cosmetics

MDPI

Article

Pickering Emulsions Stabilized by Calcium Carbonate Particles: A New Topical Formulation

Joana Marto [1,*], Andreia Nunes [1], Ana Margarida Martins [1], João Carvalheira [2], Pedro Prazeres [2], Lídia Gonçalves [1], Alexandre Marques [3,4], António Lucas [3,4] and Helena Margarida Ribeiro [1,*]

1 Research Institute for Medicine (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal; andreia.a.nunes@campus.ul.pt (A.N.); amartins@farm-id.pt (A.M.M.); lgoncalves@ff.ulisboa.pt (L.G.)

2 Paralab, Rua Dr. Joaquim Manuel Costa, 946 B, 4420-437 Valbom, Gondomar, Portugal; joao.carvalheira@paralab.pt (J.C.); pedro.prazeres@paralab.pt (P.P.)

3 Pragosa, Estrada do Tojal, Km1, 2480-188 Porto de Mós, Portugal; alexandre.marques@pragosa.pt (A.M.); antonio.lucas@pragosa.pt (A.L.)

4 Carbomin, En1, Km 86, Moita do, Poço 2460-100 Turquel, Portugal

* Correspondence: jmmarto@ff.ulisboa.pt (J.M.); hribeiro@campus.ul.pt (H.M.R.)

Received: 13 July 2020; Accepted: 29 July 2020; Published: 31 July 2020

Abstract: Pickering emulsions are systems composed of two immiscible fluids stabilized by solid organic or inorganic particles. Pickering emulsions are particularly useful in cosmetics, where the surfactants are unwanted, as well as in the pharmaceutical field, where transdermal and/or dermal drug delivery is difficult to achieve and controlled drug release is desired. Here, we studied calcium carbonate particles as stabilizers of Pickering emulsions for topical use. An optimized formulation was obtained using a Quality by Design approach. First, a screening experiment was performed to identify the formulation and process critical variables that affect the quality properties of the Pickering emulsion. The optimization of the production was then studied by establishing the design space. The final formulation was hereinafter investigated regarding the pH, rheological properties, and in vitro cytotoxicity assays. The results showed the formulation had a pH compatible with human skin and a shear thinning behavior. Moreover, this formulation showed a strong network structure, with a suitable spreadability on the skin, allowing an easy application. The in vitro assays were performed to assess the potential cytotoxicity of the calcium carbonate-stabilized emulsion and the particles themselves, and the results revealed that the formulation did not significantly affect the cell viability. In conclusion, the use of calcium carbonate particles as a stabilizer ingredient contributed to achieve an eco-friendly Pickering emulsion.

Keywords: calcium carbonate; Pickering emulsions; Quality by Design; emulsion stabilizer; eco-friendly cosmetics

1. Introduction

Pickering emulsions are liquid dispersed systems stabilized by solid particles instead of surfactants, which have been widely studied as an alternative to conventional emulsions [1]. Emulsions are widely used formulations in the pharmaceutical and cosmetic fields, but they are extremely challenging systems due to their thermodynamic instability [2]. Despite these challenges, emulsions are efficient topical drug delivery systems (DDS), which can encapsulate both hydrophilic and lipophilic actives inside the dispersed phase, protecting these from degradation, while controlling their delivery [3,4].

The instability of emulsions must be overcome, and their stabilization is usually achieved using synthetic surfactants, compounds that have been raising environmental and toxicity concerns [4,5].

Pickering emulsions are surfactant-free, thus their use is preferable to conventional emulsions. This type of emulsion was first described by Spencer U. Pickering in 1907 [6], who noticed the phenomenon that emulsions could be stabilized by small solid particles instead of surfactants. This stabilization of the emulsions' droplets is achieved due to the particles' dual wettability: because their surface is partially wetted by water and partially by oil, they spontaneously accumulate at the oil–water interface, which is stabilized against coalescence by volume exclusion and steric hindrance [7–9]. In Pickering emulsions, the continuous phase is constituted by the liquid with the best wetting properties, while the dispersed phase is made of the liquid with the poorest wetting properties [10]. The contact angle (θ) between the particle and the interface determines the type of emulsion, and quantifies the wettability of the particles at the oil–water interface. When θ is lower than 90°, O/W emulsions will be produced, while W/O emulsions originate when θ is lower than 90°. However, if the particles are very lipophilic or very hydrophilic, θ will be very high or very low, respectively, and the particles will be dispersed in either the oil or aqueous phases, producing very unstable emulsions [3].

