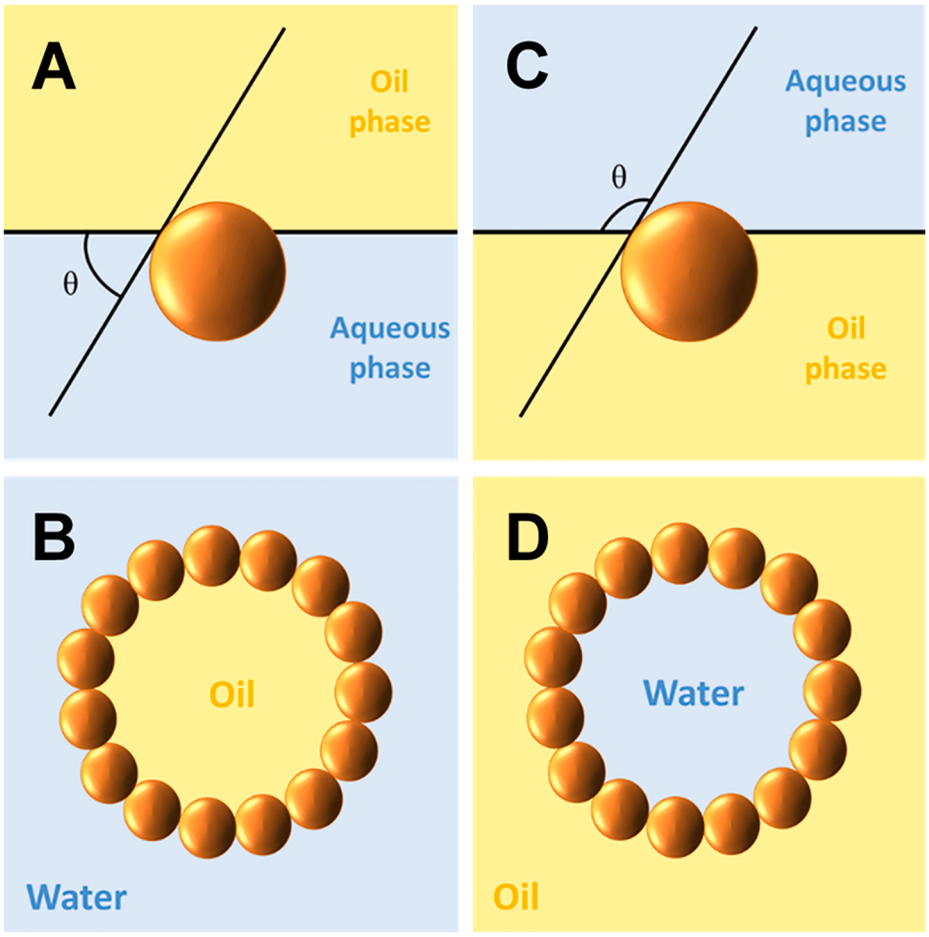

Fig. 1. Schematic representation of the wettability mechanism leading to formation of water-in-oil (W/O) and oil-in-water (O/W) Pickering emulsions. (A) Solid particle wetting and contact angle (θ) < 90° leading to formation of oil-in-water (O/W) emulsions. (B) A O/W emulsion is obtained when θ < 90° (O/W). (C) Solid particle wetting and θ greater than 90° leading to formation of water-in-oil (W/O) emulsions. (D) A W/O emulsion is obtained when θ greater than 90° (W/O).

energy. The highest adsorption energy is achieved when θ is 90°. Particles with larger size need higher adsorption energy, when compared to smaller particles with the same θ. The rate of particle adsorption is important as well because if it is not faster than the rate of coalescence, the droplets will coalesce rather than being stabilized (Albert et al., 2019).

Particle concentration is correlated with the droplet size. When the particle concentration increases, the interfacial coverage improves, and the size of the droplets decreases homogenously, a process called "limited coalescence". Nevertheless, when the particle concentration is too high, it enables a fast emulsification with heterogeneous droplet sizes or may even cause phase inversion. Conversely, at low particle concentration, the stabilization is not achieved because the particles are not sufficient to stabilize the droplets (Albert et al., 2019).

The particle size also has an impact on the stabilization of the emulsion and the size of the droplet (Albert et al., 2019). The size of the stabilizing particles can be in the nanoscale or in the microscale, thus the solid particles can be nanoparticles or microparticles (Marto et al., 2016). The increase in the size of the particle is accompanied by an increase in the size of the droplet (Albert et al., 2019). As mentioned before, the size of the particles influences the adsorption energy, which in turn, affects the stabilization. The larger particles have slower adsorption kinetics and, consequently, create barriers with high adsorption energy and weaker packing efficiency over the droplet surface (Wu and Ma, 2016).

Particles of different shapes can be used to stabilize Pickering emulsions, including dumbbell, flake, ellipsoid, cylinder, wire, (Yang et al., 2017) tube, ribbon, (Du et al., 2019) sheet, (Salerno et al., 2016) rods, cubes, and deformable nanogel; the most used are spherical particles (Albert et al., 2019). The particle shape affects the stabilization of the emulsion, with different shapes leading to different stabilization mechanisms. The particle arrangement, how they adsorbed at the interface and the aspect ratio play a role in the stabilization of non-spherical particles. The higher the aspect ratio the greater the stabilization (Albert et al., 2019). In addition, features such as flexibility, conformation, multilayer creation at the interface or all atoms of the particle contribute to stabilization, allowing 2D structures to increase the coverage around the droplet (Gonzalez Ortiz et al., 2020).

Particle surface roughness plays a significant role in the wettability. However, the effect of roughness in the stability of the emulsion needs further research, since it was observed that roughness may cause a decrease but also an increase of the contact surface of the particles with the interface (Albert et al., 2019).

Particle surface charge is a parameter with influence in the Pickering emulsions stabilization when the solid particles present a weak adsorption at the interface. Several studies have demonstrated that a reduction of particle surface charge leads to an improvement in emulsion stability (Ridel et al., 2016). On the other hand, the existence of electrostatic repulsions between particles and droplets results in a decreased of adsorption rate and increased the system instability (Albert et al., 2019).

Changes in salt concentration or pH also significantly affects the particle surface charge and their contact angle. The variation of these parameters has been widely studied, in order to control the emulsion

stability. The change of salt concentration or pH can convert repulsive into attractive interactions between particles. An increase of salt concentration can improve the efficiency of the emulsion and induce a pH change in pH-responsive particles, increasing their stability (Albert et al., 2019). Additionally, pH changes can ionize the surface groups of the particles modifying their hydrophobicity. Therefore, the pH variations can tune the adsorption mechanism or even shift the emulsion type (Gonzalez Ortiz et al., 2020).

In summary, during the Pickering emulsions' development process, it is imperative to consider all the important previously mentioned parameters related with the solid particles (i.e. size, morphology, charge, surface roughness and coverage, adsorption at the oil/water interface, and concentration) and with the emulsion phases (i.e. properties of emulsion phases, the oil phase/aqueous phase ratio, salt concentration, and pH).

3. Stabilizers of pickering emulsions for topical application

Topical application concerns the application of formulations on external surfaces such as hair, mucosa or skin to deliver the active ingredient directly to the desired site of action obtaining a local effect and thereby avoiding the potential for systemic side effects. Systemic treatment may be needed if the hair, mucosa or skin diseases are severe or fail to respond to topical therapy (Walters, 2002).

3.1. Solid particles

Several particles have been widely studied as emulsion stabilizers

Table 1
Nano-stabilizers for topical application.

Particle	Type of PE	Particle Size (nm)	Droplet size	Viscosity (mPa.s)	Zeta potential (mV)	Wettability θ (°)	Mechanism	Ref.
Inorganic								
Silica dimethyl silylate	W/O	~20 nm	9.7 ± 0.5 μm	550 ± 50 (at 20 °C)	–	151.4 ± 1.3	Hydrogen bonds are formed between the hydrophilic active ingredient and silica. Silica produces a shell around water droplets.	(Frelichowska et al., 2009)
Fumed silica nanoparticles	O/W	15–20 nm	3 ± 1 μm	6 ± 2 (at 20 °C)	–	–	Formation of silica aggregates and formation of a shell around oil droplets.	(Frelichowska et al., 2009)
		~7 nm	166.5–261.6 nm	–	−49.9–52 or + 32–35.6	–	Fumed silica nanoparticles coat oleylamine or lecithin to adsorb to the emulsion interface.	(Eskandar et al., 2009)
		~7 nm	166.5–361.5 nm	–	−39.5–40.6 or + 48.1–52.2	159 ± 1 or 162 ± 3.4		(Eskandar et al., 2010)
		20 nm	2.7 μm	26 (at 35 °C)	–	–	Fumed silica nanoparticles adsorb the oil–water interface and the decontaminant.	(Salerno et al., 2016)
Triethoxycaprylylsilane titanium dioxide	W/O	d(90) = 7.1 ± 0.3 μm	d(50) = 6.2 ± 4.1 μm or 5.7 ± 4.3 μm	non-Newtonian fluid	–	106.5 ± 0.7	Triethoxycaprylylsilane modifies TiO_2 to make it hydrophobic in order to be adsorb at droplets surface.	(Marto et al., 2016)
Fuller's Earth	O/W	10 –15 μm	d(50) = 58 μm	13 (at 35 °C)	–	–	FE adsorbs at the oil–water interface and adsorbs the decontaminant	(Salerno et al., 2016)
Organic								
PCL-*b*-PEG	O/W	32 nm	3±0.4 μm	15.2–15.3 (at 20°C)	–	–	PEG provides hydrophobic properties to polymer in the core (PCL or PLA).	(Laredj-Bourezg et al., 2017)
PLA-*b*-PEG		50 nm	2–2.7 μm	14.5–14.7 (at 20°C)	–	–		
PLGA/PSS	O/W	105–115 nm Up to 425 nm	~2 μm	–	− 30–57.5	–	PSS modifies the charge density of PLGA to adsorb and create a shell around the droplet.	(Wei et al., 2020)
Aluminium starch octenylsuccinate	W/O	d(90)= 20.85±0.02 μm	150 μm	non-Newtonian fluid	–	109.0±0.4	Aluminium octenylsuccinate confers hydrophobicity to the starch.	(Marto et al., 2015)
Starch modified by OSA	O/W	1–2 μm	30–75 μm	–	–	4.9–13	Droplet adsorption by starch monolayer or starch aggregates	(Marku et al., 2012)
Self-aggregated chitosan particles	O/W	287.3 nm	5.8±1.1 to 18.7±3.4 μm	2.07, 7.81 and 37.2 (at 25°C)	−48.1±4.7 to −78.4±4.1	42.47±1.19	At pH>pKa chitosan deprotonates and become more hydrophobic	(Asfour et al., 2017)
Chitosan and Gum Arabic	O/W	~109 nm	17.11 and 23.98 μm	non-Newtonian fluid	+56.3	89.2±0.94	Chitosan/GA nanoparticles create a well-defined layer over the oil droplet.	(Sharkawy et al., 2019; Sharkawy et al., 2020)
Cyclodextrin	O/W	30 - 250 nm	9.2–16.1 μm	non-Newtonian fluid	–	–	CD/oil complex aggregates to adsorb the emulsion interface.	(Leclercq and Nardello-Rataj, 2016)
Quercus Suber Bark	W/O	d(90)= 91.4±0.3 μm	d(90)= 182±1.3 μm	non-Newtonian fluid	–	97.3±0.3°	–	(Carrico et al., 2019)

***Abbreviations: PE – Pickering Emulsion; OSA- Octenyl Succinic Anhydride; W/O – Water in oil emulsion; O/W – Oil in water emulsion; TiO_2 – Titanium dioxide; PCL-*b*-PEG – Poly(ε-caprolactone)-block-poly(ethylene glyco)l; FE - Fuller's Earth; PLA-*b*-PEG – Poly(lactic acid)-block-poly(ethylene glycol); PEG – Poly(ethylene glycol); PCL – Poly(ε-caprolactone); PLA – Poly(lactic acid); PLGA/PSS – Polymer poly(lactide-coglycolide)/ poly(styrene-co-4-styrene-sulfonate); PSS – Poly(styrene-co-4-styrene-sulfonate); PLGA – Poly(lactide-coglycolide); GA – Gum Arabic; CD – Cyclodextrin.

(Winuprasith and Suphantharika, 2015). Nano- and microparticles are an excellent choice for topical applications because of their customizable size and surface polarity that enhance skin penetration. Also, they can create an occlusive layer and extend retention time, which is advantageous for drug delivery (Marto et al., 2016).

In this section, solid nano- and microparticles used as stabilizers for topical Pickering emulsions are divided into organic or inorganic particles, according to their chemical structure. These solid particles are summarized in Table 1. Table 2, containing interesting examples challenges and recent discoveries related to topical applications of Pickering emulsions, summarizing in the cosmetic and dermopharmaceutical fields.

3.1.1. Inorganic particles

3.1.1.1. Silica. Over the last few years, studies have been conducted on inorganic particles like silica (Lam et al., 2014). Silica, another name for silicon dioxide, can be divided into three main categories: amorphous silica, synthesized amorphous silica and crystalline silica. The synthetic process of amorphous silica generates precipitated silica, silica gel, colloidal silica and fumed silica. Only fumed silica is obtained by a thermal method, the others are obtained via wet methods. Silica nanoparticles have well-defined shapes, in a range of sizes, with tight distribution (Lam et al., 2014). These amorphous silica nanoparticles can also be surface modified (Fruijtier-Pölloth, 2012) by physical or chemical processes (Arriagada et al., 2020). Silica can be physically modified through adsorption of different compounds such as surfactants, polymers or salts, as well as polymers, pigments, natural polyphenols and other substances. Examples of chemical changes include the addition of chemical groups, such as dimethylsilyl or hydroxyl groups, in order to provide hydrophobicity or hydrophilicity, respectively. Silica can undergo modification during its synthesis process so that some properties of these nanoparticles can be improved. Modified silica particles are porous, allowing the incorporation of a great volume of active ingredient, because of the large specific surface available (Arriagada et al., 2020). Unmodified silica is most likely to stabilize O/W emulsions due to the hydrophilicity of silanol groups. Modified silica acquires hydrophobic groups at its surface and, as a result, these particles will primarily stabilize W/O emulsion (Yang et al., 2017; Fruijtier-Pölloth, 2012).

