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Pickering emulsions stabilized with chitosan/gum Arabic particles: Effect of chitosan degree of deacetylation on the physicochemical properties and cannabidiol (CBD) topical delivery

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ABSTRACT

Pickering emulsions (PEs) have recently gained increasing attention as green carriers of bioactive agents due to their surfactant-free and eco-friendly nature. Herein, the effect of the degree of deacetylation (DDA) of chitosan on the properties of chitosan/gum Arabic (CH/GA) particles and PEs was investigated. CH/GA particles with a DDA of 96% (high DDA) and 78% (low DDA) were prepared. The contact angle of water drop for the CH/GA particles prepared with high and low DDA chitosan was 84.2° and 77.5°, respectively. The dynamic interfacial tension of the particles has shown lower values with high DDA chitosan. The high DDA PE formulation exhibited higher stability after two months of storage than its counterpart prepared with low DDA chitosan. The mean emulsion droplet size was 15.6 ± 4.3 μm and 21.1 ± 2.6 μm for PEs prepared with high and low DDA chitosan, respectively. Both PE formulations demonstrated shear thinning and elastic gel-like properties. The amount of cannabidiol absorbed by the *stratum corneum*, viable epidermis and dermis was significantly higher in both PE formulations than the permeated amount, but no significant difference was observed between the amounts absorbed from both formulations.

1. Introduction

Pickering emulsions are emulsions stabilized with solid particles instead of the classical surfactants [1]. They were first reported by Ramsden in 1903, and then, they were named after S.U. Pickering who described them in 1907 [2–4]. Pickering emulsions are finding tremendous research interest nowadays [5–7]. This renewed interest is attributed to the increasing awareness towards the environmental hazards and adverse health issues related to the small molecular weight surfactants, as well as the ongoing advances in the production and characterization techniques of the nanoparticles [4].

Polymers of natural origin are considered attractive particulate emulsion stabilizers owing to their sustainability and eco-friendliness which makes them perfect candidates for the development of clean-label products [8]. Pickering emulsions stabilized with polysaccharide-based particles, aside from their cost-effective and affordable characteristics, have been reported to offer various functional properties such as, controlling lipid digestibility, reducing saturated fat and calorie content, and enhancing the delivery of bioactive agents [9].

Chitosan is a natural polymer obtained by the partial deacetylation of chitin [10]. It consists of β-(1–4)-linked D-glucosamine units and N-acetyl-D-glucosamine units [11]. It has been widely used in drug delivery due to its biodegradable, biocompatible and bioadhesive properties [11,12]. Furthermore, there has been an increasing interest in the use of chitosan-based particles as Pickering stabilizers for Pickering emulsions designed for food, pharmaceutical and cosmetic applications [13–16].

The particles that are used as Pickering stabilizers should have a proper partial wettability and amphiphilic nature in order to achieve high emulsion stability [4]. Chitosan particles have been reported to be highly hydrophilic [14,15]. Therefore, to enhance the wettability of chitosan, chitosan-based Pickering stabilizers are often formed through the interaction of chitosan with another polymer through the polyelectrolyte complexation method [17] or the polyelectrolyte complexation together with the ionic gelation method [18], or *via* the hydrophobic modification of chitosan [16].

Gum Arabic is a branched biopolymer obtained from Acacia trees [19]. It consists of polysaccharide units called arabinogalac-

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tans (which presents ~90% of the total mass), arabinogalactan-protein (AGP) units (~10% of the total gum mass), and glycoprotein (~1% of the total mass) [19,20]. The emulsification properties of gum Arabic are attributed to its amphiphilic nature that is conferred by the presence of the hydrophilic polysaccharide domains and the hydrophobic peptides moieties (present in AGP) [19,20]. Gum Arabic has been incorporated in several Pickering emulsion systems due to its ability to modify the wettability of the particles that are used as Pickering stabilizers, and achieve high emulsion stability [17,21,22].

The degree of deacetylation (DDA) of chitosan is controlled by an alkaline hydrolysis process of chitin by changing the exposure time and temperature [23]. The acid hydrolysis process has been reported to result in the hydrolysis of the polysaccharide as it affects the glycosidic linkage between the polymer units, and therefore it is not used [24,25]. The proportion of the acetylated and non-acetylated glucosamine units in chitosan affects the extent of its protonation in dilute acids, and subsequently, affects its solubility, reactivity, adsorption and bio-adhesiveness [10]. Chitosan degree of deacetylation has been reported to influence the physicochemical and rheological properties of chitosan-based particles [26]. To date, few studies investigated the effect of chitosan degree of deacetylation on emulsions prepared with chitosan-based complexes [27], but no studies have focused on the impact of chitosan degree of deacetylation on the properties and stability of chitosan-based Pickering emulsions.

The use of chitosan-based Pickering emulsions as topical delivery vehicles for bioactive agents has been investigated in very few studies in the literature [15,28]. In a previous study, we reported for the first time the production of Pickering emulsions stabilized with chitosan/gum Arabic nanoparticles that possess biodegradable nature and high stability [17]. The main objective of the present work is to investigate the influence of the degree of deacetylation of chitosan on the properties of chitosan/gum Arabic complex particles, as well as on the microstructure, rheology and stability of the produced Pickering emulsions. The work also explores the impact of the degree of deacetylation of chitosan on the encapsulation efficiency and topical delivery of cannabidiol (CBD), which was used as a model lipophilic active agent due to its high lipophilicity and skin benefits [29,30]. To the best of our knowledge, this is the first study that evaluates the impact of chitosan degree of deacetylation on the physicochemical properties of Pickering emulsions stabilized with chitosan-based particles, and compares their potential as dermal delivery vehicles of lipophilic active agents.