The solid particles used as stabilizers in Pickering emulsions can be of organic origin such as polymer latex or starch, or inorganic such as silica, titanium dioxide, and clay particles [7]. Additionally, nanoparticles and cyclodextrins (CD) are also being studied as emulsion stabilizers [3,11–14]. Several parameters influence the effectiveness of the solid particles as Pickering emulsion stabilizers, namely their shape and size, concentration, wettability, and interactions between particles. In all cases, the size of the particles used as stabilizers have to be significantly smaller than the size of the emulsion droplets, which reduces the amount required to stabilize a given emulsion droplet interface [3,9,15].

Inorganic-based particles such as silica and titanium dioxide (TiO_2) are the most widely used as Pickering emulsion stabilizers. There are several different commercially available particles that can be used as stabilizers, with sizes ranging from nanometers to microns, different surface areas, and hydrophobicity, which can be adjusted by changing the particle coating, the extent of chemical modifications, or the degree of substitution by functional groups [3].

The current trend in the pharmaceutical and cosmetics markets is to formulate, using green and natural compounds, renewable resources mainly derived from plants or microorganisms to meet the demand for eco-friendlier products. Organic particles such as starch and CDs are examples of biocompatible and environmental-friendly compounds that can be used for stabilizing Pickering emulsions [1,8], constituting a great eco-friendly development in healthcare, pharmaceutical, and cosmetic products [16,17]. However, some inorganic particles also offer sustainable and eco-friendly solutions as stabilizers of these emulsions. One example is calcium carbonate (CC), which is widely used in the cosmetics industry as a buffer in formulations, opacifying, bulking, and absorbent agent in foundations and oil-free moisturizers, abrasive in toothpastes and exfoliants, among others [18]. Calcium carbonate is an inert chemical compound mined from marble, limestone, and other mineral rock foundations, and can be supplied in different purity grades (industrial, chemical, pharmaceutical) [18]. Calcium carbonate particles were first used as one of the model stabilizers for Pickering emulsions by Tambe and co-workers [19]. The group of Cui et al. [20–22] later studied the influence of anion surfactants, fatty acids, and impurities present in triglyceride oils on the stabilizing impact of CC particles in Pickering emulsions. Recently, Huang et al. [23] investigated the effect of the morphology of CC particles (cubic, spherical, rod-like) on the stabilization of O/W Pickering emulsions.

The objective of the research work described herein was to develop and optimize a Pickering emulsion with high skin compatibility using biocompatible CC particles as a stabilizer to meet the consumer demands for green and natural products, whilst profiting from CC properties. A Quality by Design (QbD) approach was used for the optimization process. The developed Pickering emulsion was characterized at the physicochemical level, namely droplet size distribution, and its in vitro safety was inferred from cytotoxicity studies using the human keratinocyte HaCaT cell line.

2. Materials and Methods

2.1. Materials

Calcium carbonate (Carbo 125), derived from crushing aggregates of limestone, was obtained from Carbomin (Turquel, Portugal). The caprylic/capric acid triglyceride (Tegosoft® CT) (CT), used as the oil phase, was a kind gift from Evonik Industries AG (Essen, Germany). Purified water was obtained by reverse osmosis and electrodeionization using a Millipore, Elix 3 (Burlington, MA, USA) and was filtered (pore 0.22 μm) prior to use. The 2′,7′-dichlorodihydrofluorescein diacetate (H_2-DCFDA) was obtained from Life Technologies (Carlsbad, CA, USA). The spontaneously immortalized human keratinocyte cell line HaCaT used in the antioxidant and cytotoxicity assays was purchased from CLS (Eppelheim, Germany).

2.2. Methods

2.2.1. Characterization of Calcium Carbonate (CC) Particles

Particle Morphology

Morphology analysis of $CaCO_3$ was conducted using a scanning electron microscope, Phenom ProX, produced by Thermo Fischer Scientific—FEI (Eindhoven, The Netherlands). Micrographs were acquired using a backscattered electron detector (BSED) with a beam acceleration voltage of 10 kV and 15 kV at 1.0 Pa. Sample treatment was not required.

Particle Size Distribution

The particle size distribution was determined using a Malvern Mastersizer 2000 (Malvern Instruments, Malvern, UK), coupled with a Hydro S accessory, with a default refractive index of 1.52, as described previously. The span was also calculated as described [24].