Frelichowska et al. (2009) described the modification of silica by addition of dimethylsilyl groups for hydrophobicity, and hydroxyl groups for surface hydrophilicity (Fig. 2). This modification allowed silica particles to bind caffeine, a hydrophilic drug, via hydrogen bonding. The modified silica particles created a shell around the droplets containing caffeine, acting like a barrier, which resulted in a much slower caffeine release into the skin in comparison with conventional emulsions (Fig. 3C). This phenomenon is only possible if the amount of silica particles is enough to cover all the droplets. Additionally, the modification permits interactions between surface hydrophobic groups from silica and lipids from the outermost layer of the skin, promoting an easier skin penetration and caffeine transport (Fig. 3A). The physicochemical characteristics of the silica particles used in this study (20 nm of size, 9.7 ± 0.5 μm droplet size, 151.4 ± 1.3° wettability in a W/O Pickering emulsion with 550 ± 50 mPa.s at 20 °C), allowed a sustained release behaviour. As a result, the emulsion skin permeation was increased 3-fold and there were 2-fold more caffeine accumulated in the receptor fluid when compared to a conventional emulsion, which proves that this hydrophilic active ingredient permeates properly through the epidermis and dermis (Fig. 3) (Frelichowska et al., 2009).

In a subsequent study, Frelichowska et al. (2009) used fumed silica nanoparticles ranging from 15 to 20 nm to stabilize O/W emulsions with 6 ± 2 mPa.s at 20 °C, with the aim to deliver the lipophilic *all-trans* retinol to the skin. The silica was hydrophobized by the addition of dichlorodimethylsilane grafts, to provide partial wetting in oil and water phases. A large amount of silica was employed in this formulation which led to a full coverage of oil droplets by silica aggregates. This phenomenon occurs because the silica nanoparticles have a strong affinity for the interface of the emulsion. The rigid shell that the modified silica creates around the droplets stabilizes the emulsion even when it is applied to the skin. Moreover, the improved stability enables the intact droplets to cross the SC, like microcapsules of 3 ± 1 μm, in size. The penetration of retinol from Pickering emulsions and conventional emulsions was similar but increased approximately 5-fold in both emulsions, in comparison with the retinol solution. Retinol present in the Pickering emulsion was mostly distributed and retained in the outermost layer of the skin, 5-fold more than with the conventional emulsion. Therefore, the Pickering emulsion provided an improvement in active ingredient retention in the SC, as well as a sustained release behaviour (Frelichowska et al., 2009).

Eskandar et al., 2009; Eskandar et al., 2010) also showed that Pickering emulsions can be stabilized by fumed silica particles. In two different works, fumed silica was used to cover oleylamine or lecithin, two surfactants, in order to achieve a synergistic effect in the O/W emulsion stabilization as well as in the emulsification efficiency. The interactions between fumed silica and oil charged droplets led to a partial coverage of the negatively charged lecithin, and a strong coverage of the positively charged oleylamine. In these two studies, the fumed silica had a mean diameter of 7 nm. In the first study, the droplet size ranged from 166.5 to 261.6 nm, while the zeta potential was between −49.9 and −52 mV for the lecithin emulsion, and between + 32 and + 35.6 mV for the oleylamine emulsion (Marku et al., 2012). In the second study the droplet size range was from 166.5 to 361.5 nm, while the zeta potentials varied between −39.5 and −40.6 mV, or from + 48.1to + 52.2 mV, for the lecithin and oleylamine emulsions, respectively (Eskandar et al., 2009; Eskandar et al., 2010). In the first study the skin delivery of *all-trans* retinol was evaluated. The retinol skin retention in Pickering emulsion with lecithin was 12.6 times higher than the control emulsion of lecithin, while the Pickering emulsion with oleylamine provided a 3 to 4-fold higher retinol skin retention, compared to the lecithin Pickering emulsion. The use of fumed silica nanoparticles enabled a sustained release of the active ingredient from both Pickering emulsions, in the Pickering emulsion with lecithin the release decreased 3.6 times and with oleylamine reduced 1.28 times. Also, this inorganic particle-stabilized the emulsions in the long-term and had an impact on the skin hydration (Eskandar et al., 2009).

In the second study, the Pickering emulsions were used to deliver acridine orange 10-nonyl bromide (AONB), a fluorescent probe. The contact angle was 159 ± 1° for lecithin and 162 ± 3.4° for oleylamine. These results oppose the statement that only particles with contact angle <90° stabilize O/W emulsion. The results also showed an improvement of AONB skin retention, 2-fold higher for lecithin and oleylamine compared to their controls, and 5- to 10-fold higher for oleylamine when compared to lecithin. Furthermore, when fumed silica particles were used, the skin penetration was enhanced 2-fold in lecithin and 1.18 times in oleylamine (Eskandar et al., 2010).

Arslan Azizoglu et al. (2017) used silica nanoparticles to stabilize an O/W Pickering emulsion with melatonin as the active ingredient component, to be used as a sunscreen (Arslan Azizoglu et al., 2017). UV radiation, especially the UVB type, can modify or induce dermal cells (e.g., keratinocytes, melanocytes and fibroblasts) to synthesize melatonin. This substance has anti-inflammatory, immunomodulatory and antioxidant action. It is also a scavenger of reactive oxygen species (ROS), radicals that may induce mutagenicity and carcinogenicity. The addition of melatonin to the sunscreen provides therapeutic properties to this product (Marto et al., 2016). The formulation developed was composed by a Pickering emulsion with octyl methoxycinnamate (Sarker et al., 2017), a UV filter, and silica as a stabilizer, to which melatonin-loaded niosomes were added. The beneficial properties of melatonin are achieved when it reaches the deeper layers of the epidermis, while OMC should remain in the epidermis outer layers. *Ex vivo* studies showed a 5.57-fold increase in melatonin penetration and 1.7-fold increase of skin

Table 2
Pickering emulsions for topical application.

Therapeutic application	Nano-stabilizer	Type of PE	Active ingredient	Output	Ref.
Active ingredient delivery	Silica dimethyl silylate	W/O	Caffeine	PE increased skin permeation 3-fold; Caffeine accumulated twice more in receptor fluid when in PE Caffeine had good skin permeation.	(Frelichowska et al., 2009)
	Fumed silica nanoparticles	O/W	AONB	Lecithin and oleylamine PEs increased 2-fold when compared to their controls; Oleylamine PE was 5 to 10-fold higher in comparison with lecithin PE.	(Eskandar et al., 2010)
		O/W	*All-trans* retinol	Retinol penetration in PE was 5-fold higher when compared to retinol in solution; PE retinol distribution was 5-fold higher in the *stratum corneum* when compared to conventional emulsion.	(Frelichowska et al., 2009)
				Retinol skin retention in PE with lecithin was 12.6 times higher than the control emulsion of lecithin; PE with oleylamine had 3-fold to 4-fold higher retinol skin retention compared with lecithin PE; Lecithin PE drug release was 3.6 times slower; Oleylamine PE drug release 1.28 times slower.	(Ghouchi Eskandar et al., 2009)
	PCL-*b*-PEG	O/W	*All-trans* retinol	PE enhanced retinol skin penetration 1.77-fold when compared to the conventional emulsion; Retinol distribution in the second method was 1.27-fold higher than conventional emulsion.	(Laredj-Bourezg et al., 2017)
	PLA-*b*-PEG			PE enhanced retinol skin penetration 2.22-fold when compared to the conventional emulsion; Retinol distribution in the second method was 1.4-fold higher than conventional emulsion.	
Skin decontamination	Fumed silica Nanoparticles Fuller's Earth	O/W	Fumed silica nanoparticles Fuller's Earth	PE showed equivalent decontamination efficiency in comparison to water suspension. PE decreased the contaminant amount by 2-fold when compared to the suspension; PE decreased 3.8-fold when compared with the skin without treatment.	(Salerno et al., 2016)
Wound healing	Self-aggregated chitosan particles	O/W	Rutin	PE drug release efficiency improved between 1.45 and 1.6 times compared with rutin in suspension; PE with rutin enhanced wound healing 1.17 times when compared with PE without rutin and 2-fold compared to the untreated wound; PE without rutin increased the wound healing process 1.7-fold when compared to the untreated wound; PE decreased 1.2-fold the MDA amount and increased 1.6 times the GSH levels and 35 times the CAT levels; HA concentration increased 1.46-fold, and Col I increased 1.2-fold in comparison with untreated wound.	(Asfour et al., 2017)
Antibacterial	Aluminium starch octenylsuccinate	W/O	Minocycline hydrochloride	The PE with MH increased the inhibition zone up to 1.7-fold in comparison with MH in solution and significantly decreased the colony-forming units (CFU)/mL when compared with untreated skin; ASt particles proved an enhanced of wound healing; all PE led to epidermis re-epithelization and decreased inflammatory activity, but this are more accentuated in PE with MH.	(Marto et al., 2019)
Antifungal	Modified starch	O/W	Thymol oil	α-amylase enhanced the amphotericin B and thymol release; MFC decreased up to 0.5 for thymol and 0.8 for amphotericin B, when α-amilase was 100 U/mL.	(Cossu et al., 2015)
Antifungal and antibacterial	Cyclodextrin	O/W	Econazole nitrate	The commercial formulation with econazole nitrate and PE stabilized by α-CD and β-CD had identical action; PE with γ-CD as stabilizer exhibit minimal or no biocidal activity, due to inability of econazole nitrate to diffuse from the CD.	(Leclercq and Nardello-Rataj, 2016)
			Miconazoctylium bromide	PE was 2-fold more effective against *C. albicans* and methicillin-resistant *S. aureus* than the commercial formulation; PE had high activity on *E. coli*, in contrast to the commercial one that is inactive for this bacterium.	(Leclercq et al., 2020)
	Silica nanoparticles	O/W	Chamomile oil	PE MIC90 results showed a mean of 14 times lower concentration in comparison with ethanolic solution; PE MIC90 results showed a range from 1.5 to 2.36 times of lower concentration in bacteria and from 1.85 to 3.67 times of lower concentration in fungi when compared to conventional emulsion: MEC10 outcome exhibited 1.04–2.64 times lower	(Das et al., 2019)

(*continued on next page*)

Table 2 (*continued*)

Therapeutic application	Nano-stabilizer	Type of PE	Active ingredient	Output	Ref.
				concentration in bacteria and 1.79–4-fold in fungi when compared to conventional emulsion.	
Sunscreen	Triethoxycaprylylsilane titanium dioxide	W/O	Triethoxycaprylylsilane titanium dioxide Zinc oxide Aluminum starch octenylsuccinate Green coffee oil	Formulation with starch increased the SPF value about 2-fold; both formulations were water resistant.	(Marto et al., 2016)
			Triethoxycaprylylsilane titanium dioxide Zinc oxide Aluminum starch octenylsuccinate Green coffee oil Melatonin	Formulation with melatonin had a 1.09-times lower SPF value; melatonin potentiated photoprotection by eliminating ROS from cells.	(Marto et al., 2016)
Sunscreen	Silica Nanoparticles	O/W	Melatonin Octyl methoxycinnamate	PE enhanced 5.57 times the melatonin penetration when compared to melatonin in niosomes; PE improved the skin accumulation 1.7 times compared to melatonin in niosomes; Melatonin in PE had high antioxidant activity.	(Arslan Azizoglu et al., 2017)
Antioxidant activity	PLGA/PSS	O/W	Tocopheryl acetate	The EC50 of free TA or in the PE were similar, thus, the TA in PE preserves its activity; The antioxidant activity retention was higher for PE when compared to conventional emulsion; PE decreased 1.18-fold the intensity of cellular fluorescence in comparison with free TA, indicating a greater antioxidant activity.	(Wei et al., 2020)
	Solid core-mesoporous shell silica nanoparticles		Carminic acid Tocopheryl acetate	In acetone CA linked to CSSNPs increased the antioxidant activity over free CA by 2-fold; in deuterium oxide CA linked to CSSNPs improved the antioxidant activity over free CA by 11-fold; PE stabilized with CSSNPs and CA had a 2.59 times lower vitamin E oxidation when compared to a conventional emulsion.	(Arriagada et al., 2020)
	Quercus Suber Bark	W/O	Quercus Suber Bark	The percentage of reduction of ROS was slightly lower, in comparison to ascorbic acid; The formulation exhibited anti-elastase activity.	(Carrico et al., 2019)
Anti-ageing	Chitosan and Gum Arabic	O/W	*Trans*-resveratrol	The resveratrol retention increased up to 3.76-fold and permeation decreased up to 5.28-fold when compared with the solution; PE photodegradation was zero while the remaining ingredients in the control solution decreased 1.47-fold	(Sharkawy et al., 2020)
	Modified silica dioxide	W/O	5′-AMP	MIPs had a maximum adsorbed capacity 4.7-fold higher and a binding constant 5.18 times higher than NIPs; MIPs retained the 5′-AMP allowing a sustained release by diffusion.	(Ayari et al., 2019)
j	Fumed silica nanoparticles	O/O/W	Rutin	Sustained release 1.2 times lower than from the ethanol solution; skin permeation increased 2.394-fold in comparison with aqueous solution.	(Wang et al., 2017)
Skin penetration vehicle	OSA quinoa starch	O/W	Paraffin Oil	The skin penetration in PEs with methyl salicylate was 2-fold higher when compared to methyl salicylate in buffer solutions.	(Marku et al., 2012)

****Abbreviations: PE – Pickering Emulsion; PCL-*b*-PEG – poly(ε-caprolactone)-block-polyethyleneglycol; PLA-*b*-PEG - Poly(lactic acid)-block-polyethyleneglycol; PLGA/PSS - Polymer poly(lactide-coglycolide)/ poly(styrene-co-4-styrene-sulfonate); OSA- Octenyl Succinic Anhydride; W/O – Water in oil emulsion; O/W – Oil in water emulsion; O/O/W - Oil-in-Oil-in-Water; AONB - Acridine orange 10-nonyl bromide; 5′-AMP – 5′-Adenosine Monophosphate; MDA – Malondialdehyde; GSH - Reduced Glutathione; CAT – Catalase; HA - Hyaluronic Acid; Col I - Collagen Type I; MH - Minocycline Hydrochloride; CFU - Colony-Forming Unit; ASt - Aluminum Starch Octenylsuccinate; MFC - Minimum Fungicidal Concentration; CD – Cyclodextrin; MIC - Minimum Inhibitory Concentration; MEC - Minimum Effective Concentration; SPF – Sun Protection Factor; ROS - Reactive Oxygen Species; EC50 - Median Effective Concentration; TA - Tocopheryl Acetate; CA - Carminic Acid; CSSNPs - Solid core-mesoporous shell silica nanoparticles; NIP - Non-Imprinted Polymer

accumulation in comparison with the formulation composed only by niosomes and melatonin. The antioxidant activity of melatonin in Pickering emulsions was similar to that of the melatonin solution dispersed in ethanol, both significantly high (Arslan Azizoglu et al., 2017).