2. Materials and methods

2.1. Materials

Chitosan with a high (96%) and low (78%) degree of deacetylation and a molecular weight of 120–135 kDa was a kind gift from Primex ehf, Iceland. Gum Arabic (Molecular weight = 250 kDa), olive oil, Nile Red and Nile Blue were obtained from Sigma Aldrich. Cannabidiol (CBD) was obtained from THC Pharm (Frankfurt, Germany). Methanol and acetonitrile (HPLC grade) were supplied by Carlo Erba Reagents. Acetic acid (0.1 N) was purchased from Alfa Aesar. The porcine skin samples were a kind gift from Grupo Primor, Portugal.

2.2. Production of chitosan/gum Arabic (CH/GA) particles

Two types of CH/GA particle dispersions were produced depending on the used degree of deacetylation (DDA %) of chitosan; high DDA CH/GA particles in which the DDA% of chitosan is 96% and low DDA CH/GA particles in which the DDA% is 78%. The particles were prepared through polyelectrolyte complexation between chitosan and gum Arabic following a method reported by Tan and co-workers [31], and modified by our research group [17]. In brief, 1 g of chitosan was dissolved in 50 ml of acetic acid (0.1 N), followed by the addition of gum Arabic solution (consisting of 1 g of gum Arabic dissolved in 50 ml of deionized water) in a dropwise manner, with constant magnetic stirring (at 800 rpm) for 30 min. The resultant CH/GA particle dispersions (2% w/v) had a pH of 4.9 ± 0.1.

2.3. Production of CBD-loaded Pickering emulsions stabilized with CH/GA particles

The cannabidiol-loaded Pickering emulsions were prepared by dissolving 0.6 g of cannabidiol (CBD) in 60 ml of olive oil. The oil phase was then added portion-wise to the CH/GA particle dispersion with continuous homogenization at 13500 rpm for 7 min using a high-speed homogenizer (Ultra-Turrax Digital T25, IKA, Germany). The final volume of the emulsion was 100 ml, with an oil volume fraction (φ) of 0.6. Two types of Pickering emulsions were produced; high DDA and low DDA Pickering emulsions in which the DDA% of the chitosan used in the preparation of the particle dispersion is 96% and 78%, respectively.

2.4. Characterization of the produced CH/GA particles

The wettability of the particles was determined by measuring the contact angle using Dataphysics OCA15 Plus equipment (Dataphysics, Germany). Briefly, homogenous films of the particles were prepared by the deposition of CH/GA particles dispersion (2% w/v) onto glass slides that were dried at room temperature. The contact angle was measured by injecting water droplets (of a volume of 4 μl each) on the surface of the particles films. The droplets were photographed at zero seconds and after 30 s to allow equilibrium to take place [32]. The Young-Laplace equation was used to fit the profile data of the imaged droplets to calculate the contact angle. The measurements were conducted at least three times.

The size and zeta potential of the produced CH/GA particles dispersions were measured using a Zetasizer Nano ZS ZEN3600 (Malvern Instruments, UK). The results were reported as the average of three consecutive runs.

The morphology of the particles was examined by transmission electron microscopy (TEM) using a JEOL JEM 1400 TEM at 80 kV (Tokyo, Japan). Samples of the particles dispersions prepared with high and low DDA were both diluted with deionized water (1/1000). Afterward, 10 μl of each sample was mounted on Formvar/carbon film-coated mesh nickel grids (Electron Microscopy Sciences, Hatfield, PA, USA). The negative staining was then performed by the addition of 10 μl of 1% uranyl acetate on the prepared grids. The images were obtained using a CCD digital camera Orious 1100 W (Tokyo, Japan).

The dynamic interfacial tension between the CH/GA particles dispersions and olive oil was determined using a Dataphysics OCA15 Plus device (Dataphysics, Germany). The experiments were conducted by the pendant drop method, which entailed the formation of a drop of the CH/GA particle dispersion by a syringe submerged inside a cuvette filled with olive oil. The dynamic interfacial tension was measured over 2400 s and was calculated using the Young-Laplace equation according to the pendant drop shape [18]. The dynamic interfacial tension was also assessed between pure deionized water and olive oil under the same experimental conditions.

2.5. Characterization of the produced Pickering emulsions

The morphology and microstructure of the high and low DDA Pickering emulsions were examined by optical microscopy using a Leica DM 2000 optical microscope.

The interfacial microstructure of the produced Pickering emulsions was investigated by confocal laser scanning microscopy (CLSM) using a Leica TCS SP5 CLSM (Leica Microsystems Inc., Germany). Nile Red dye (0.1 w/v %) was used to stain the oil droplets of the emulsions, while Nile Blue dye (0.1 w/v %) was used to stain the CH/GA particles. The staining of Pickering emulsion samples was done by mixing 1 ml of the fluorescent dye mixture with 35 ml of the emulsion [18]. The excitation wavelengths were 488 nm for Nile Red and 633 nm for Nile Blue.

The mean droplet diameter was determined by laser diffraction using LS230 Beckman Coulter equipment. The refractive index was set as 1.46 and 1.33 for the oil phase (olive oil) and water phase, respectively.

The creaming index (CI%) was used to evaluate the stability of the produced PEs and was calculated according to the following equation [22]:

$$CI\% = \frac{Hs}{Ht} * 100$$

where Hs is the serum layer height and Ht is the total emulsion height.

The encapsulation efficiency (EE%) was investigated to determine the distribution of CBD within the oil and aqueous phases through accelerated phase separation via centrifugation according to a previously reported method [33]. The CBD concentration was determined by HPLC (as will be described in Section 2.7). The EE% was calculated using the following equation:

$$EE\% = \frac{Total\ \ CBD - Free\ \ CBD}{Total\ \ CBD} * 100$$

where the "Total CBD" is the total amount of CBD incorporated in the emulsion, whereas the "Free CBD" indicates the amount of CBD that was not encapsulated in the oil phase (i.e., the amount present in the aqueous phase).