Wettability Measurements

The measurement of the contact angle of water and CT was performed by means of the Sessile Drop technique, using an Optical Tensiometer Theta Flex (Biolin Scientific, Gothenburg, Sweden). The solid surfaces were prepared pressing suitable amounts of $CaCO_3$ powder between two microscope glass slides. The volume of water or CT used for the wettability measurements was ca. 3 μL. The contact angle was measured by the sessile drop method using the Young–Laplace equation with OneAttension version 4.0.4 software from Biolin Scientific (Gothenburg, Sweden). All measurements were performed in triplicate (n = 3). The images were collected by a high-speed camera at 332 fps.

2.2.2. Quality by Design Optimization Studies

Identification of Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs)

To assist the formulation and process design, a QbD approach was used, and the first and most important step was the pre-definition of the desired final QTPP (Table 1), which describes the product quality and forms the basis for defining the CQAs and critical process parameters (CPPs). The required QTPP is dependent on scientific, regulatory and practical considerations, as well as on previous results. The droplet size distribution of emulsions, namely, d(10), d(50), d(90), and span, is one of their most important features, influencing other characteristics such as viscosity and physical stability.

Table 1. Quality target product profile of calcium carbonate (CC)-stabilized emulsions.

Quality Target Product Profile Element	Target	Reference
Route of Administration	Cutaneous	[25]
Dosage Form	Oil-in-water Pickering emulsion	[1]
	Droplet size distribution: d(10): 6–10 µm d(50): 12–18 µm d(90): 20–26 µm Span: 1.1–1.2	[1]
Stability	Homogeneous without phase separation	[1]
In Vitro Studies	Safety: Cytotoxicity assay	[26]

Risk Analysis of CQAs

For risk assessment, a list of all the possible factors that can influence product quality was considered. The identification of critical variables and the levels used in DoE were based on available literature and previous work. Based on this information, Ishikawa diagrams were built to identify the potential risks related to emulsion stability as well as the process parameters, and the CQAs that had the greatest chance of leading to product failure. The droplet size was defined and further outlined to identify potential risks. In this analysis, two variables were identified for optimization in subsequent studies.

Design of Experiments (DoE)

A two-factor central composite design (CCD) was used to optimize the formulation. The independent variables for the process optimization were not analyzed in this work because they had been previously studied [1]. For formula optimization, two independent variables were evaluated: the oil phase percentage and the percentage of CC particles.

To investigate the variables affecting the responses, a central composite design composed of five levels (coded as $-\alpha$, -1, 0, 1, and $+\alpha$) was used, as described in our previous work (Table S1, Supplementary Materials) [24]. The software MODDE®Pro 11 (Umetrics, Umeå, Sweden) was used for data analysis, and results were considered statistically significant for $p < 0.05$.

2.2.3. CC-Stabilized Emulsion: Preparation and Characterization

The emulsions were prepared by a cold process, as previously described [24]. Briefly, the CC particles were dispersed in the CT oil phase, and then mixed with the aqueous phase using an UltraTurrax®homogenizer (IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) at 12,000 rpm for 5 min at 25 °C. The emulsions' droplet size distribution of the 11 experimental runs at 25 °C was calculated from images obtained by optical microscopy using the software ImageJ®, 1.52v (NIH, Bethesda, MD, USA). The sizes were expressed in terms of relative distribution as diameter values (of approximately 150 droplets for each emulsion) corresponding to percentiles of 10, 50, and 90, and span [27].

2.2.4. Physicochemical Characterization of the Final Formulation

The pH was determined using a pH-meter (WTW inoLab®pH 730, Xylem Inc., Rye Brook, NY, USA) at room temperature.

Structural experiments were performed with a controlled stress Kinexus Lab+ Rheometer (Malvern Instruments, Malvern, UK). The flow curve method was performed using a destructive measurement and increasing the shear rate from 0.1 s^{-1} to 100 s^{-1}, with a ramp time of 5 min and 15 samples per decade.

In the oscillatory method, an amplitude sweep test was performed first, with an applied shear strain of 0.01 to 100%, a frequency of 5 Hz, and seven samples per decade, followed by a frequency sweep test with a shear strain of 0.1%, frequency between 0.1 and 10 Hz, and 10 samples per decade. The measurements were performed at 25 °C, using cup and bob geometry.