Ayari et al. (2019) developed an anti-ageing formulation using molecularly imprinted polymers (MIPs) in a W/O Pickering emulsion to regulate the release of 5′-adenosine monophosphate (5′-AMP). This nucleotide is widely distributed in the human body and, when released in the epidermal layer, it accelerates its turnover. This turnover is linked to the increased amount in 5′-adenosine triphosphate (5′-ATP), required for cell division and renovation, which are processes that slow as skin ages. Through a process of derivatization, silica dioxide acquires hydrophobic properties, allowing the preparation of the Pickering emulsion by inverse polymerization. Trials were performed to evaluate MIPs activity and the results showed that Pickering emulsions with these polymers had a 4.7-fold higher maximum adsorbed capacity, and a 5.18-fold increased binding constant, compared to the formulation with non-imprinted polymers (NIPs). The high value of the binding constant suggests a possible controlled release. The bioactive compound release was studied, and it was confirmed that the MIPs retained the 5′-AMP in their cavities allowing a controlled release by diffusion. This sustained-

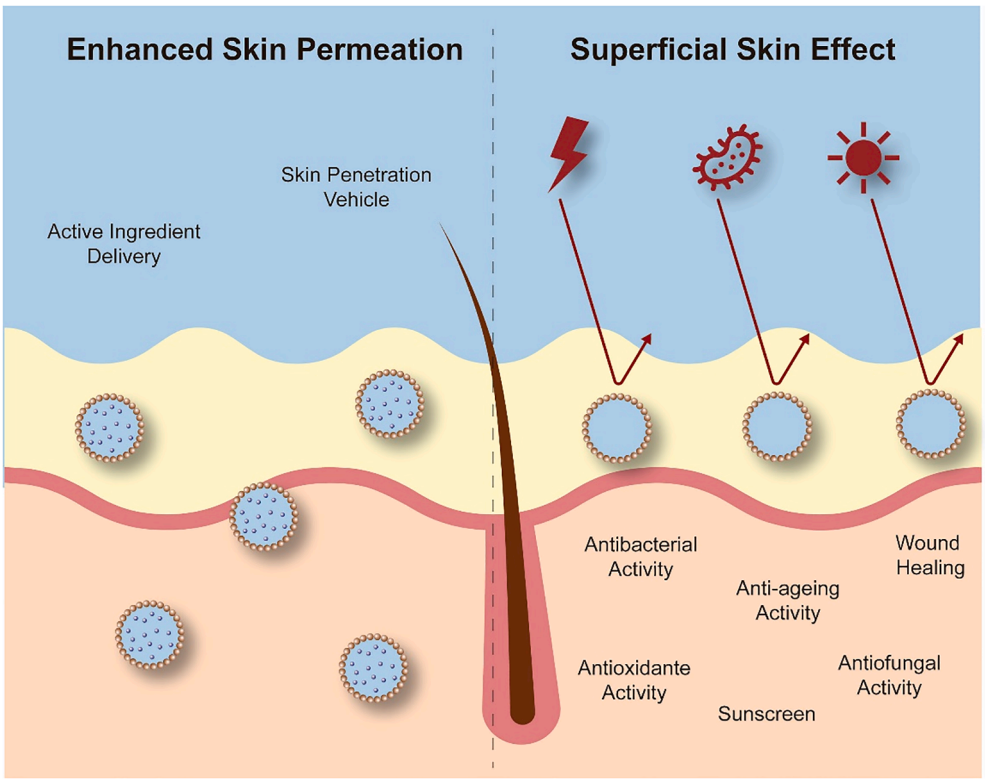

Fig. 2. Comparative scheme of an enhanced skin permeation (left) and a superficial skin effect (right) application based on Pickering emulsions. The left side of the illustration shows a dermal deep action of Pickering emulsions, such as water-in-oil (W/O) emulsion stabilized by silica nanoparticles (orange spheres) to deliver caffeine (purple spheres). The right side of the figure exemplifies a dermal superficial action like, for example, the mechanism of the protective action of physical sunscreens, in which the W/O emulsion is stabilized by triethoxycaprylylsilane titanium dioxide (orange spheres). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

release prevented the burst phenomenon, which occurred with the NIPs (Fig. 4**)** (Ayari et al., 2019).

Arriagada et al. (2020) developed solid core-mesoporous shell silica nanoparticles (CSSNPs) to stabilize Pickering emulsions. First, the silica nanoparticles formed dense cores, which were coated by mesoporous silica. Then, the CSSNPs obtained were functionalized by (3-aminopropyl)triethoxysilane, and then carminic acid (CA) was linked to the CSSNPs shell. CA is a known pigment commonly used in the food, cosmetic and pharmaceutical fields, that confers different shades of red to the formulation by changing pH. In addition, this compound has an antioxidant activity. The synthesis of mesoporous shell and dense cores of silica enables obtaining an ideal particle density for adsorption at the O/W emulsion interface because these cores have the area required to incorporate a large number of molecules. The antioxidant activity was evaluated by the quenching rate of singlet oxygen and the results showed that CA linked to CSSNPs had a 2-fold increased quenching rate compared to free CA, when in acetone. Furthermore, when in deuterium oxide, the quenching rate improved 11-fold. The Pickering emulsion stabilized with CSSNPs and CA also showed a superior protective effect on vitamin E, decreasing its oxidation 2.59 times in comparison with a conventional emulsion. (Arriagada et al., 2020)

A study by Das et al. (2019) described the stabilizing effect of modified silica nanoparticles on an O/W Pickering emulsion with chamomile essential oil. Essential oils have antifungal and antibacterial activity, so the aim of this study was to study the effect of the stabilization on the antimicrobial activity of the Pickering emulsions. This activity was evaluated by the minimum effective concentration (Abu Samaan et al., 2019) and minimum inhibitory concentration (MIC). The MIC results showed a 14-fold lower concentration of chamomile oil in Pickering emulsion in comparison with chamomile oil in ethanolic solution, and from 1.5 to 2.36 times less concentration for bacteria and 1.85 to 3.67 times less concentration for fungi when compared to a conventional emulsion. The MEC results showed that a 1.04 to 2.64 times lower concentration of chamomile oil in Pickering emulsion was needed to achieve the same antibacterial action of the oil in the conventional emulsion. The same antifungal activity was obtained with a 1.79 to 4-fold lower concentration of chamomile oil in Pickering emulsion, compared to the conventional emulsion. Therefore, chamomile oil Pickering emulsion had an effective antimicrobial activity, predicting an interesting application for this formulation. However,

in vitro and *in vivo* trials should be done in order to prove their efficiency as a therapeutic formulation (Das et al., 2019).

Silica was also studied to be a stimuli-responsive stabilizer. Stimuli-response Pickering emulsions are stable emulsions in certain conditions and unstable in others, simplifying demulsification processes and enabling the control of these formulations. In a study by Zhang et al. (2017) the silica particles were functionalized by selenium-containing 11-(benzylselanyl)-*N,N*-dimethylundecan-1-amine, in order to be redox- and CO_2-responsive. In the absence of CO_2, the oil phase was not emulsified into the water phase, while in the presence of CO_2 an O/W Pickering emulsion was formed. Additionally, when CO_2 was removed the formulation was demulsified and after CO_2 replacement it emulsified again. The same phenomenon was observed for the redox stimulus: in the presence of H_2O_2, a very stable Pickering emulsion was obtained. These stimuli-responsive silica nanoparticles represent a very interesting and potential application for the cosmetics and pharmaceutical fields Zhang et al. (2017).

3.1.1.2. Titanium dioxide. Titanium dioxide (TiO_2) contains hydroxyl groups which confer hydrophilicity to its surface (Rossano et al., 2014). TiO_2 comprises three polymorphic forms named rutile, anatase and brookite. One of the parameters that may affect the physicochemical properties of the particles, hence their activity, is the variation in surface characteristics. Anatase is one of the crystal forms of TiO_2, chemically more reactive than rutile and brookite and generating more ROS than rutile, which is also a crystalline form of TiO_2. Anatase also presents a higher toxicity potential, when compared to rutile (Marto et al., 2016). TiO_2 has been a common ingredient in sunscreens for over 25 years, due to its safety and effectiveness. It is included in formulations with SPF and used by people with a high tendency to present skin irritation. Thus,

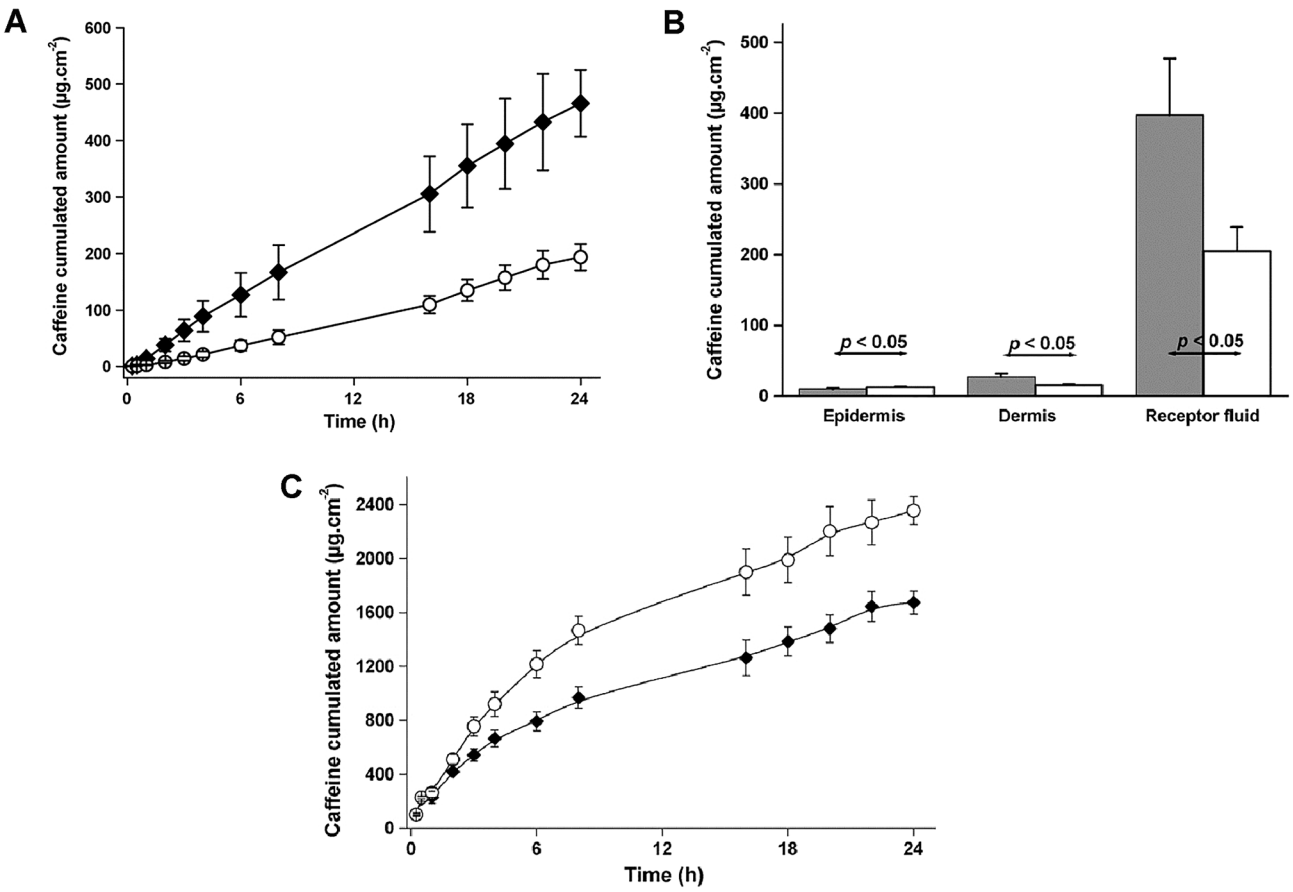

Fig. 3. (A) Permeation profile of caffeine (µgcm − 2) from Pickering emulsion (◆, n = 6) and classical emulsion (○, n = 6) over 24 h. Each point represents the mean ± S.E. of six determinations. (B) Distribution of caffeine in the skin layers from both emulsions in the excised pig skin after 24 h. Grey bars: Pickering emulsion and white bars: classical emulsion. Mean ± S.E., n = 6. (C) Release profile of caffeine from Pickering emulsion (◆, n = 6) and classical emulsion (○, n = 6). Plot of the amount of caffeine (µgcm − 2) permeated from water droplets to bulk aqueous phase vs. time (h); mean ± S.E. n. Reproduced with permission from (Frelichowska et al., 2009). Copyright Elsevier, 2021

using TiO_2 as a stabilizer of Pickering emulsions, will add SPF properties to the formulation (Marto et al., 2016). The modification of TiO_2 particles further improves their performance and compatibility. Two main modifications are used in cosmetic applications: the inclusion of aluminium oxide graft, and the inclusion of silane, stearic acid or dimethicone. The first modification decreases the production of ROS and photocatalytic activity by TiO_2, while the second one allows the incorporation of hydrophobic groups into the surface (Rossano et al., 2014).

Marto et al. (2016) used triethoxycaprylylsilane TiO_2 as a stabilizer in a Pickering emulsion (Fig. 2). This formulation was composed by triethoxycaprylylsilane TiO_2, zinc oxide, aluminum starch octenylsuccinate (ASt) and green coffee oil. The TiO_2 was modified by triethoxycaprylylsilane, in order to stabilize the W/O emulsion. Once incorporated in an emulsion, the stabilization with TiO_2 particles occurs by adsorption of these particles at droplet's surface. The corresponding to 90% percentile of the size distribution of the particles was 7.1 ± 0.3 µm and the corresponding to 50% percentile for the droplets' size was 6.2 ± 4.1 µm for the emulsion without (ASt), and 5.7 ± 4.3 µm for the emulsion with ASt. The contact angle of TiO_2 for this emulsion was 106.5 ± 0.7° and the formulation acquired a non-Newtonian behaviour. A non-Newtonian fluid is characterized by having a viscosity dependent on the shear rate. The TiO_2 also showed a UVB filter action, while the zinc oxide present in the formulation protected more against UVA radiation. *In vivo* and *in vitro* studies demonstrated that this sunscreen formulation improved the protection against both UV radiations, in particular when the antioxidant green coffee oil and ASt were added. In this case, ASt only acted as a promoter and not as a stabilizer. The SPF value of the formulation with ASt increased about 2-fold. Moreover, ASt ensures skin spreadability of the formulation, which further contributes to better protection against the harmful effects of UV radiation. The formulations with and without ASt were considered water-resistant and proved to be safe and stable for topical application (Marto et al., 2016).