The rheological properties of the produced Pickering emulsions were examined using a Kinexus Pro Rheometer (Malvern, UK). The apparent viscosity was measured *versus* the shear rate using a cone plate (with a diameter 40 mm, an angle of 4° and a fixed gap of 0.15 mm). The storage modulus and loss modulus were determined over a frequency of 0.01–10 Hz using a parallel plate (with a diameter of 20 mm and a fixed gap of 1 mm). All measurements were conducted in triplicate at 25 °C.

2.6. Ex-vivo skin absorption studies

The dorsal porcine skin samples were prepared according to the OECD guidelines [34]. In brief, the skin samples were carefully shaved with an electric shaver (Grundig Intermedia GmbH, Germany), and the subcutaneous layer was removed. The samples were then rinsed with water and stored at −20 °C until the time of the experiment.

The skin membranes were left to thaw at room temperature for one hour before their use. They were then placed between the donor and the receptor compartments of the Franz diffusion cells (PermeGear, USA), with the *stratum corneum* (uppermost skin layer) facing the donor compartment. The receptor medium consisted of ethanol and ultrapure water (1:1), and was kept under continuous magnetic stirring (600 rpm) at 32 ± 1 °C using a thermostatic water bath. An amount of 250 μl of the Pickering emulsion sample was added to the donor compartment and was uniformly spread on the skin. The experiment was performed under occlusive conditions to prevent water evaporation. Thereafter, samples of 400 μl were collected from the receptor compartment at predetermined time intervals (0, 2, 4, 8 and 24 h), and were protected from light until analysis to prevent any possible degradation of CBD by light. The collected samples were replaced immediately by equal volumes of fresh receptor medium to keep sink conditions.

The total mass recovery of CBD was determined based on the mass balance of CBD distributed in the skin and the donor and receptor compartments following a previously reported method [33]. Briefly, at the end of the experiment, the residual emulsion sample was collected from the donor compartment. The skin surface was washed with methanol and the washing solution was added to the residual sample. The final solution was filtered and analyzed by HPLC to determine CBD in the residual emulsion sample. Tape stripping of the *stratum corneum* was then performed with twenty adhesive tapes (Scotch®, 3 M, USA), which were cut into small pieces to which methanol was added, and placed in an ultrasonic water bath for CBD extraction from the cut tapes. The solution was then analyzed to determine the amount of CBD present in the *stratum corneum*. The skin sample representing the viable epidermis and dermis (after stripping off the *stratum corneum*) was cut into small pieces, added to methanol, and placed in an ultrasonic water bath to extract the CBD from the viable epidermis and dermis. The experiment was performed four times (n = 4) for each formulation.

2.7. Quantification of CBD

CBD was quantified by HPLC using a reversed-phase column ACE 5 C18-pentafluorophenyl group (250 mm × 3 mm, 5 μm). The mobile phase consisted of acetonitrile/methanol/water (7:1:2 v/v) [35]. The analysis was performed at room temperature at a flow rate of 0.4 ml/min, and UV detection at 220 nm.

2.8. Statistical analysis

The results of the measurements were presented as the mean ± standard deviation using Microsoft Excel 365. The statistical comparisons were performed by the Student's t-test, and one-way analysis of variance (ANOVA) (with the level of significance at p values <0.05).

3. Results and discussion

3.1. Effect of chitosan degree of deacetylation (DDA) on the physicochemical properties of the produced CH/GA particles

3.1.1. Wettability of the CH/GA particles

The wettability of the particles was assessed by measuring the contact angle. It has been reported that the degree of deacetylation of chitosan influences its surface wettability. However, the results on this issue are somehow contradictory. Some studies reported that the hydrophilicity increases with the increase in the DDA of chitosan [36], while other studies showed that the hydrophilicity of chitosan decreased with the increase of DDA [23]. These differences could be related to the chitosan film preparation methods, and also to the changes in the surface availability of the chitosan amine groups [23]. In the present work, it was observed that the contact angle value of the water drop was 84.2° and 77.5° for the CH/GA particles prepared with high and low DDA chitosan, respectively, indicating that the hydrophilicity of the produced CH/GA particles was higher when the low DDA chitosan was used (as shown in Fig. 1). It is suggested that the higher degree of polyelectrolyte complexation between the positively charged amino groups