2.2.5. In Vitro Cytotoxicity Assays

The cytotoxicity of the final emulsion was evaluated in the spontaneously immortalized human keratinocyte cell line HaCaT (CLS, Eppelheim, Germany), as previously reported [8]. Briefly, cells were incubated for 24 h with CC-stabilized emulsion (100 µg/mL) or CC particles (6.25 µg/mL) and cell viability was calculated using the general cell viability endpoint MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) reduction assay [8].

3. Results and Discussion

3.1. Characterization of Calcium Carbonate Particles

3.1.1. Particle Morphology and Particle Size Distribution

In the cosmetic industry, it is very important to work with particles bigger than 100 nm to avoid regulatory issues such as the need to prevent nano-ethical problems [28]. The size distribution of CC particles is defined as a Normal–Gaussian and monomodal, stable, and uniform distribution [29]. The size of the CC particles used in this work ranged from 1.76 ± 0.03 (d10) to 10.11 ± 0.70 µm (d90), with a main peak at 3.95 ± 0.09 µm. These results provide evidence that most CC particles were smaller than the targeted emulsion droplet size (Figure 1), suggesting their use as effective stabilizers of emulsions. These micromolar size particles are still sufficiently small to allow the reduction of the amount required to stabilize the emulsion droplet interface, while also giving a smaller droplet size to the emulsion.

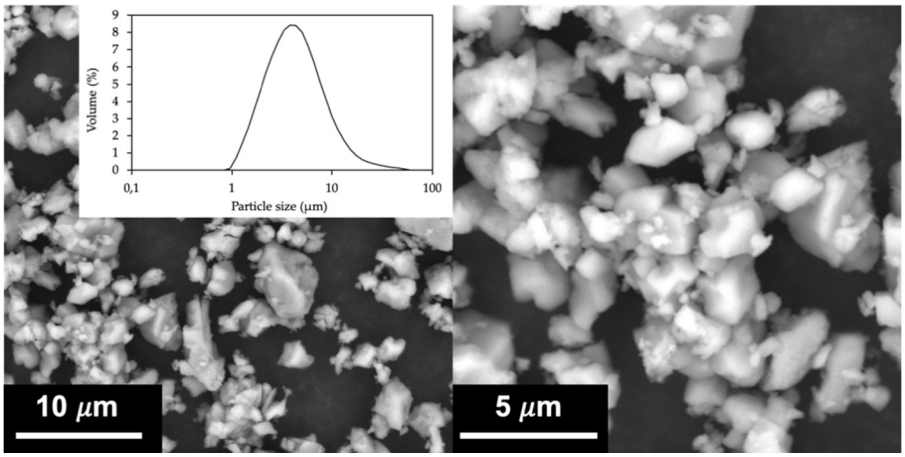

Figure 1. Scanning electron microscopy (SEM) micrographs and corresponding particle size distribution of calcium carbonate (mean, n = 6).

3.1.2. Wettability Measurements

The Bancroft Rule states that the emulsion type is related to the preferential solubility of the emulsifying agent in one of the phases. The phase in which the stabilizer is more soluble is the continuous phase, and those that preferentially solubilize in water stabilize O/W emulsions, and vice versa [30].

For many researchers, the particle wettability is the key parameter for the stabilization [31]. On the other hand, Maestro et al. [32] emphasized that the wetting determination of particles is a complex process, which is associated with the chemical nature of both the particles and the fluid phases,

and with the shape and size of the particle. Although most studies have determined the contact angle of spherical particles, non-spherical particles such as CC are providing an interesting alternative to replace spherical ones.

In a surfactant-free system stabilized by solid particles, these will be more wetted by one of the liquid phases than by the other, the latter being considered the dispersed phase. The importance of the wettability of the particles at the oil–water interface is quantified by the contact angle (θ) between the particle and the interface, which determines the type of emulsion obtained, as stated in the introduction. In this work we aimed to obtain an O/W emulsion because it is more pleasant from a cosmetic point of view. The results obtained for the CC-stabilized emulsion (mean ± SD, n = 3) were θ = 31.2 ± 0.5° in water (Figure 2a), and θ = 99.5 ± 1.3° in CT (Figure 2b). Since the contact angle measured through the aqueous phase was lower than 90°, the CC particles will more effectively stabilize O/W emulsions. This result suggests that the external phase of the formulation is composed by water with dispersed oil droplets.