Marto et al. (2016) also developed a sunscreen formulation with melatonin. This W/O Pickering emulsion was stabilized by triethoxycaprylylsilane TiO_2, like in the previously described study. The formulation with melatonin enhances sunscreen photoprotection by eliminating ROS from the skin cells, which prevents cell damage, stopping skin cancer induced by UV radiation. *In vitro* and *in vivo* studies showed that melatonin delivery was accomplished with a high protection against UV radiations (UVA and UVB) and ROS. Thus, this active ingredient might be advantageous for sun protection (Marto et al., 2016).

3.1.1.3. Clays. Clays are another popular type of solid particles used for the stabilization of Pickering emulsions. The use of clays present several advantages, such as being non-pollutant, having low-cost, and being easily accessible. However, its strong surface hydrophilicity demands a surface modification, in order to adsorb properly the two phases at the interface (Yang et al., 2017).

In a study by Salerno et al. (2016), the solid particles used as stabilizers were fumed silica nanoparticles and Fuller's earth (FE), which is mainly composed of montmorillonite (MMT) (Salerno et al., 2016). MMT is a clay particle characterized by environmental sustainability, a high specificity in surface area and good dispersibility. MMT is

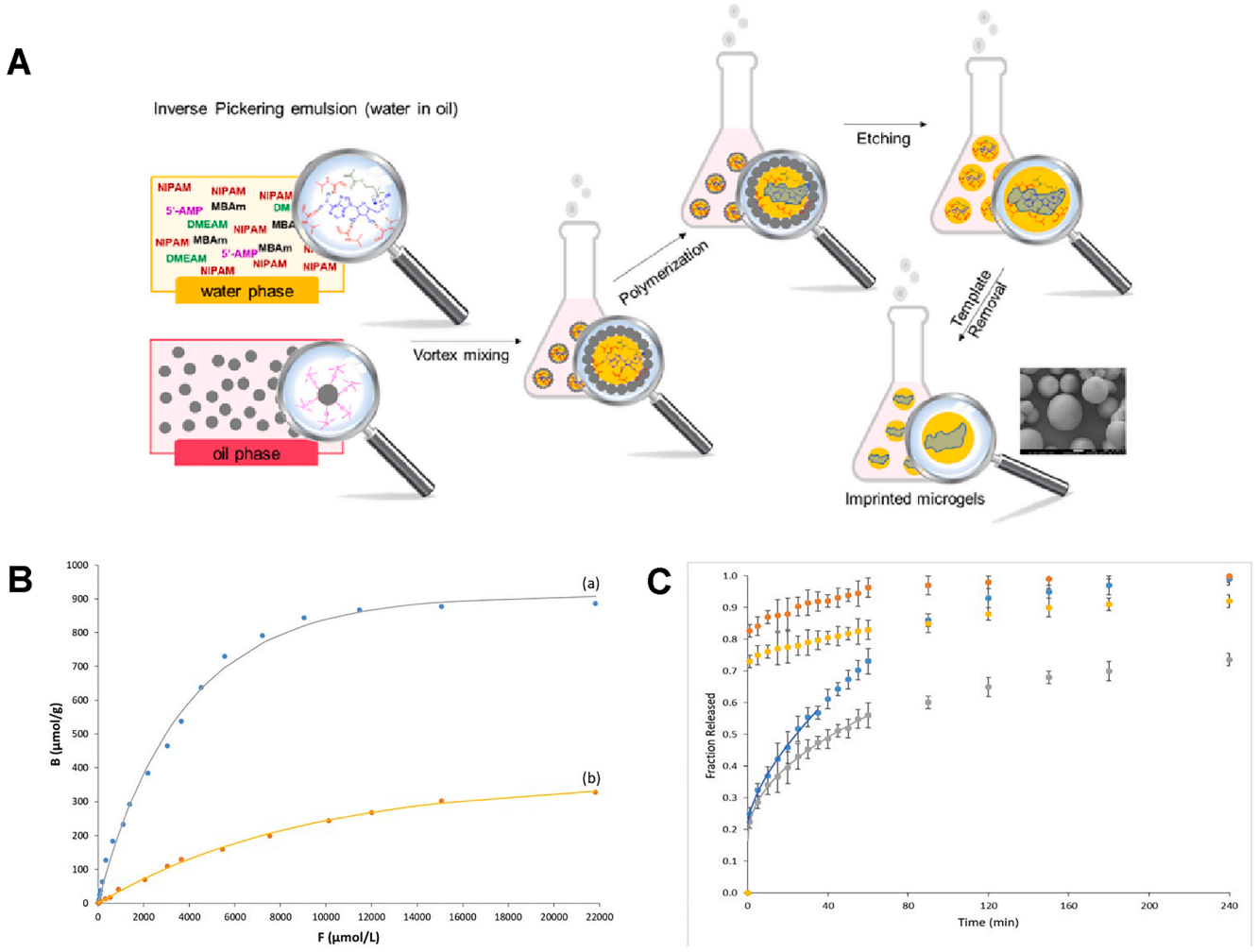

Fig. 4. (A) Scheme of preparation of inverse Pickering emulsion to synthesize molecularly imprinted polymers-based (MIPs). (B) Comparison between experimental adsorption data of adenosine 5′-monophosphate (5′-AMP) and values calculated using the Hill model. Blue points represent B values measured for SHMIP and Orange points represent B values for SHNIP. The molecularly imprinted polymers-based (MIPs) non-imprinted polymers (NIP) synthesized from inverse Pickering method are named SHMIP and SHNIP, with SH as spherical hydrogel. Curves (a) and (b) are based on theoretical values obtained by Hill model for SHMIP and SHNIP, respectively. (C) Calculated fraction release of 5′-AMP in lactate buffer (5 mM, pH = 5) by SHMIP at 25 °C (blue circle filled), SHMIP at 35 °C (grey circle filled), SHNIP at 25 °C (orange circle filled) and SHNIP at 35 °C (yellow circle filled). Curves in blue and grey are based on theoretical values obtained by Korsmeyer-Peppas model (KP) for fractional release values ≤ 0.6 for SHMIP at 25 °C and 35 °C, respectively. Reproduced with permission from (Ayari et al., 2019). Copyright Elsevier, 2021 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

composed of two sheets of tetrahedral silica with an octahedral sheet of aluminum hydroxide or magnesium hydroxide in between (Wang et al., 2020). The FE particles used in this study had a size ranging from 10 to15 µm and formed O/W Pickering emulsions with a viscosity of 13 mPa·s at 35 °C and a droplet diameter of 58 µm, when the 50% percentile was applied. Fumed silica nanoparticles had 20 nm size, with 26 mPa·s at 35 °C and 2.7 µm, respectively. The aim was to use the formulation as an efficient skin decontaminating agent of the highly toxic chemical warfare nerve agent VX. In this study, two water suspensions and two O/W Pickering emulsions were tested. Both solid particles adsorbed at the oil–water interface in order to stabilize the emulsion. Additionally, these particles were used as decontaminant systems due to their ability to adsorb the contaminant. The results showed that all formulations decreased the VX amount in the skin. Pickering emulsions stabilized by FE enhanced the decontamination efficiency by decreasing the contaminant by 2-fold, comparatively to the use of the suspension, and 3.8-fold when compared to the nontreated skin. However, Pickering emulsion stabilized by silica showed a decontamination efficiency equivalent to that of the water suspension (Fig. 5) (Salerno et al., 2016).

In a study by Wang et al. (2020) the MMT was also used as a Pickering emulsion stabilizer in combination with alginate. MMT is extremely hydrophilic, and cannot be used as an effective emulsion stabilizer, unless it is modified. The modification of MMTs surface wettability by physical adsorption of the surfactant or chemical reaction with a hydrophobic molecule increases the adsorption at the oil–water interface. However, the substances used for the modification can be toxic, environmentally hazardous and/or expensive, preventing the application on a large scale. To overcome this difficulty, salt or polymers can be added in order to increase viscosity and create a network composed of clay particles in water. Since alginate is biocompatible and its properties are a combination of salt and polymer, this substance is a good candidate for combination with MMT, as stabilizers of Pickering emulsions. Furthermore, alginate has a tunable rheological behaviour with salt and pH variations. Alginate is also adsorbed on the surface of MMT by hydrogen binding and through electrostatic interactions, adjusting MMT dispersibility, therefore promoting the stabilization of the emulsion. Moreover, alginate increases viscoelasticity, providing a gel-like consistent phase that hinders droplet coalescence. Thus, MMT and alginate are two promising compounds that can be easily combined

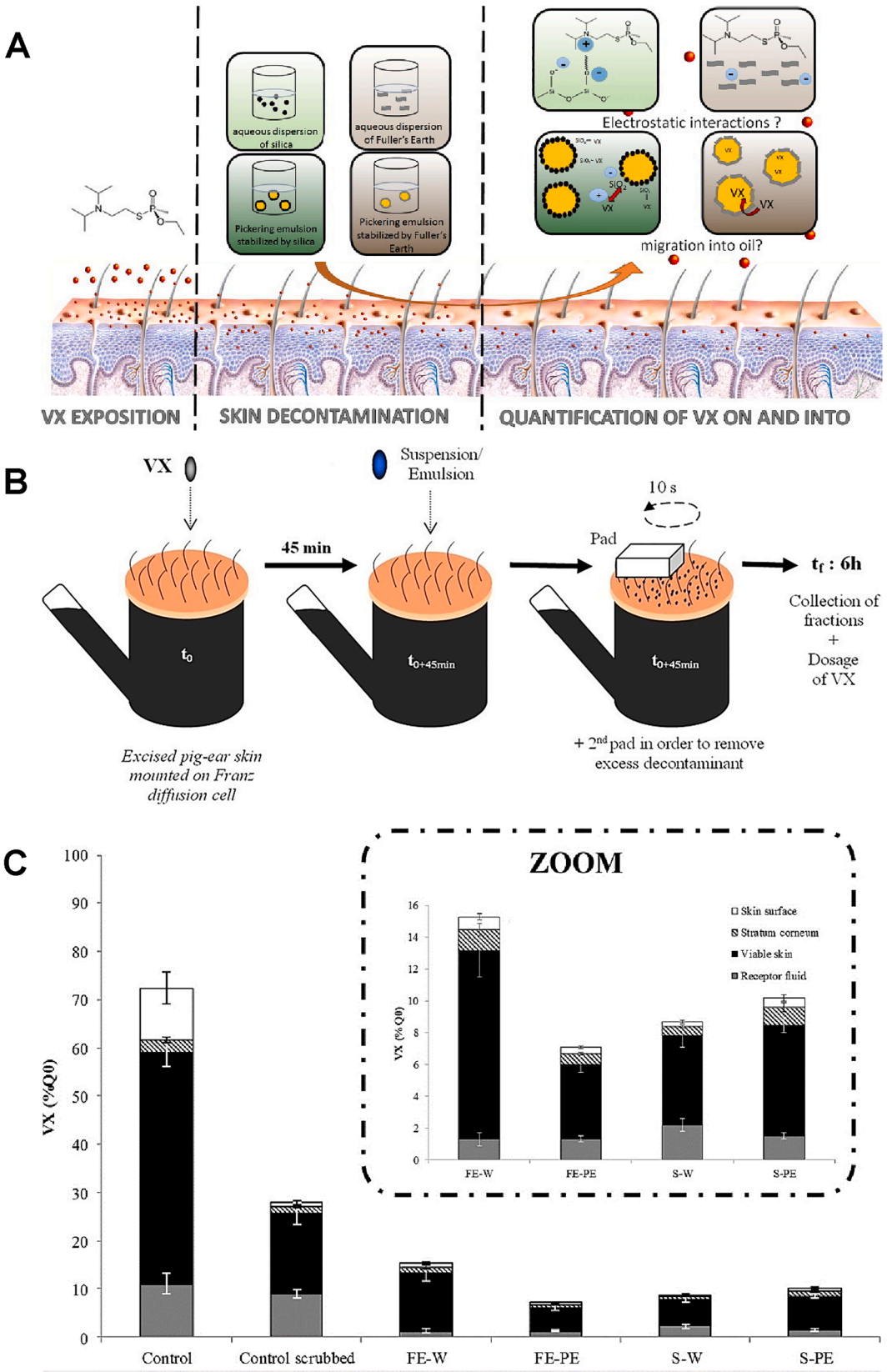

Fig. 5. (A) Schematic representation of the Fuller's Earth (FE) particles and silica nanoparticles as VX skin decontamination agents. (B) Schematic procedure for the skin decontamination. (C) *In vitro* distribution of VXeq ($Q_0 = 5$ mg·cm − 2) 6 h after skin decontamination at 45 min through pig-ear skin, using only a pad (control scrubbed), the aqueous dispersion of FE and silica and the corresponding Pickering emulsion. Results expressed as percentage of applied dose are means ± S.E.M (n = 6). Reproduced with permission from (Salerno et al., 2016). Copyright Elsevier, 2021

to improve emulsion stability, with the advantages of being cost-efficient and biocompatible (Wang et al., 2020).