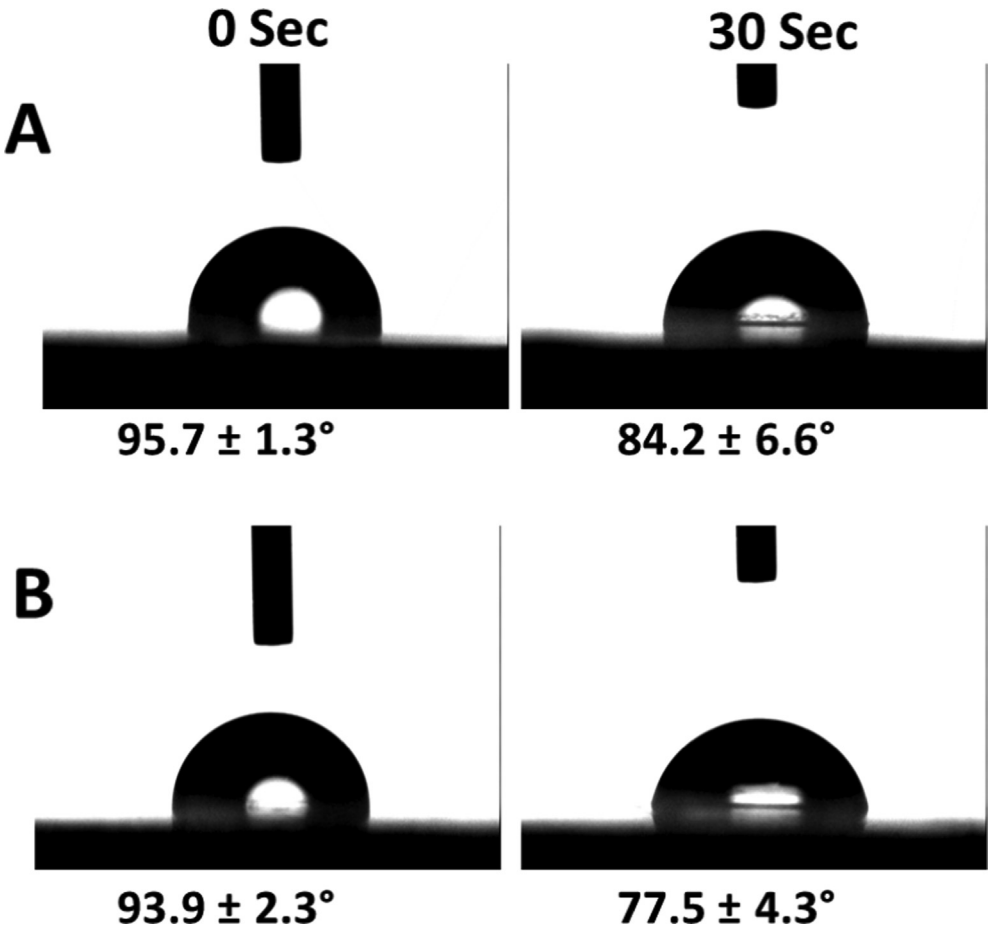

Fig. 1. Contact angle of CH/GA particles prepared with chitosan of (A) High DDA (96%), and (B) Low DDA (78%). The measurements were conducted in the air environment at zero seconds and after 30 s.

of chitosan and the negatively charged groups of gum Arabic in the case of high DDA chitosan (due to the presence of a higher number of amino groups) resulted in greater surface exposure of the hydrophobic groups (of both polymers), leading to a higher contact angle value.

3.1.2. Morphology of the CH/GA particles

The TEM examination of the particles revealed that they have spherical morphology. It can be observed that the particles prepared with the higher DDA chitosan were more spherical and had a denser appearance than those prepared with lower DDA chitosan (Fig. 2), similarly to the findings of Yang et al. [37] who reported that chitosan/tripolyphosphate particles prepared with higher DDA chitosan (90%) were more spherical than their counterparts produced with the lower DDA chitosan (75%). It has also been reported that the degree of deacetylation of chitosan determines its degree of interaction with other polymers as it affects the charge balance. Chitosan with a higher degree of deacetylation possesses more reactive groups within the chain (i.e., more positively charged amino groups), which consequently increases its

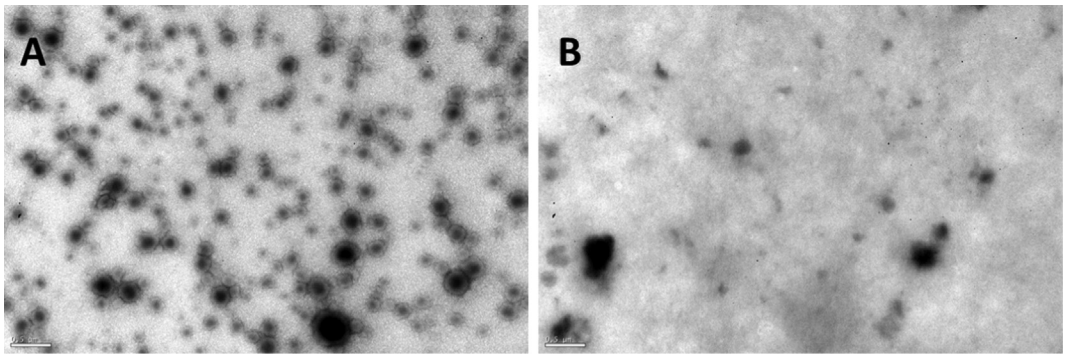

Fig. 2. TEM images of CH/GA particles (2% w/v) prepared with chitosan of (A) high DDA, and (B) low DDA at a magnification of 25,000× and same dilution (1/1000). Scale bars = 500 nm.

reactivity with the oppositely charged polymer chain of gum Arabic resulting in a higher polymer inter-connection and a more pronounced complexation [38,39].

3.1.3. Average size and zeta potential

Fig. 3 shows that the CH/GA particles prepared with chitosan of high DDA exhibited a monomodal size distribution, whereas their counterparts produced with chitosan of low DDA had a bimodal size distribution. A similar observation was described by Yang and co-workers who reported that chitosan nanoparticles prepared using chitosan with DDA of 75% had a bimodal size distribution, which could be possibly due to the presence of a lower number of amino groups resulting in uneven interactions with tripolyphosphate (TPP) ions [37]. The hydrodynamic average particle size of the particles prepared with the high DDA chitosan was reported to be 787.7 ± 36.8 nm, while the particles prepared with the low DDA chitosan had average sizes of 45.0 ± 8.6 nm and 433.7 ± 30.6 nm according to the peaks in Fig. 3.

The zeta potential of the produced CH/GA particles was +68.5 ± 4.9 mV and +46.7 ± 1.7 mV for the particles formulated with chitosan of high DDA and low DDA, respectively. Previous studies showed that CH/GA complex particles had a positive zeta potential [17,31]. It is suggested that the particles produced with the high DDA chitosan exhibited higher zeta potential due to the presence of a greater number of cationic amino groups compared to those available in particles prepared with chitosan of low DDA.