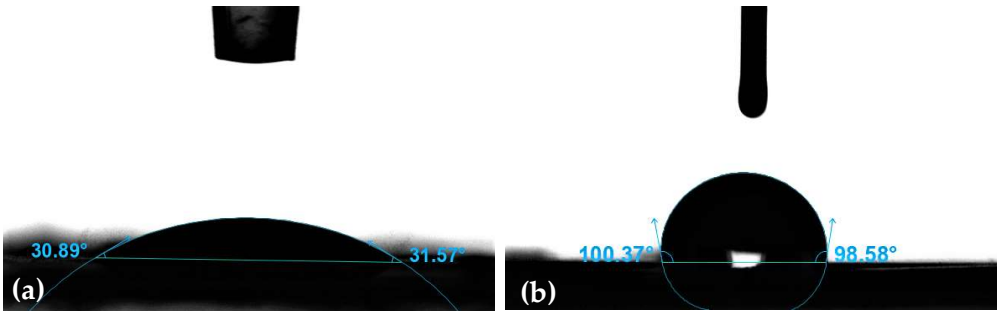

Figure 2. Contact angles of CC in (**a**) water and (**b**) caprylic/capric acid triglyceride (CT).

3.2. Optimization Studies

3.2.1. Risk Analysis of CQAs

The factors affecting the CQAs of the CC-stabilized emulsions were found to be formulation- and process-related. The critical factors were identified and their effects on the emulsion droplet size were studied (Figure 3). The results obtained by risk analysis, reported in the literature and obtained in previous work, and the data acquired in the laboratory during the experiment suggested that the major CQAs were the percentage of CC particles and the volume (percentage) of internal (oil) phase, thus these were the studied variables. The process variables, which also have an impact on product quality, were optimized previously, in the conditions described elsewhere [1].

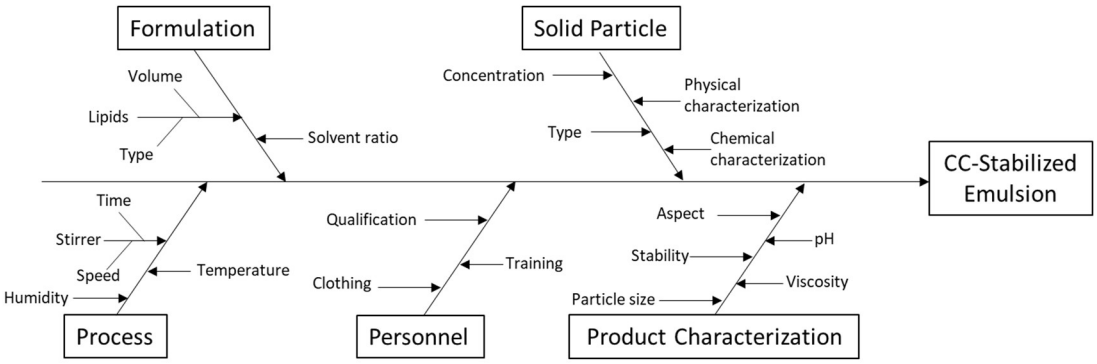

Figure 3. Ishikawa diagram showing factors that may impact the droplet size of a CC-stabilized emulsion [33].

Five percent of solid particles was selected as the CCD "0" level and 2.5% and 7.5% as the "$-\alpha$" and "α" levels, respectively, based on previous work [1].

3.2.2. Establishment of Design Space

Response Surface Analysis

Polynomial models were obtained by the analysis of the data obtained by the experimental design using MODDE®software. Analysis of Variance (ANOVA) was also performed, and the p-value was calculated for each variable, with the effects considered statistically significant for $p < 0.05$. The information resulting from the models was expanded graphically using isoresponsive curves. The observed and predicted values showed good correlation, as indicated by R^2, which ranged from 0.92 to 0.99 for all variables in the optimization study.

In Table 2, the coefficient column reflects the relative strength of each factor; the higher the absolute value, the greater the effect on the response.

Table 2. Results of regression analysis for measured responses.