Halloysite is another clay material that belongs to the family of aluminosilicates and presents a hollow structure with a tubular shape, being considered the safest clay (Lvov et al., 2019; Santos et al., 2018). The surface of halloysite clay nanotubes (HNTs) is composed of tetrahedral bonds between silica and oxygen, while the inner lumen is composed of octahedral bonds between alumina and oxygen (Peixoto et al., 2021; Pereira et al., 2021). Due to its layered structure, halloysite preserves most of its hydroxyl groups on the inner surface (Lisuzzo et al., 2019). When in contact with halloysite, water binds to the layered walls, functioning as a glue. In Pickering emulsions, HNTs are stabilizing elements, positioning themselves laterally in the interface in order to reduce surface tension. The contact with the non-aqueous phase can be increased by hydrophobizing halloysite's surface. There are several different topical formulations containing HNT, such as creams, bandages and sprays. HNTs in creams are especially useful in cosmetics, allowing the sustained release of the active ingredients, which in turn enables controlled absorption by the skin. HNTs can also be used in sprays due to their high zeta potential. They are used, for example, in the development of antibacterial sprays by the formation of stable colloids, enabling loading of antiseptics. Another potential topical application of HNTs is in bandages, where HNTs are loaded with antibacterial substances and embedded in a matrix of gel (Santos et al., 2018). Despite their numerous applications in the cosmetic and therapeutic fields, these particles have yet to be thoroughly studied as Pickering emulsion stabilizers.

3.1.2. Organic particles

3.1.2.1. Synthetic polymeric particles. Synthetic polymers are derived from petrochemicals, but recently their biodegradable versions started being considered as stabilizers for Pickering emulsions. Despite their acceptance as stabilizers, their use is still limited for healthcare applications (Calabrese et al., 2018). Some examples of biodegradable polymers are poly(lactic acid) (Ayari et al., 2019), a glassy and solid material, and poly(ε-caprolactone) (PCL), an amorphic substance of soft consistency (Laredj-Bourezg et al., 2015). These polymers are only considered biodegradable under strict industrial composting, thenceforth the importance of their end of life conditions (Calabrese et al., 2018). However, all synthetic polymers are biocompatible molecules and they have better stability than bioparticles (Wei et al., 2020). Additionally, they have different chain conformations and behaviours, a feature that can be explored for the development of Pickering emulsions (Wang and Wang, 2016).

3.1.2.1.1. Poly(lactic acid). PLA is a bio-based polymer with thermoplastic properties, which belongs to the alpha-hydroxy acid group. This polymer is manufactured by direct polycondensation of naturally extracted lactic acid or by a process of ring-opening polymerization of the lactide dimer, using an appropriate catalyst. Recently, an enzymatic process using lipase was developed for the manufacture of PLA, eliminating the need for metallic catalysts. However, the major process of synthesis is still the condensation technique. PLA is mainly used due to its multiple advantageous properties such as biodegradability, biocompatibility, processability and mechanical strength, which gives it the flexibility to fulfil multiple needs and a wide spectrum of possible applications. However, this compound has limitations, namely its hydrophobicity, degradation rate and impact toughness. To mitigate these disadvantages PLA can be combined with other polymers, in order to enhance some properties and/or generate new ones, to target applications without having to develop new materials (Saini et al., 2016).

3.1.2.1.2. Poly(ε-caprolactone). PCL is a synthetic polyester with adjustable biodegradable and tunable drug release behaviour, processability and good biocompatibility. However, PCL has low cell adhesion, low bioactivity and high hydrophobicity (Hu et al., 2018). In spite of these disadvantages, PCL has a high permeability to hydrophobic and hydrophilic drugs, is hydrolytically more stable than PLA and poly (glycolic acid) (PGA), and is currently under investigation for possible applications in the synthesis of biomolecules to be employed in drug delivery to specific targets. Due to its small melting and glass transition temperatures, PCL eases active ingredient integration without compromising properties such as the active ingredient potency and chemical stability. Additionally, PCL is cheaper than other polymers (Ivanova and Bazaka, 2014).

In a study performed by Laredj-Bourezg et al. (2017), nanoparticles composed by PCL- poly(ethylene glycol) (PEG) block copolymer (PCL-*b*-PEG) or PLA-PEG block copolymer (PLA-*b*-PEG) were used as stabilizers of O/W Pickering emulsions, with triglycerides as an oil phase (Laredj-Bourezg et al., 2017). Pickering emulsions with excess of these copolymers can be combined to enhance the delivery of compounds with hydrophobic properties to the upper layers of the skin (Calabrese et al., 2018). From a drug delivery perspective, block copolymers are attractive due to their small size, ranging from 20 to 100 nm, and tunable amphiphilicity (Wang and Wang, 2016; Laredj-Bourezg et al., 2015). The amphiphilic properties of block copolymers are similar to those of surfactant molecules because of the separation between hydrophilic and hydrophobic components in their chemical structure. Their core is made of the hydrophobic PLA or PCL, surrounded by a shell of the hydrophilic PEG, which can swell when in contact with water. Another reason for the use of block copolymers in drug delivery is the capacity of these particles to solubilize active ingredients with hydrophobic characteristics and deliver them to the skin while avoiding the penetration of nanoparticles into the skin. In classical emulsions, on the contrary, surfactants permeate the skin and interact with the components of the SC (Laredj-Bourezg et al., 2015). In the study by Laredj-Bourezg et al. (2017), the Pickering emulsions with both copolymers were developed by two different methods, a classical process and spontaneous emulsification. The PCL-*b*-PEG nanoparticles had a size of 32 nm with a droplet size of 3 $\pm$ 0.4 μm and the emulsion viscosity ranged between 15.2 and 15.3 mPa.s, while the PLA-*b*-PEG nanoparticles had a size of 50 nm, with a droplet size from 2 to 2.7 μm and an emulsion viscosity of 14.5–14.7 mPa.s. The differences in droplet size and viscosity are due to the use of the different preparation methods. The two block copolymers were used to encapsulate *all-trans* retinol in an O/W formulation. *In vitro* studies were conducted to compare the retinol absorption and distribution of the Pickering emulsions obtained by the two different preparation methods. The results showed that all Pickering emulsions enhanced retinol skin penetration when compared to the conventional emulsion; the increase was 2.22-fold for the PLA-*b*-PEG Pickering emulsion and 1.77-fold for the one using PCL-*b*-PEG copolymer. The retinol distribution was mainly observed in the outermost skin layer for all formulations, showing a 1.4-fold increase when PLA-*b*-PEG was used and 1.27-fold increase for PCL-*b*-PEG, compared to the conventional emulsion (Fig. 6) (Laredj-Bourezg et al., 2017).

3.1.2.1.3. Poly(lactic-co-glycolic acid. Poly(lactic-co-glycolic acid) (PLGA) is a frequently used polymer, manufactured using PLA and glycolic acid. Lactic acid and glycolic acid have different hydrophobicities and crystallinities, allowing the synthesis of particles with characteristics that can be adjusted to different applications, depending on the synthesis process. This adjustment is made by varying the composition of the copolymer, the ratio of the components or by modification of the surface of the polymer. Yet another advantage is that lactic acid and glycolic acid are not toxic, which is important when these compounds decompose through enzymatic reactions and hydrolysis (Whitby et al., 2012). PLGA has been used in the medical field for many years, as a polymer for biodegradable sutures, and has potential application in different targeting, therapy and imaging technologies. This polymer is a great candidate as a nano-stabilizer due to its biodegradability, biocompatibility, FDA and EMA approval as a viable delivery system, possibility of designing the molecule in order to provide sustained release as well as surface modification to grant new properties like

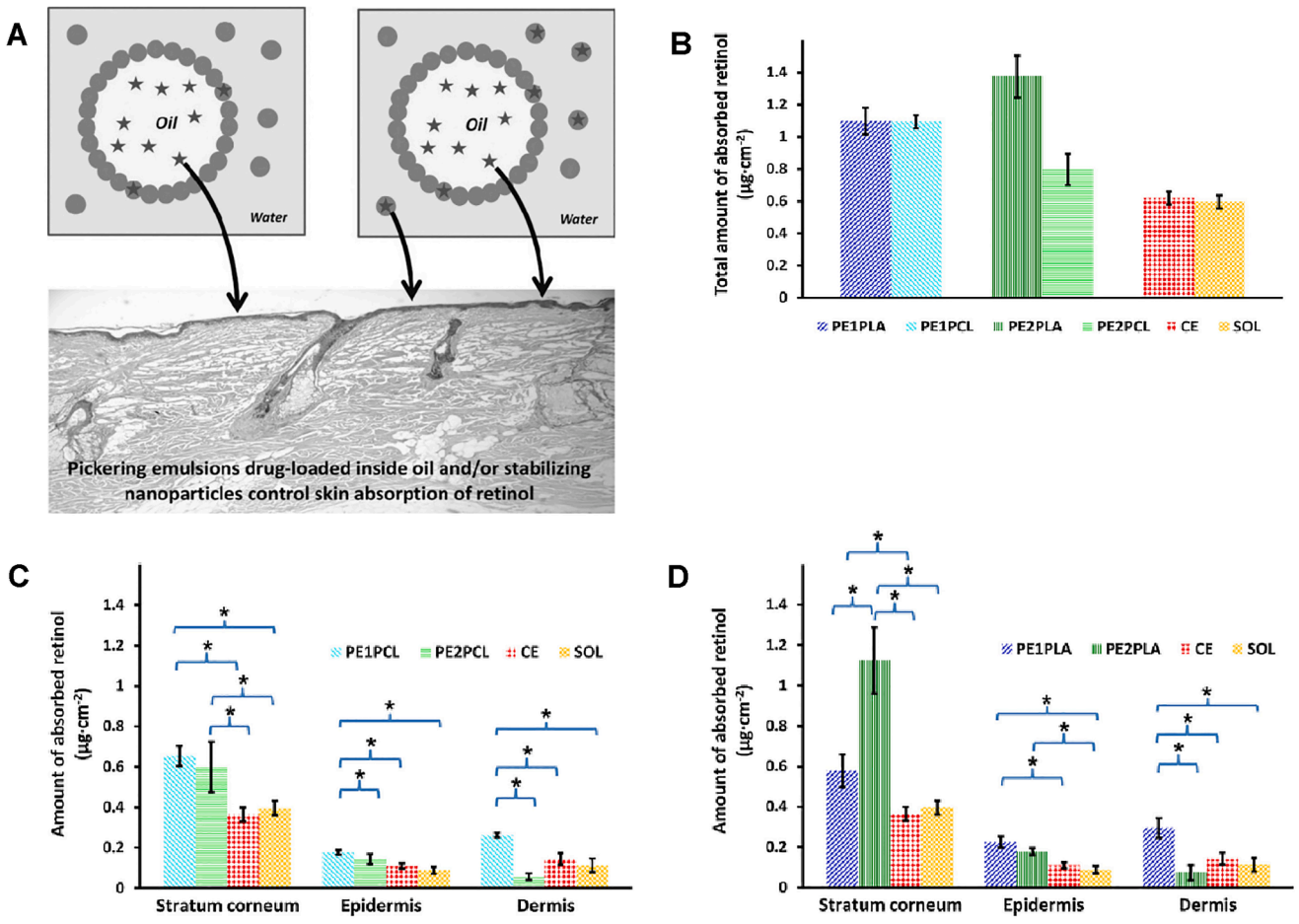

Fig. 6. (A) Schematic representation of Pickering emulsions drug-loaded inside oil and/or stabilizing nanoparticles control skin absorption of retinol. (B)Total retinol (µg cm^{-2}) retained in skin for each sample. Mean ± *sem*, n = 10. All amounts show statistically significant differences with $p < 0.05$ excepted between PE1PLA and PE1PCL and between classical emulsion (CE) and oil solution (Hozayen et al., 2019). (C) Effects of formulation type, PE1PCL, PE2PCL, classical emulsion (CE) and oil solution (Hozayen et al., 2019), on skin distribution of retinol in the excised pig skin after 24 h exposure. Mean ± *sem* values in µg cm^{-2} are shown (n = 10). For each skin layer, absorbed amounts appear from left to right as PE1PCL, PE2PCL, CE and SOL. Stars mark statistically significant differences with $p < 0.05$. (D) Effects of formulation type, PE1PLA, PE2PLA, classical emulsion (CE) and oil solution (Hozayen et al., 2019), on skin distribution of retinol in the excised pig skin after 24 h exposure. PE1PLA, PE2PLA, CE and SOL. Mean ± *sem* values in mg µg cm^{-2} are shown (n = 10). For each skin layer, absorbed amounts appear from left to right as PE1PLA, PE2PLA, CE and SOL. Stars mark statistically significant differences with $p < 0.05$. Reproduced with permission from (Laredj-Bourezg et al., 2017). Copyright Elsevier, 2021.

stealthiness and biological interactions (Mir et al., 2017).

Wei et al. (2020) developed a PLGA/poly(styrene-co-4-styrene-sulfonate) (PSS) polymer to stabilize an O/W Pickering emulsion and to encapsulate the lipophilic component tocopheryl acetate (TA), a form of vitamin E with antioxidant activity (Wei et al., 2020). PSS is a polystyrene partially sulfonated with sulfonic acids. When added to PLGA, PSS has the function of charged density modification, allowing the design of a nanocarrier with a negative charge and adjustable charged density. To prepare the PLGA/PSS nanoparticles, the polymers were first dissolved in an organic solvent and afterwards in water. PSS and PLGA created a polymer film, after organic solvent evaporation. Moreover, the use of different amounts of PSS showed that this polymer increased the particle diameter due to its swelling properties (Cai et al., 2008). The PLGA/PSS nanoparticles developed by Wei et al. (2020) showed sizes ranging from 105 to 115 nm and a zeta potential ranging from −57.5 to −30 mV, both physicochemical parameters dependent on the pH value, which was varied between 2.52 and 10.14. When the nanoparticles were set in the emulsion with 5% of the oil phase, their size increased up to 425 nm. This phenomenon suggests that the nanoparticles were adsorbed at the interface of the emulsion. The droplets formed in this emulsion had a shell/shaped structure with approximately 2 µm of diameter. The stability of the Pickering emulsion was achieved at pH 4.29 to 7.07, due to the decreased electrostatic repulsion, which led to a better adsorption of the nanoparticles. In addition, this pH range is also suitable for the pH of the skin, which is nearly 5.5. The stability of TA was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and calculating the median effective concentration (EC50) of free TA and TA encapsulated in the Pickering emulsion, by UV irradiation of both Pickering and conventional emulsion. The EC50 values obtained for both samples were similar, suggesting that TA, when encapsulated in Pickering emulsions, preserves its activity. The retention of antioxidant activity decreased with increasing UV irradiation time but it was higher for the Pickering emulsion, compared with the conventional one. Thus, the Pickering emulsion enhanced TA stability. The cellular antioxidant activity (CAA) results showed a 1.18-fold decrease of cellular fluorescence intensity when using the Pickering emulsion compared to free TA, indicating a greater antioxidant activity of TA in the emulsion. Overall, the results suggest that the utilization of Pickering emulsion to encapsulate TA can be beneficial in cosmetic products (Wei et al., 2020).