3.1.4. Dynamic interfacial tension

The dynamic interfacial tension was assessed to compare the emulsification ability of CH/GA particles prepared with chitosan of high and low DDA. Fig. 4 shows that both types of particles decreased the interfacial tension in a time-dependent manner to a greater extent in comparison with a control experiment that was run using deionized water and olive oil for the same time period (2400 s). The results also show that the particles prepared with high DDA chitosan have slightly better emulsification properties than their counterparts prepared with chitosan of low DDA. This observation could be related to the presence of a greater number of particles in the first sample which allowed higher adsorption of the particles at the interface. Moreover, it is suggested that the higher positive charge of the particles prepared with chitosan of high DDA, due to the presence of a greater number of amino groups (as discussed in Section 3.1.3), resulted in better interaction with the free fatty acids present in olive oil. This electrostatic interaction between olive oil and chitosan has been discussed in previous studies [33,40].

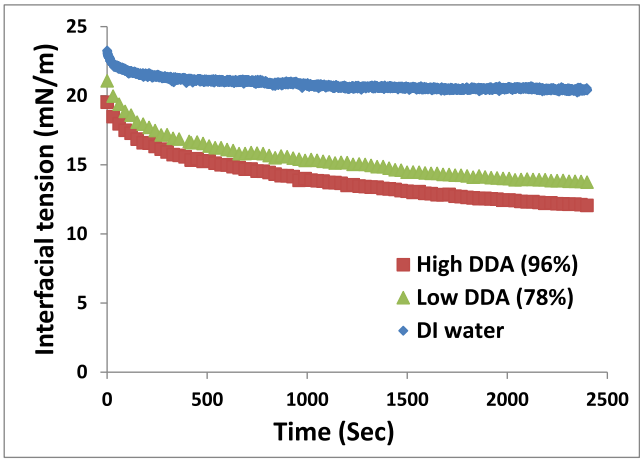

Fig. 4. The dynamic interfacial tension between chitosan/gum Arabic particles (prepared with chitosan of high and low DDA) and olive oil, and between deionized water (DI water) and olive oil.

3.2. Effect of chitosan degree of deacetylation on the physicochemical properties of the produced Pickering emulsions (PEs)

3.2.1. Microstructure

Optical microscopy and CLSM were both used to visualize the microstructure of the produced PEs. The optical microscopy images of the emulsions (Fig. 5) show that the emulsion droplets are intact and spherical. The images also reveal that the emulsion droplets of the PE prepared with chitosan of high DDA (high DDA PE) were smaller in size than the droplets of the emulsion prepared with chitosan of low DDA (low DDA PE). CLSM images (Fig. 6) confirm the adsorption of the particles which appear as red halos on the surface of the oil droplets containing the encapsulated CBD (stained in green). Fig. 6 also shows that the high DDA PE had smaller droplet sizes than the low DDA one, which is in agreement with the optical microscopy images. It can be also observed that the fluorescence intensity of the adsorbed CH/GA particles is higher in the case of the high DDA PE formulation (Fig. 6A) than that of the low DDA PE one (Fig. 6B), indicating the presence of a greater number of particles.

3.2.2. Emulsion droplet size, storage stability and encapsulation efficiency

The emulsion droplet size was measured for both high and low DDA Pickering emulsions directly after preparation and after stor-

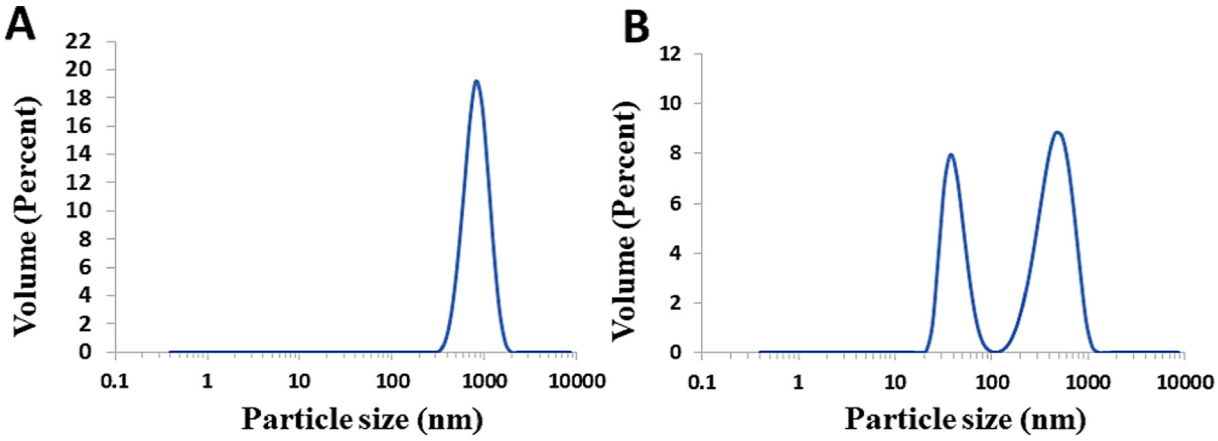

Fig. 3. Size distribution in volume of CH/GA particles prepared with chitosan of (A) high DDA, and (B) low DDA.

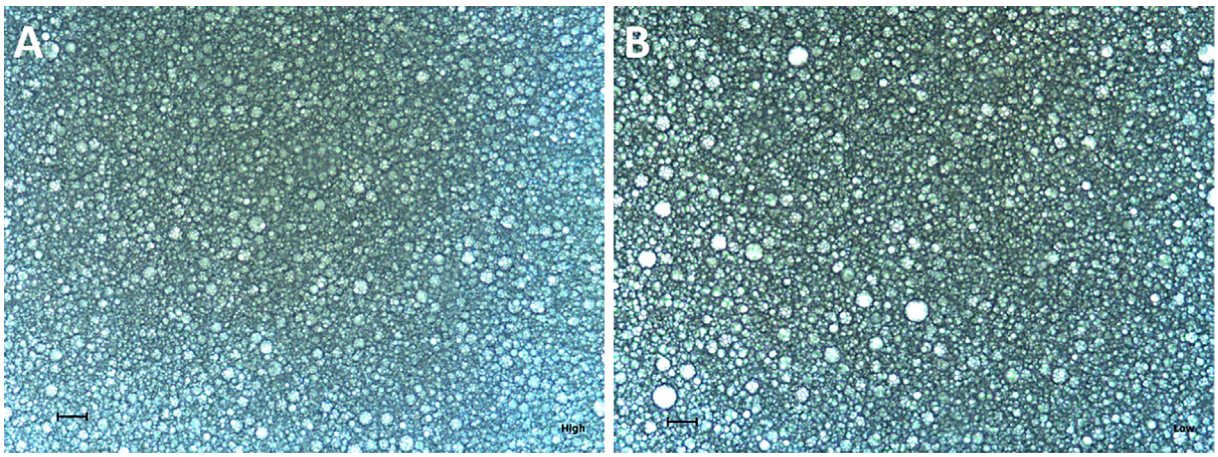

Fig. 5. Optical microscopy images of CBD-loaded Pickering emulsions stabilized with CH/GA particles (2% w/v) formulated with chitosan of (A) High DDA, and (B) Low DDA. Scale bars = 50 μm.

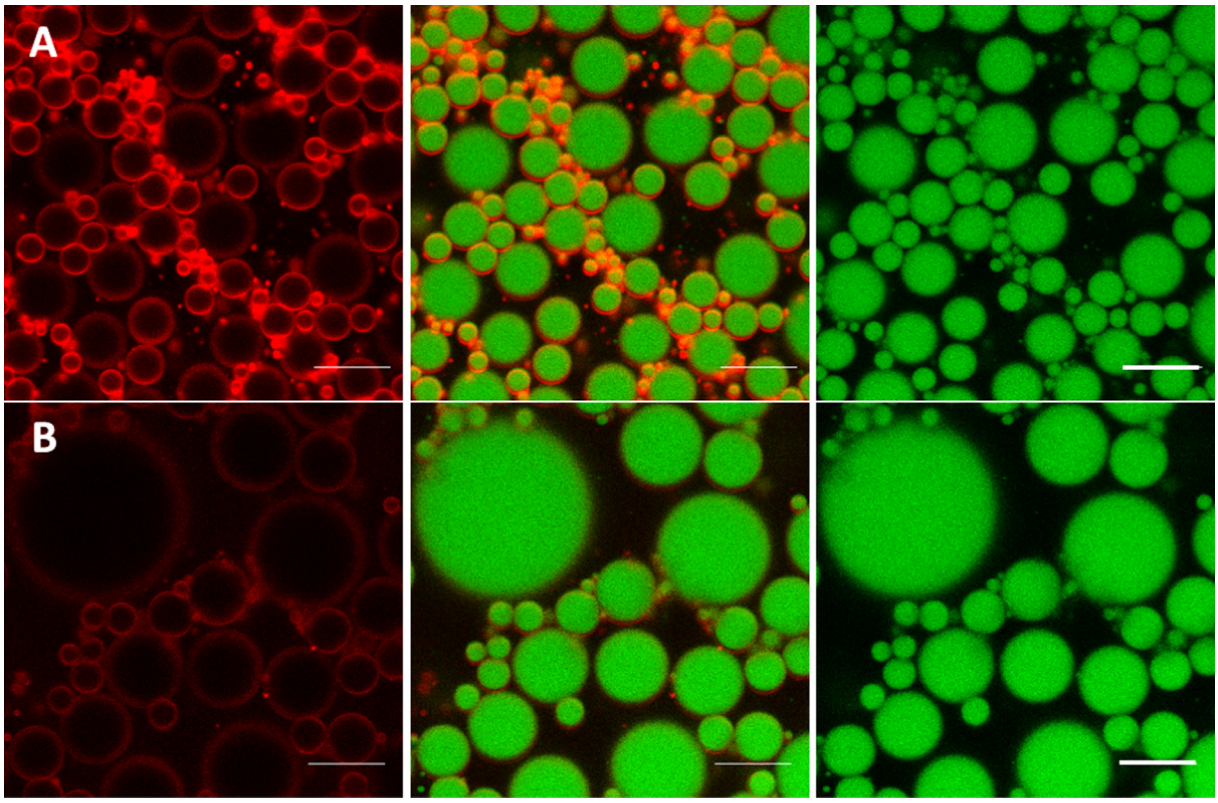

Fig. 6. CLSM images of CBD-loaded chitosan/gum Arabic Pickering emulsions formulated with chitosan of (A) High DDA (96%), and (B) Low DDA (78%). The images on the left show the particles as red rings stained with Nile Blue, whereas the images on the right represent the emulsion oil droplets stained with Nile Red, and the images in the middle are an overlay of these images. Scale bars = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

age for 60 days. Table 1 shows that the formulation prepared with high DDA chitosan had a smaller droplet size than its counterpart prepared with low DDA chitosan. This observation could be explained by the presence of a higher amount of CH/GA particles prepared with high DDA chitosan resulting in larger interfacial area coverage and smaller droplets [17]. Table 1 also shows that there was also a slight increase in the droplet size of both formulations after storage for 60 days, with a noticeably less change in the high

Table 1
The mean droplet diameter, creaming index (CI%) for fresh formulations (d0) and after 60 days of storage (d60), and encapsulation efficiencies of the PE formulations prepared with chitosan of high and low DDA.