PLGA, as a tunable particle, can also be combined with poly(vinyl alcohol) (PVA), which is a non-toxic polymer with stabilizing properties. PVA/PLGA has potential in a wide variety of applications in pharmaceutical and cosmetic fields (Albert et al., 2018). Moreover, polymers can be designed at molecular levels and synthesised by different

methods, improving the control over the emulsion compared with inorganic emulsifiers. Among different polymeric particles, polymeric self-assembled micelles are recognized as holding great potential due to their architectural tailorability. In some studies, self-assembled polymeric micelles using amphiphilic copolymers showed great emulsifying performance and proved surface active ingredient. However, further studies need to be performed (Zhu et al., 2016).

3.1.2.2. Bioparticles. Nowadays, consumer demands, legal requirements and corporative sustainability aims are pressuring the market in the direction of sustainable, environmentally friendly and natural products with natural ingredients instead of synthetic components, with plant-based formulations also preferred over animal-based ones. These products are also known as "clean label" products (Santos et al., 2019). Substituting synthetic surfactants for renewable emulsifiers such as biosurfactants, amphiphilic proteins, phospholipids, polysaccharides and bioparticles (McClements and Gumus, 2016), can also provide low toxicity, specific activity and high selectivity at high temperature, salinity, and pH. For these reasons, cosmetic, food and pharmaceutical industries have been investing in research to identify natural alternatives, from microorganisms and plants Pickering emulsions using bioparticles as stabilizers are a very promising sustainable and environmental-friendly approach (Bai et al., 2019).

3.1.2.2.1. Polysaccharides. Polysaccharides are polymers of monomers called monosaccharides. They can be found in natural sources such as plants, algae, crustacean shells and bacteria. The use of polysaccharides is an alternative to some synthetic or inorganic particles because they are biodegradable and biocompatible, making these particles valuable candidates as Pickering emulsions stabilizers (Albert et al., 2019).

Starch

Starch is one of the most used polysaccharides to stabilize Pickering emulsions. It can be obtained from several plants such as rice, potato, corn or wheat. This polysaccharide is extracted from different parts of the plant like roots, tubers, seeds and fruits. Starch presents several advantages that make it an attractive option for food, pharmaceutical and cosmetic applications. (Marto et al., 2016; Albert et al., 2019) Like other polysaccharides, it is biocompatible and biodegradable, it is inexpensive, edible, non-irritant and non-toxic. The wide range of starch sources allows obtaining particles with different physicochemical characteristics, such as the size range, that will affect the emulsion stabilization. Frequently, it is necessary to modify the starch granulates, by physical or chemical methods, in order to increase their hydrophobicity (Yang et al., 2017; Marto et al., 2015).

Innovative studies showed that starch modified with octenyl succinic anhydride (OSA) is an adequate candidate to be used as a stabilizer in Pickering emulsions (Zhai et al., 2019). Marto et al. (2015) exploited the effect of OSA-modified starch particles, ASt, on the stabilization of W/O emulsions. The addition of OSA to starch granulates conferred hydrophobicity properties to the particles because of their hydrocarbon chains length and coverage density. ASt particles are intensely adsorbed at the interface of the emulsion and constitute a barrier against instability. The ASt granules used in this formulation had 20.85 ± 0.02 μm size when the 90% percentile was applied and formed a W/O emulsion with droplets of 150 μm in diameter and with a contact angle of 109.0 ± 0.4°. The emulsion showed a non-Newtonian behaviour, with the viscosity dependent on shear rate, and an increase in ASt resulted in increased formulation viscosity. Additionally, this system exhibited viscoelastic behaviour. The change of external phase or ASt concentration also had an impact on skin adhesion and drug release (Marto et al., 2015).

In a subsequent study, the use of ASt particles was tested in antibiotic topical delivery. The antibiotic used in this W/O Pickering emulsion was minocycline hydrochloride (Carter et al., 2019), which has an action against Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*). *In vitro* studies showed that Pickering emulsions stabilized by ASt particles led to a significant accumulation of MH in the outermost skin layer, and a skin penetration with a slow-release of the antibiotic. MH did not reach the innermost layers, which suggests a reduced effect by systemic antibiotic absorption. The antibacterial activity was evaluated *in vitro* by determining the MIC and *in vivo* by Tape-Stripping Infection Model, both on *S. aureus*. The Pickering emulsions with MH increased the inhibition zone up to 1.7-fold in comparison with MH in solution and significantly decreased the colony-forming units (CFU)/mL when compared with untreated skin. In addition, ASt particles showed enhanced wound healing properties in a scratch test. The skin histology evaluation showed epidermis re-epithelization and decreased inflammatory activity by all Pickering emulsions, which was more accentuated in the presence of MH. Thus, this work emphasized the potential therapeutic application of starch as a stabilizer and an external bacterial infection treatment (Marto et al., 2019).

Marku et al. (2012) developed an OSA-modified quinoa starch, with a small size of 1 to 2 μm, to stabilize an O/W Pickering emulsion. The formulation had a droplet size of 30 to 75 μm and a contact angle of 4.9 to 13°. The range of these results depends on the type of oil used, namely paraffin oil, sheanut oil, or Miglyol oil® 812 (medium chain triglyceride ester composed of saturated caprylic (C_8) and capric (C_{10}) fatty acids). The stabilization by starch particles occurred mainly by monolayer adsorption on the surface of the droplet, but starch aggregates could also be formed. Methyl salicylate was incorporated in all formulations and the skin penetration was investigated *in vitro* and showed that the steady state fluxes were 2-fold higher than that of methyl salicylate in buffer solutions. There were no significant skin penetration differences between the Pickering emulsions with the three different oils, thus it was considered that all systems allowed a high skin penetration (Marku et al., 2012).

In a study performed by Cossu et al. (2015), modified starch was used in an O/W emulsion, with the aim of developing an antifungal topical treatment for oral candidiasis. Topical oral treatments have the disadvantage of reduced active ingredient bioavailability in the oral cavity, due to the potential for drug digestion, making it challenging to design formulations with adequate drug release. In this study, a Pickering emulsion with starch nanoparticles was designed with the objective of inducing the controlled release of hydrophobic antifungal substances, such as thymol oil. The flat disc shape of starch particles allows them to stack above each other and around the droplet, preventing coalescence and providing stability. The oral α-amylase enzyme can then enhance the release of amphotericin B and thymol, contributing to a higher bioavailability of these compounds to eradicate *Candida albicans*. The amphotericin B and thymol antifungal activity was evaluated in *C. albicans*, using the minimum fungicidal concentration (MFC) as a parameter. The addition of α-amylase to the emulsion only affected the MFC of both substances when a concentration of 100 U/mL was used. The decrease of MFC was up to 0.5 for thymol and 0.8 for amphotericin B. These findings indicate that the antifungal activity of these substances can be greater and controlled by the concentration of the enzyme. Additionally, *C. albicans* growth inhibition was measured and, for both components, the enzyme did not modify the effective concentration of inhibition but decreased the inhibition zone. Thus, these starch particles exert a stabilizing action and have the potential to deliver hydrophobic active ingredients in the oral cavity (Cossu et al., 2015).

Some studies suggest that starch can be used to encapsulate tea polyphenols in Pickering emulsions stabilized by nanoparticles of taro starch. Starch nanoparticles can also be used as thermo-responsive stabilizers, however, none have been tested for topical applications (Zhai et al., 2019).

Chitosan

Chitosan is another considerably abundant polysaccharide. Chitosan

is obtained from chitin, a water-insoluble polysaccharide that can be extracted from shrimp, crabs, insects, and microorganisms. Chitosan is formed when chitin undergoes a sufficient degree of deacetylation to obtain, under acidic conditions, a soluble polymer. The amine groups in chitosan give it a pH-dependent solubility compound is biodegradable, biocompatible, non-toxic, non-irritant and has antibacterial properties. In Pickering emulsions, chitosan can form complexes with other particles or self-aggregate (Albert et al., 2019; Yang et al., 2017; Lam et al., 2014).

In a study by Asfour et al. (2017), chitosan was used as self-aggregated chitosan particles (SACP). In an acidic environment, the amine groups of the chitosan are protonated, forming ammonium groups respirable for inducing a polycationic behavior. This generates a poor surface activity, leading to a suboptimal emulsifier performance. However, at pH higher than the chitosan pKa, ammonium groups are deprotonated increasing the intermolecular attraction. This phenomenon creates gel nanoparticles that can stabilize O/W emulsions. These nanoparticles had an average diameter of 287.3 nm, with a contact angle of 42.47 ± 1.19° and a zeta potential between −48.1 ± 4.7 and −78.4 ± 4.1 mV. The oil droplets formed had a diameter of 5.8 ± 1.1 to 18.7 ± 3.4 μm with three different viscosities at 25 °C, 2.07, 7.81 and 37.2 mPa. s, depending on the chitosan percentage, 0.2%, 0.3% and 0.4%, respectively (all having the same 20% of oil). Additionally, chitosan proved to have bioadhesiveness which was beneficial for the aim of the formulation as well as for drug release efficiency (Asfour et al., 2017). The aim of the formulation was to deliver rutin for wound healing. Chitosan was chosen not only for its stabilization attributes but also for its bactericidal activity and tissue regeneration properties, which contribute to the wound healing process. *In vitro* studies showed that drug release efficiency of the three Pickering emulsions with different chitosan concentration improved 1.45 to 1.6 times compared with rutin in suspension. *In vivo* studies were performed only with Pickering emulsion with 0.4% chitosan because it showed enhanced healing properties and higher wounded tissue adhesion. After 10 days of treatment, the Pickering emulsion with rutin significantly enhanced wound healing 1.17 times when compared with Pickering emulsion without rutin, and 2-fold compared to the untreated wound. However, despite the significant efficacy of Pickering emulsion with rutin in wound healing treatment, the Pickering emulsion without rutin also showed the ability to improve the healing process, with an increase of 1.7-fold when compared to the untreated wound at day 10. This result indicates that the formulation itself plays an active role in the therapeutic properties of this Pickering emulsion. Moreover, the oxidative stress markers such as malondialdehyde (MDA), catalase (CAT) and reduced glutathione (Liu et al., 2019) were evaluated. The presence of MDA is due to the reaction of ROS with fatty acids, that occurs at high levels of oxidative stress, while CAT and GSH are free radical scavengers. The results showed that Pickering emulsion decreased the amount of MDA by 1.2-fold and increased 1.6 times the levels of GSH and 35 times the levels of CAT. The evaluation of collagen type I (Col I) and hyaluronic acid (HA) was also considered in this study due to activity of these two substances in the wound healing process, in particular connective tissue formation. The concentration of HA increased 1.46-fold and Col I improved 1.2-fold in comparison with the untreated wound. These results suggest that Pickering emulsions stabilized by chitosan are promising formulations for wound healing (Asfour et al., 2017).

Gum Arabic

Gum Arabic (GA) is a dried exudate extracted from *Acacia* tree, mainly composed by branched polysaccharides (Sharkawy et al., 2019), and it is biodegradable and biocompatible. At an approximately neutral pH, carboxyl groups dissociate, leading to an expanded structure and highly charged molecule. This conformation provides a better surface activity and viscoelastic behaviour with the formation of a film. In comparison with other polysaccharides, GA presents more interactions sites as well as a negative charge, which is beneficial to establish electrostatic interactions with chitosan, since it is a polycationic molecule. (Tan et al., 2016) GA has applications in food, cosmetic, pharmaceutical, textile and biomedical industries (Sharkawy et al., 2019).

The use of GA to stabilize an O/W Pickering emulsion to be topically applied was explored by Sharkawy et al. (2020) In this work, chitosan was used in association with GA to make Pickering emulsion stabilizers. The chitosan/GA nanoparticles had a mean size of 109 nm with a zeta potential of + 56.3 mV. The oil droplet generated had a mean diameter of 17.11 μm for 0.5% of nanoparticles and 23.98 μm for 1.5% of nanoparticles (Sharkawy et al., 2020). In a previous work by the same authors, the chitosan/GA nanoparticles showed a contact angle of 89.2 ± 0.94° for the same weight ratio (1:1). The viscosity of the formulation with 1.5% of nanoparticles was measured and exhibited a non-Newtonian fluid and a shear-thinning performance. The nanoparticles create a well-defined layer over the oil droplet interface, restricting their free movement and avoiding the formation of separate phases, which ensures the stability (Sharkawy et al., 2019). *Trans*-resveratrol is a polyphenolic phytoalexin that has been widely investigated attending to its recognized biological activities (Santos et al., 2019; Santos et al., 2019). The aim of the developed formulation was to increase the topical delivery and photostability of *trans*-resveratrol, to enhance its anti-ageing effect. In cosmetic, *trans*-resveratrol induces the production of elastin and collagen, improving skin thickness and elasticity and minimising wrinkles. *In vitro* release studies of *trans*-resveratrol showed a slow release for Pickering emulsion in comparison with the component in the solution. Furthermore, *ex vivo* experiments were performed to evaluate the skin permeation and retention of resveratrol. The resveratrol retention was higher in the viable epidermis and dermis (Chaturvedi et al., 2013), instead of in the SC, with an increase of 3.76-fold compared to the solution, while permeation decreased up to 5.28-fold when compared with the solution. The photostability of *trans*-resveratrol was also tested, because its photosensitive properties decrease its effectiveness. The photodegradation of Pickering emulsion after 4 h was zero, while the remaining ingredients in the control solution decreased 1.47-fold. In summary, the results showed an increase of the *trans*-resveratrol skin delivery and photoprotection by Pickering emulsion, which makes this formulation more effective and stable for longer periods of time (Fig. 7) (Sharkawy et al., 2020).

3.2. Cyclodextrin

Cyclodextrin (CD) is a cyclic oligosaccharide in a cone shape form, composed by glucose hydroxyl groups that provide hydrophilic properties on the outside and a hydrophobic cavity on the inside (Leclercq and Nardello-Rataj, 2016). It is obtained by starch hydrolysis carried out by cyclodextrin glycosyltransferase (CGTase), a bacterial enzyme. Native CDs can be α-CD, a CD with six glucose units, β-CD with seven and γ-CD with eight (Albert et al., 2019). The amphiphilic properties of CD make them suitable for drug delivery due to their ability to encapsulate hydrophobic molecules in their cavity by non-covalent interactions (Marto et al., 2016; Albert et al., 2019). These nano-stabilizers are characterized for being biocompatible, biodegradable and non-toxic, which makes them a suitable candidate for skincare and pharmaceutical applications (Yang et al., 2017; Leclercq and Nardello-Rataj, 2016).