Formulation	Mean diameter (day 0)	Mean diameter (day 60)	CI% (d0)	CI% (d60)	EE %
High DDA	15.8 ± 4.3 μm	17.0 ± 3.7 μm	0%	0%	99.6 %
Low DDA	21.1 ± 2.6 μm	25.1 ± 2.2 μm	0%	10%	95.8 %

DDA formulation. Moreover, the high DDA formulation did not exhibit phase separation after storage (the CI% remained 0), whereas the low DDA formulation demonstrated a small phase separation after 60 days with the CI% reaching 10% reflecting the higher stability of the high DDA formulation as shown in Table 1.

Table 1 also shows that the encapsulation efficiency (EE%) of CBD in both Pickering formulations was high. This is attributed to the high lipophilic nature of CBD (Log P = 6.3) [41], which favors its presence in the oil phase (emulsion droplets) rather than in the external phase [33]. It was also observed that the EE % of CBD in the formulation prepared with chitosan of high DDA was slightly higher than the low DDA formulation, which is related to the presence of a denser layer of particles surrounding the emulsion droplets (as was revealed by the CLSM images in Fig. 6). This denser/thicker layer guarded against the diffusion of CBD to the emulsion external phase which led to a higher value of EE%.

3.2.3. Rheological properties

The apparent viscosity of the CBD-loaded PEs prepared with high and low DDA chitosan exhibited a gradual decrease with the increase of shear rate (from 0.1 to 100 s^{-1}) as shown in Fig. 7A, reflecting a non-Newtonian and shear-thinning behavior [17]. This observation is attributed to the breaking/disruption of the internal microstructure of the Pickering emulsions with increasing the shear rate, leading to a decrease in the apparent viscosity [42]. Fig. 7A also shows that the viscosity of the high DDA PE is slightly higher than the low DDA formulation throughout the tested range of shear rate, which is suggested to be due to the presence of a stronger internal three-dimensional network structure owing to the higher degree of interactions between the particles formed with high DDA chitosan and olive oil (as has been discussed earlier in Section 3.1.4). It has been reported that the apparent viscosity fundamentally increases with the increase in the interparticle interactions between the solid particles and the oil droplets [43], which is in agreement with the findings in the current study. It is also suggested that the higher apparent viscosity of the high DDA formulation led to higher stability and no phase separation after storage (CI% = 0) as shown in Section 3.2.2.

The frequency sweep curves of the formulations (Fig. 7B) show that the values of the storage modulus (G′) were always higher than the values of the loss modulus (G″) over the tested frequency range. This observation reflects the elastic gel-like composition of the produced PEs [17], and their tendency to store energy rather than lose it upon applying strain [44]. It is suggested that the presence of unadsorbed particles in the continuous phase of the Pickering emulsions, which were visible in the CLSM images (Fig. 6), have resulted in the formation of three-dimensional networks in the continuous phase entrapping the emulsion droplets, thus contributing to the gel-like viscoelasticity [45,46].

3.3. Effect of chitosan DDA on the ex-vivo skin absorption of CBD from PEs

Pickering emulsions have been reported to promote the accumulation of highly lipophilic compounds in the *stratum corneum* which acts as a reservoir for the sustained release of the active agent to deeper skin layers [47,48]. Chitosan has been reported to enhance the dermal delivery of bioactive agents because of its cationic nature that allows it to interact with negatively charged skin cells [49]. The DDA is an important parameter that has been reported to influence the biological properties of topically applied chitosan, such as wound healing, antibacterial activity and hemostasis [50]. However, the effect of the DDA on the dermal bioavailability of bioactive agents is scarcely reported in the literature.

Fig. 8 shows that the amount of CBD absorbed by the *stratum corneum*, viable epidermis and dermis was significantly higher in

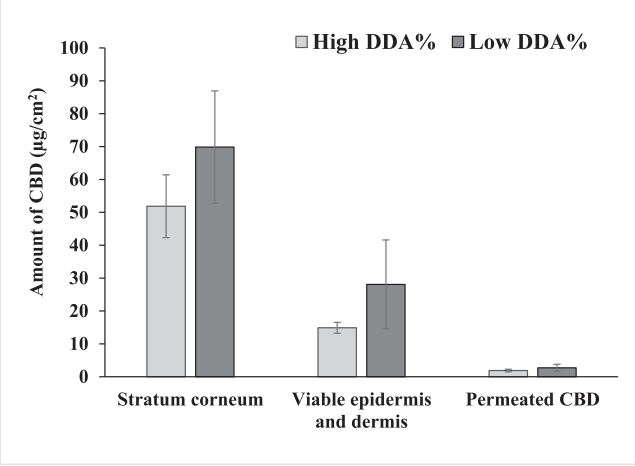

Fig. 8. Distribution of CBD in skin layers and receptor fluid (permeated CBD) from Pickering emulsions stabilized with CH/GA particles formulated with chitosan of high and low DDA.