Currently, several topical formulations containing CDs are available for the prevention and treatment of skin disorders (Patravale and Mandawgade, 2008; Ascenso et al., 2013). CDs have consistently demonstrated an ability to improve several physicochemical and pharmacokinetic properties (e.g. cutaneous permeability, active ingredient solubility, chemical stability, photostability, cutaneous irritability) for an effective topical active ingredient delivery (Del Valle, 2004; Ascenso et al., 2011). The unique structure of CDs enables the formation of inclusion complexes with a host–guest active ingredient, which may display improved physicochemical and pharmacokinetic properties compared to the free active ingredient (Del Valle, 2004; Ascenso et al.,

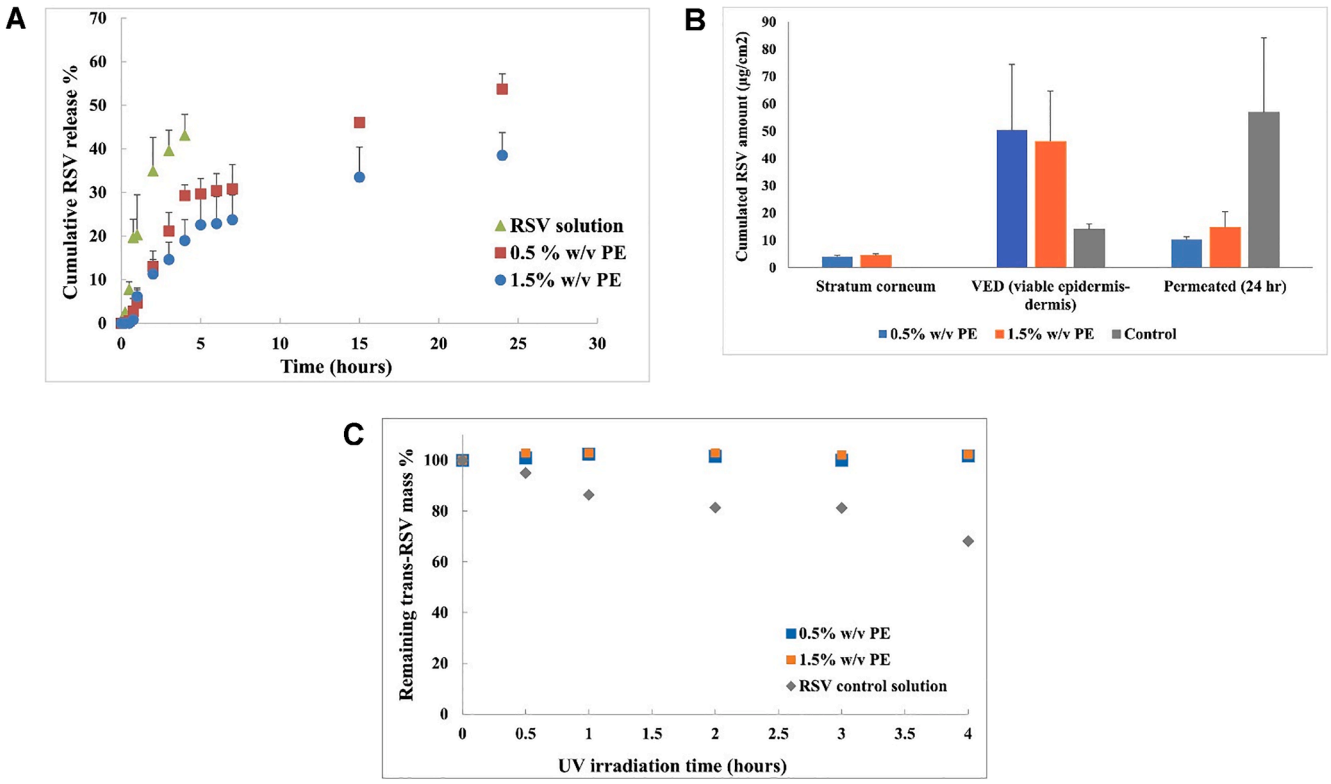

Fig. 7. (A) *In vitro* resveratrol release profiles of Pickering emulsion formulations prepared with 0.5% w/v and 1.5% w/v chitosan/Gum Arabic (GA) nanoparticles, and resveratrol solution in diluted alcohol (20% v/v) through cellulose acetate membrane. (B) *Trans*-resveratrol distribution in the skin layers and receptor fluid after 24 h of exposure to Pickering emulsion formulations stabilized by chitosan/GA (0.5% w/v and 1.5% w/v), and the resveratrol control solution. Values are represented as mean ± SD (n = 3). Note: No *trans*-resveratrol was detected in the *stratum corneum* for the control solution. (C) Percentages of remaining *trans*-resveratrol mass in Pickering emulsions stabilized by 0.5% and 1.5% w/v chitosan/GA nanoparticles, and in *trans*-resveratrol control solution upon exposure to UV radiation. Reproduced with permission from (Sharkawy et al., 2020). Copyright Elsevier, 2021.

2011). Recent evidence suggests that CDs can act as emulsion stabilizers of W/O or O/W Pickering emulsions by forming low soluble inclusion complexes with hydrophobic active ingredients, which are able to decrease the interfacial tension and modify the interfacial rheology (Leclercq and Nardello-Rataj, 2016; Patravale and Mandawgade, 2008).

The development of CDs as Pickering emulsion stabilizers was studied by Leclercq and Nardello-Rataj (Leclercq and Nardello-Rataj, 2016). The mechanism of stabilization of the Pickering emulsion with CDs was different from that of other particles used as stabilizers. Before stabilizing the emulsion, CDs form a complex with the oil, which acts as a surfactant on the emulsion interface, with polar and nonpolar parts, and with properties similar to conventional emulsions. An increase in CDs concentration causes the clustering of the complexes in particles, thus creating a Pickering emulsion. The Pickering emulsion was stabilized by CDs with particle diameters between 30 and 250 nm, which originated an O/W emulsion with a droplet diameter range from 9.2 to 16.1 µm, resulting in a Non-Newtonian fluid. Furthermore, the Pickering emulsions obtained with CDs showed thixotropy, the phenomenon of changing viscosity, where the emulsion has high viscosity in storage and has low viscosity when it is applied. The formation of this Pickering emulsion aimed to encapsulate econazole nitrate, an antifungal active ingredient which also has *in vitro* antibacterial activity, particularly on *S. aureus*. The *in vitro* antibacterial and antifungal (*C. albicans*) studies showed microbial growth inhibition by econazole nitrate. Three types of Pickering emulsions were prepared, each stabilized by a different type of CD (α, β and γ) and the antimicrobial activity was tested. The results obtained with the econazole nitrate commercial formulation, and the Pickering emulsions stabilized by α-CD and β-CD, were identical meaning that the formulations have, at the very least, the same biocidal effectiveness as the commercial formulation. However, the Pickering emulsions with γ-CD as stabilizer exhibit minimal or no biocidal activity, because econazole nitrate cannot diffuse from the CD. In summary, Pickering emulsions stabilized by α- and β-CD can be a great alternative to the commercially available formulations. (Fig. 8) (Leclercq and Nardello-Rataj, 2016).

In a recent study from the previously referred authors, another azole-based active ingredient, miconazoctylium bromide, was encapsulated in the O/W Pickering emulsion stabilized by CDs (Leclercq et al., 2020). The active ingredient was used in association with two phytochemical oils, carvacrol and terpinen-4-ol, to achieve a synergy between anti-biofilm and antimicrobial activities. The formulation with miconazoctylium bromide and carvacrol was 2-fold more effective against *C. albicans* and methicillin-resistant *S. aureus* than the marketed formulations and had high activity against *E. coli*, in contrast to the commercial formulation which was inactive against this bacterium. This type of formulation may be beneficial for clinical purposes because it is more effective in killing the pathogens in a shorter time, which results in decreased proliferation of resistant pathogens.

In a different study, the CD was modified by a short linear glucan (SLG) to achieve a hybrid CD/SLG, in order to enhance the native CD properties as a stabilizer. The hybrid with a 1:1 ratio provided better adsorption, with an improved film at the interface and enhanced stability. However, the physicochemical parameters, such as wettability, did not match the stability standards, since the contact angle was not close to 90°, suggesting that a different stabilization mechanism was involved. This needs further investigation but can provide a different perspective for the development of improved stabilizers with accurate mechanisms (Hu et al., 2020).

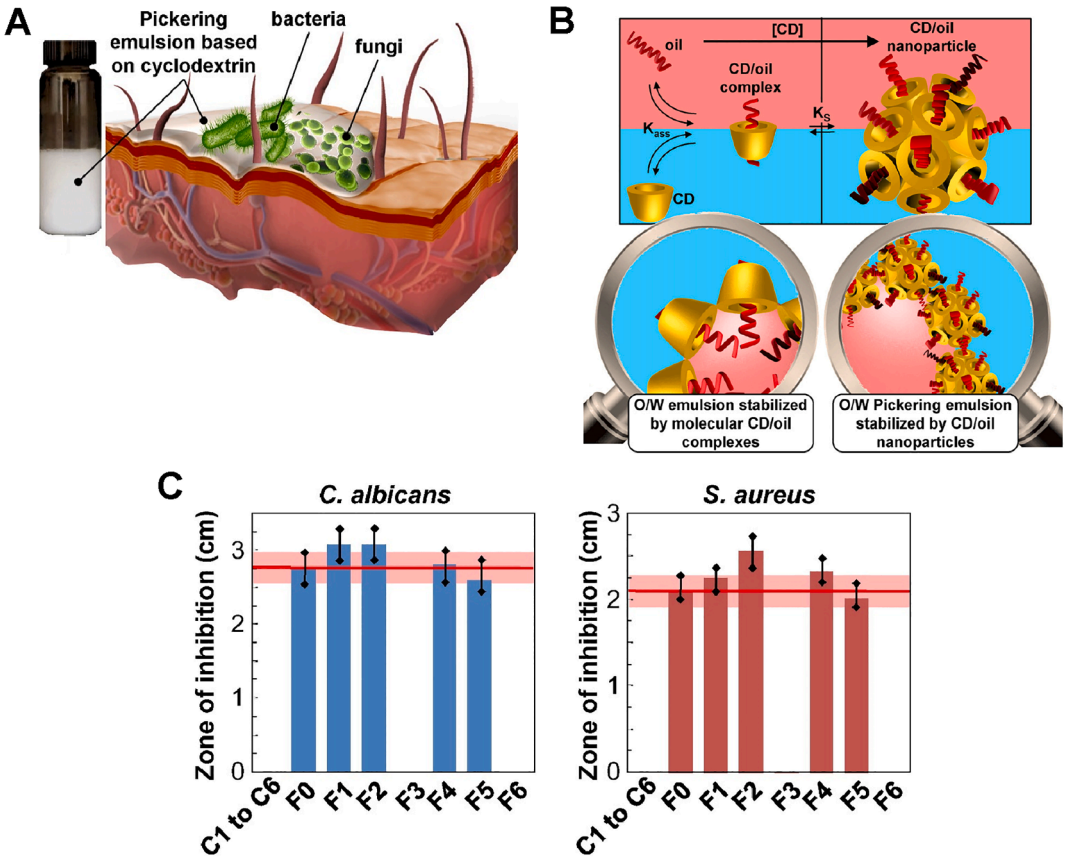

Fig. 8. (A) Schematic representation of Pickering emulsion stabilized by cyclodextrins (CDs) with antibacterial and antifungal activity. (B) Schematic illustration of the stabilization of oil-in-water O/W emulsions depending on the total CD concentration (Kass and Ks are the binding and the solubility constants, respectively). (C) Zone of inhibition obtained by Kirby–Bauer tests against *Candida albicans* (left) or *Staphylococcus aureus* (right) of the formulated Pickering emulsions with econazole nitrate (F1 to F6) or without econazole nitrate (C1 to C6) compare to the commercial formulation (F0). This study was performed in triplicate for each formulation and the red area is the acceptable biocidal activity based on the standard deviation of F0. Reproduced with permission from (Leclercq and Nardello-Rataj, 2016). Copyright Elsevier, 2021. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.1. *Quercus suber* Bark

Quercus suber Bark (QSB) also known as cork is extracted from *Quercus suber* L. bark, predominantly found in the Mediterranean region. QSB is mostly composed by cellulose, lignin and suberin. Additionally, it has saccharides, terpenes, fatty acids and aliphatic compounds, in small amounts. Due to its complex composition, it has multiple functions such as anti-inflammatory, antioxidant, antimicrobial, antifungal, anti-ageing and radical scavenger actions. In a study by Carrico et al. (2019), this organic material was used to stabilize a W/O Pickering emulsion. The QSB particles used in this study had a particle size of 91.4 ± 0.3 μm and a droplet size of 182 ± 1.3 μm, both when the 90% percentile was applied. The contact angle of these particles was 97.3 ± 0.3° and the formulation acquired a thixotropic, as well as a non-Newtonian behaviour. In order to assess the antioxidant activity of the formulation, *in vitro* studies were carried out, and the results showed that the percentage of ROS reduction was slightly lower, in comparison to ascorbic acid, a potent antioxidant. Besides, the formulation exhibited anti-elastase activity in *in vitro* studies, which prevents elastin degradation. In a sensorial study, the formulation was characterized as cosmetically appealing during and after application (Carrico et al., 2019).

In summary, these studies described a wide diversity of Pickering emulsions used on topical application, which have been developed in the past few years. The results clearly suggest that Pickering emulsions improve active ingredient skin stability and permeation and provide better topical application for skin decontamination, antimicrobial treatments, sunscreens and anti-ageing formulations. Additionally, Pickering emulsions proved to be a beneficial alternative to conventional emulsions in therapeutic and cosmetic applications.

3.3. Other promising stabilizers

Pickering emulsions are the subject of several research studiesrelated with topical active ingredients'delivery. This section presents recent findings focusing on particles, either organic or inorganic, that have been shown potential as stabilizers of Pickering emulsions for topical applications.