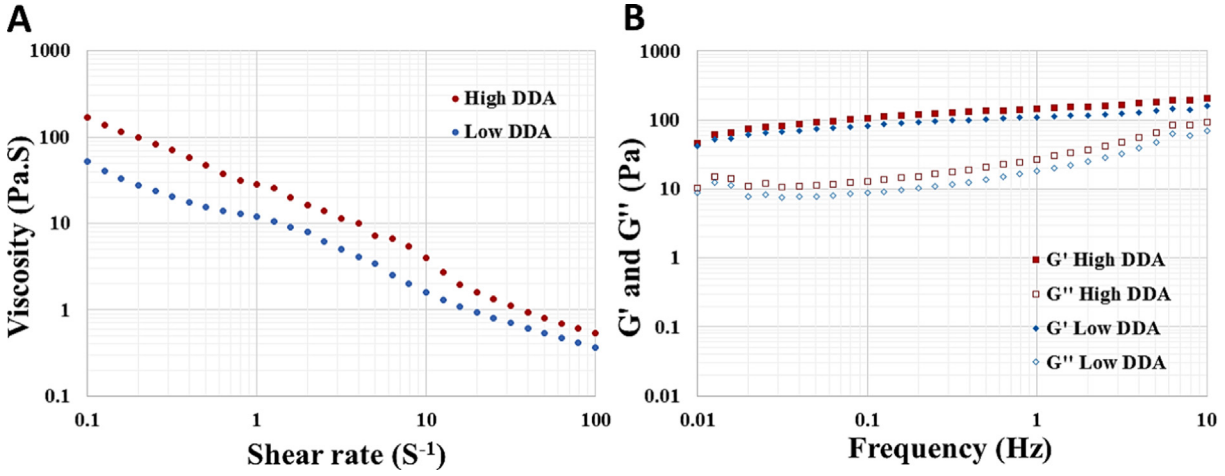

Fig. 7. The rheological profiles of CBD-loaded Pickering emulsions stabilized with CH/GA particles prepared with chitosan of high and low DDA; (A) Apparent viscosity *versus* shear rate, and (B) Storage modulus (G′) and loss modulus (G″) *versus* frequency.

Table 2
Distribution of CBD in the *stratum corneum*, viable epidermis and dermis, receptor fluid, and residual sample on the skin surface after 24 h of exposure to the formulations prepared with chitosan of high and low DDA%. The amount of CBD is shown in μg/cm^2 ± SD (n = 3), as well as percentages of the applied dose.

	Applied dose	*Stratum corneum*	Viable epidermis &dermis	Permeated CBD	Residual sample	Mass recovery
High DDA% (96%)						
μg/cm^2	2328.81 ± 18.07	51.86 ± 9.55	14.89 ± 1.66	1.89 ± 0.40	2187.31 ± 152.87	2255.95 ± 161.64
% of the applied dose		2.23%	0.64%	0.08%	93.92%	96.87%
Low DDA% (78%)						
μg/cm^2	2278.36 ± 248.08	69.84 ± 17.09	28.09 ± 13.51	2.71 ± 1.07	2134.38 ± 330.80	2235.03 ± 327.39
% of the applied dose		3.07%	1.23%	0.12%	93.68%	98.10%

both PE formulations in comparison with the permeated amount ($p < 0.05$), suggesting that both formulations are suitable for the cosmetic delivery of CBD (Log P = 6.3). These findings are in agreement with previous reports available in the literature which showed that oil-in-water Pickering emulsions boost the penetration and accumulation of highly lipophilic drugs, such as retinol (Log P = 5.7) and CBD, in the *stratum corneum* [33,47]. It is worth noting that drugs of moderate lipophilicity (i.e., with lower Log P values), such as resveratrol (Log P = 3.1) and bifonazole (Log P = 4.7), have been reported to demonstrate higher accumulation in the viable epidermis and dermis than in the *stratum corneum* from oil-in-water Pickering emulsions [28,51,52]. Fig. 8 also shows that the amount of CBD retained in the skin layers is slightly higher in the case of low DDA PE than the high DDA PE. However, this difference is not statistically significant ($p > 0.05$). It is suggested that the presence of a denser layer of CH/GA particles surrounding the emulsion droplets (containing the encapsulated CBD) in the high DDA PE did not hinder the diffusion of the CBD from the emulsion droplets to the skin. This could be explained by the higher zeta potential of the CH/GA particles prepared with chitosan of high DDA that allowed good interaction and adhesion to the skin cells.

Table 2 shows that the mass recovery percentage of CBD from both formulations had high values as more than 95% of CBD was recovered, which is considered a very satisfactory value according to the OECD guidelines for the *ex-vivo* skin absorption testing of unstable compounds [34]. Table 2 also shows that the total amount of CBD absorbed by the skin is 2.87% and 4.30% of the total applied dose for the high and low DDA PEs, respectively. These results are comparable with the amounts of highly lipophilic drugs delivered by o/w topical PE formulations [47]. Moreover, it is noteworthy that the amounts of CBD absorbed by the skin from the PEs in the current study are in the range of the amounts that have been reported in recent studies on CBD cosmetic delivery [30,33], reflecting the suitability of the PE formulations stabilized with CH/GA particles to act as potential eco-friendly cosmetic carriers for CBD.

4. Conclusions

The effect of chitosan DDA on the physicochemical properties of CH/GA complex particles and the produced PEs was evaluated. The DDA had an impact on the wettability, interfacial tension, average size and zeta potential of the produced particles. CLSM revealed that the PE formulation produced with high DDA chitosan had a denser layer of CH/GA particles around the emulsion droplets. Moreover, the PE formulation produced with high DDA chitosan has demonstrated higher stability (no phase separation) and higher CBD encapsulation efficiency compared with its counterpart produced with low DDA chitosan. The *ex-vivo* skin absorption assessment showed high CBD retention in the *stratum corneum* and very low permeation to the receptor fluid. It was also observed the CBD deposition in the *stratum corneum*, viable epidermis and dermis was not significantly different between the PE formulations prepared with high and low DDA chitosan. The results obtained in this study are particularly useful in the preparation of chitosan-based PEs. The findings also unveil the effect of the chitosan DDA on the stability, physicochemical properties and dermal delivery of drugs encapsulated in chitosan-based PEs.

CRediT authorship contribution statement

Asma Sharkawy: Conceptualization, Methodology, Investigation, Writing – original draft. **Filomena Barreiro:** Supervision, Writing – review & editing. **Alírio Rodrigues:** Supervision, Writing – review & editing.

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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