3.3.1. Organic materials

Proteins are stabilizers widely used in food formulations as biocompatible biopolymers and have also been studied as stabilizers of cosmetic formulations (Albert et al., 2019). They have several advantages such as biodegradability, biocompatibility, simple structure, sustainability, cost-effectiveness, easy access and chemical diversity (Du et al., 2019). In addition, protein nanoparticles have several shapes, such as tubes, spherical micelles, vesicles or even ribbons, and are characterized by unique binding through non-covalent forces, such as hydrogen bridges, electrostatic and hydrophobic bonding (Du et al., 2019). Numerous proteins present a great balance between hydrophilicity and hydrophobicity (Albert et al., 2019) but several others have a heterogeneous charge surface and are considered vulnerable to high temperatures or pH changes (Xu et al., 2019). The main disadvantages of organic particles is their polydispersity and the need for surface modification in order to increase the activity. Environmental conditions,

namely temperature, ionic strength, storage conditions and pH can easily change the surface activity of organic particles (Sarker et al., 2017), which can be a challenge in the emulsion preparation process. In order to overcome these adversities, a few different approaches have been considered. One example is the use of protein nanobarrels from *E. coli*, which have robust structure and stabilization efficiency. These nanobarrels are characterized by high chemical denaturation or thermal resistance. In addition, this structure has a hydrophobic cavity which provides binding sites for hydrophobic molecules or surfaces. This is a promising approach to achieve enhanced protein stability as well as a bioactive compound carrier functionality (Xu et al., 2019). Another strategy is the use of whey protein isolate (WPI) as a heat-resistant particle. This protein has significant surface activity and amphiphilic properties, which makes it an excellent stabilizer. Therefore, WPI could be a feasible option in applications that require heat treatment (Wu et al., 2015). Another alternative is the use of E2 pyruvate dehydrogenase multienzyme complex, a protein extracted from the thermophilic bacterium *Geobacillus stearothermophilus*. The E2 protein unit has a dodecahedron cage framework, which can encapsulate active ingredient, dyes and oils. This protein induced emulsion stability even at different ionic strength, storage temperatures and pH (Sarker et al., 2017). Egg yolk peptides (EYPs) were also used as stabilizers of Pickering emulsions, with promising results. These particles are characterized by having an acceptable surface activity and an intermediate wettability, which improve the emulsion stabilization. These features, alongside the versatility of the emulsion, can be an advantage for cosmetic, pharmaceutical and food applications (Du et al., 2019).

Cellulose nanocrystals (CNC) are promising Pickering emulsion stabilizers, due to their size and rod-like morphology (Gong et al., 2017). These are regarded as an ideal material due to reduced carbon footprint and density, environmental, sustainability and low cost. Their main applications are in the pharmaceutical, medical and cosmetic fields (Xie et al., 2019), as drug carriers, emulsifiers for Pickering emulsions and nanofillers (Tang et al., 2018). Additionally, they are biocompatible and biodegradable (Ren et al., 2019). Cellulose is a highly adaptable molecule, that can undergo multiple modifications. These potential modifications occur due to the presence of anhydrous glucose groups and numerous functional groups, such as hydroxyl and aldehyde groups, which can be easily modified (Tang et al., 2018). Several studies have suggested hydrophobilized microfibrillated cellulose and cellulose nanocrystals solid particles as a strategy for Pickering emulsions stabilization (Kalashnikova et al., 2011; Andresen and Stenius, 2007). However, it is difficult to keep these cellulose-derived materials stable for long periods due to their tendency to aggregate in aqueous medium. The aggregation of hydrophobilized microfibrillated cellulose and cellulose nanocrystals solid particles can result from the establishment of enhanced hydrophobic interactions between them and microfibrillated cellulose, or the establishment of weakened electrostatic repulsions between between them and cellulose nanocrystals *via* acid hydrolysis (Kalashnikova et al., 2011). This limitation can be surpassed by consecutive oxidation of cellulose nanocrystals, resulting in cellulose nanocrystals more homogeneous and electrostatically stable in aqueous medium than cellulose nanocrystals obtained via acid hydrolysis (Yang et al., 2012). The particle size and rod-like shape of oxidized cellulose nanocrystals make them suitable to be adsorbed at the oil–water interface, making them relevant Pickering emulsions stabilizers (Madivala Gurappa et al., 2009; Noble et al., 2004; Alargova et al., 2004).

CNC are versatile biomaterials that can also be apllied as stabilizers in stimuli-response Pickering emulsions. These are emulsions with manageable stability through a directed response to different stimuli, with a more precise response being obtained when multiple stimuli are applied. Wettability is the main factor in stability control and, since wettability is altered when an external stimulus is applied, there is a possibility of controlling the Pickering emulsion through its stabilizer (Ren et al., 2019).

Hydroxyapatite (HAp) is a mineral that exists in the human body. This mineral has been exploited as a stabilizer of Pickering emulsions in many applications, due to its excellent adsorbability, biocompatibility and osteoinductivity (Song et al., 2018), as well as a simple synthesis procedure (Yang et al., 2017). Recently, a study described the concomitant use of HAp and a surfactant, in order to create a synergetic effect and maximize the stabilization of the emulsion. The surfactant changed the HAp physicochemical characteristics, improving its hydrophobicity and stabilizing the emulsion. Additionally, the increasing amount of surfactant led to an inversion of the emulsion type, which changed from O/W to W/O. The coating of HAp with poly(L-lactic acid) (PLLA) also enhanced the emulsion stabilization (Song et al., 2018).

Calcium carbonate (McClements and Gumus, 2016) is an inorganic particle obtained from mineral rocks, such as limestone and marble, used in the cosmetic formulations as absorbent agent, bulking, opacifying, buffer or abrasive. This ingredient can be found in oil-free moisturizers, foundations, exfoliants and toothpastes. It is characterized by its biocompatible and eco-friendly properties. In a recent study, CC was exploited as a stabilizer in a O/W Pickering emulsion. The formulation showed in *in vitro* trials to be safe as well as to have great stability and spreadability into the skin, which suggests that CC can be a promising stabilizer for topical formulations (Marto et al., 2020).

4. Toxicity issues

The increasing interest in cosmetic and dermopharmaceutical applications of Pickering emulsions requires more toxicological studies to confirm the suitability for topical active ingredients delivery. Numerous studies using Pickering emulsions are currently at lab research stages or under experimental trials (Wu and Ma, 2016). However, toxicological profiles of these formulations must be considered and evaluated to ensure human safety.

Dermatotoxicology is the field which evaluates the toxicity of ingredients and formulations for topical applications. Several tests are required in order to classify an active ingredient or formulation as toxic or non-toxic, including the evaluation of the eye and skin irritation potential (Ngo and Maibach, 2010).

The sunscreen formulation stabilized by TiO_2 previously discussed was assessed by *in vitro* (EpiSkin® model) and *in vivo* studies (Human Repeat Insult Patch Test (HRIPT)). The results showed that no skin reactions occurred, thus the formulation was classified as non-irritating and non-sensitizing (Marto et al., 2016). However, the potential of dermal absorption and/or penetration of TiO_2 nanoparticles from sunscreens formulations has been a controversial and much disputed subject within the cosmetic field. Although several studies demonstrate the contrary (Senzui et al., 2010; Crosera et al., 2015), there is conclusive evidence that the penetration of TiO_2 nanoparticles in healthy and in damaged skin (e.g. scarring, sunburn and depilated skin) happens, with damaged skin being more prone to penetration of TiO_2 nanoparticles (Gulson et al., 2015; Gontier et al., 2009; Lekki et al., 2007; Schneider et al., 2009; Larese Filon et al., 2013; Touloumes et al., 2020; Monteiro-Riviere et al., 2011; Lin et al., 2011; Shakeel et al., 2016).

The sunscreen containing melatonin was also tested by HRIPT, and was classified as non-irritating and non-sensitizing (Marto et al., 2016). The sunscreen with melatonin stabilized by silica nanoparticles was tested using cell viability and cell proliferation as parameters to assess toxicity and showed no negative outcomes (Arslan Azizoglu et al., 2017).

The Pickering emulsion stabilized by PLGA/PSS was evaluated by cellular uptake and cytotoxicity (Wei et al., 2020). The cellular uptake was similar in Pickering emulsion and free TA, thus the encapsulation by Pickering emulsion did not affect this parameter. The cytotoxicity was assessed in human keratinocyte HaCaT cells, and the results showed that the cells exposed to the Pickering emulsion had a survival rate higher than 90%, therefore it was considered non-toxic for these cells.

In vitro cytotoxicity studies were also performed on the antibiotic (Carter et al., 2019) formulation stabilized by ASt particles (Marto et al.,

2019). The cells exposed to the Pickering emulsion had a viability percentage higher than 50% thus the formulation was considered non-irritating. Furthermore, the literature mentions that starch up to 30.5% of concentration is non-irritating and non-sensitizing (Nair and Yamarik, 2002).

The formulation stabilized by QSB was evaluated by *in vitro* cytotoxicity and *in vivo* studies (HRIPT). In the cytotoxicity assay, the cell viability was greater than 50% therefore, the formulation was considered non-irritating. The QSB concentration in the Pickering emulsion can be claimed safe. In HRIPT, the formulation did not cause reactions or even skin sensitization/irritation (Carrico et al., 2019).

In Pickering emulsions, it is also important to evaluate the toxicity of nanoparticles due to the nanoscale of some of the particles. Generally, several parameters are responsible for the toxicity of nanoparticles, including size, shape, electrical charge, surface area, biocompatibility, stability, solubility in water, and biodegradability. According to the Nanotoxicological Classification System, nanoparticles are classified in four main classes (I to IV), ranging from low/no risk to high risk. This classification system is based on particle size, biodegradability, and biocompatibility of nanoparticles (Keck and Müller, 2013).

Particle size of nanoparticles plays an important role in shaping their toxicity profile, since cellular uptake of nanoparticles depends on their particle size. The cellular uptake of particles larger than 100 nm and up to 10000 nm can only occur *via* phagocytosis. Thus, it has been suggested that the toxic risk of these particles is limited, given that there are a limited number of cells with phagocytic activity, and some of these cells are difficult to access. In contrast, particles with a particle size inferior to 100 nm can be considered as high-risk nanoparticles, since they can be taken up by any cell *via* endocytosis (Muller et al., 2011). Although the particle size of particles plays an important role in their toxicity profile, it is important to consider the intrinsic toxicity of particles and the administrated concentrations (Albert et al., 2019).

Concerning to biodegradability or particles, it is characterized by their ability to naturally be degraded in the body. A biodegradable material has reduced toxicity because the potential activation of the immune system will be brief, due to its ability to degrade in the body (Su and Kang, 2020). As for biocompatibility, it is a parameter that refers to the ability of the particle to be compatible with living systems, not producing a toxic or immune response (Keck and Müller, 2013).

Inorganic particles have a narrow size distribution and defined shape, but they present low biocompatibility and low biodegradability (Sarker et al., 2017). Silica nanoparticles are of synthetic origin, thus there are concerns about their topical application and their effects on the environment (Marto et al., 2016). Thus, inorganic particles present some limitations (e.g. poor sustainability) (Lam et al., 2014) that may limit possible applications in pharmaceutical and cosmetic fields (Wu et al., 2015).

For this reason, natural particles that are biodegradable and biocompatible are ideal candidates (Bai et al., 2019 Beyond their biocompatibility and biodegradability, these particles are also desirable because of their environmentally friendly characteristics (Wu et al., 2015 The attractive characteristics of these biomaterials are causing a shift from using inorganic particles to using bioparticles, (e.g., modified starch, chitosan, cellulose, among others) as stabilizers of Pickering emulsions (Sarker et al., 2017 The solid particles mentioned in Section 3.1 were all biocompatible, but only the organic particles were biodegradable.

There are only a few reports on cytotoxicity, distribution or *in vivo* metabolic concerns in Pickering emulsions. Currently, most of the research is still focused on the design and *in vitro* evaluation of new formulations (Wu and Ma, 2016).

5. Conclusions and future perspectives

Topical and transdermal delivery of active ingredients can be challenging due to the natural and protective barrier function of the skin. Numerous methods have been explored to increase the permeation of active ingredients to improve cutaneous permeation or retention on superficial skin layers of active ingredients. Concerning the topical applications, formulations containing active ingredients are developed to locally produce the desired effects in the epidermal tissue.

The development of topical formulations has been receiving increasing attention from cosmetic and pharmaceutical industries in order to develop safer and more sustainable products.

Pickering emulsions have been widely investigated as an innovative strategy for the development of topical formulations. Several types of solid particles, either inorganic and organic, have been employed to stabilize Pickering emulsions, in different approaches and applications. These particles present the ability to create a mechanical barrier around the droplets, by adsorbing irreversibly at the interface of the immiscible fluids. This ability of solid particles ca be advantageous in providing greater stability to the emulsion and reducing the risk of coalescence and flocculation. Moreover, the use of solid particles as stabilizers of Pickering emulsions instead of the conventional hazard surfactants can be beneficial in reducing the toxicity of the formulation.

Particle size, wettability, surface charge, surface roughness, pH, salt concentration and particle adsorption were some of the physicochemical characteristics addressed as main parameters for Pickering emulsion preparation. Several particles acting as stabilizers have been explored for topical applications, namely silica, titanium dioxide, clay, polymers (PCL, PLA and PLGA), starch, chitosan, gum arabic and cyclodextrin. In addition, other promising stabilizers have also been investigated with the aim of stabilizing cosmetic and/or pharmaceutical formulations, such as proteins, cellulose nanocrystals and hydroxyapatite. Thus, pickering emulsions are highly promising formulations in a wide range of fields, presenting advantages such as high stability, biocompatibility and ease of preparation.

New approaches for the stabilization of Pickering emulsions are being pursued and different techniques for the preparation of these emulsions are being studied. There are still a number of challenges to be overcome, including the scale-up to industry and the need for more *in vivo* experiments on topical formulations intended to treat skin diseases. Moreover, the use of particles with tunable characteristics or stimuli-responsive, the association of different solid particles or solid particles with other emulsifiers in the same emulsion, open way for new applications.

CRediT authorship contribution statement

Sofia Peito: Conceptualization, Writing – original draft. **Diana Peixoto:** Writing – original draft, Visualization. **Inês Ferreira-Faria:** Writing – original draft, Visualization. **Ana Margarida Martins:** Writing – review & editing. **Helena Margarida Ribeiro:** Supervision. **Francisco Veiga:** Conceptualization, Supervision, Project administration. **Joana Marto:** Conceptualization, Supervision, Writing – review & editing. **Ana Cláudia Santos:** Conceptualization, Supervision, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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