Critical Quality Attributes	d(10)		d(50)		d(90)		Span	
	Coeff	**±SE**	**Coeff**	**±SE**	**Coeff**	**±SE**	**Coeff**	**±SE**
K	−1.953	0.003	−1.923	0.005	−1.862	0.005	1.076	0.070
CC	−0.009	0.005	−0.018	0.007	−0.046	0.006	−0.174	0.090
Oil	0.013	0.005	0.022	0.007	0.044	0.006	-	-
Oil*Oil	-	-	-	-	-	-	-	-
CC*CC	-	-	-	-	-	-	0.06	0.01
CC*Oil	-	-	-	-	−0.014	0.008	-	-

Oil—% Oil Phase; CC—% Calcium carbonate particles. Coeff—Coefficient scaled and centered; SE—Standard error; k—Constant; - (no significant)—$p > 0.05$.

The data obtained show that an increase in CC concentration contributes more strongly to a narrow droplet size distribution than a decrease in the oil phase.

Figure 4a–d shows the response surface plots concerning formula optimization. The variables that significantly affected ($p < 0.05$) the droplet size distribution were the concentration of CC particles and percentage of oil phase. A negative correlation was observed for the percentage of CC particles in the dependent variables d(10), d(50), and d(90), meaning that an increase in the concentration of particles in the emulsion led to a decrease in droplet size. It has been described that increasing concentrations of solid particles will decrease the droplet size, producing stable emulsions throughout the entire volume of the mixture, without coalescence or phase separation. However, if the droplet and the solid particles are similar in size, flow oscillations are induced and the droplets tend to fragment, increasing polydispersity [34]. Concerning the influence of oil phase percentage on the droplet size distribution, a positive correlation was observed. In this case, an increase in this phase percentage led to the increase in droplet size by aggregation of smaller droplets into larger ones. Both CC particle concentration and percentage of oil phase show a positive correlation, thus an increase in any of these two parameters might lead to a wider droplet size distribution.

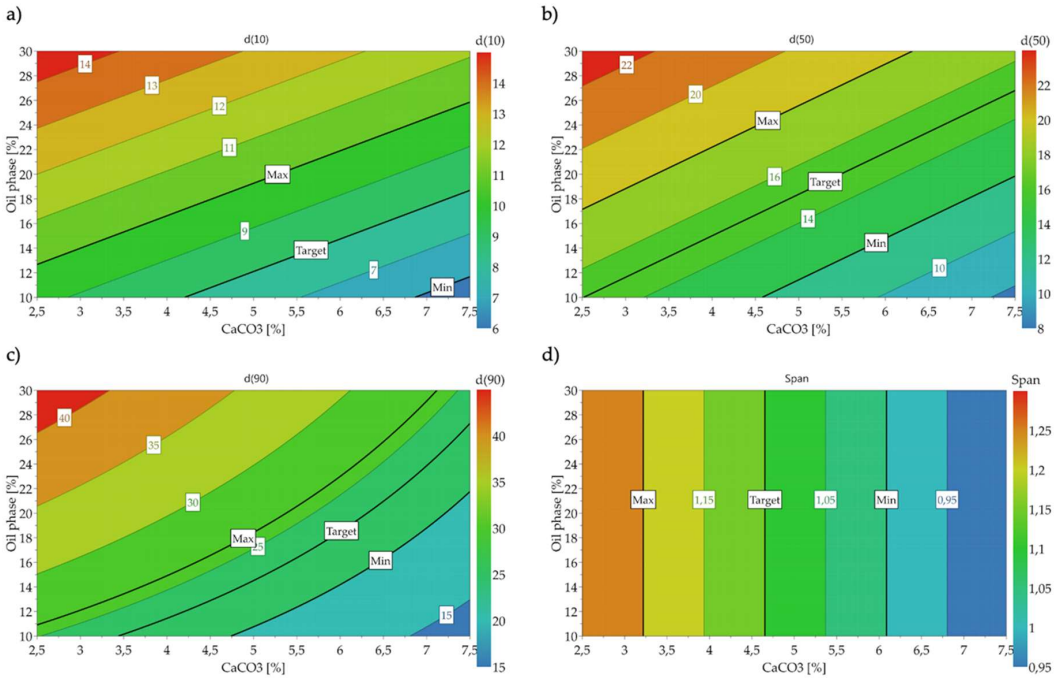

Figure 4. Isoresponse surface plots of relative size distribution (μm), respectively: (**a**) d(10), (**b**) d(50), (**c**) d(90), and (**d**) span for formula optimization. $CaCO_3$ [%]—% Calcium carbonate particles.

Design Space

In this study, the SS and DS were established by response surface methodology [33]. The process key parameters that had been shown to affect the quality of the emulsions were used to construct the DS (Figure 5).

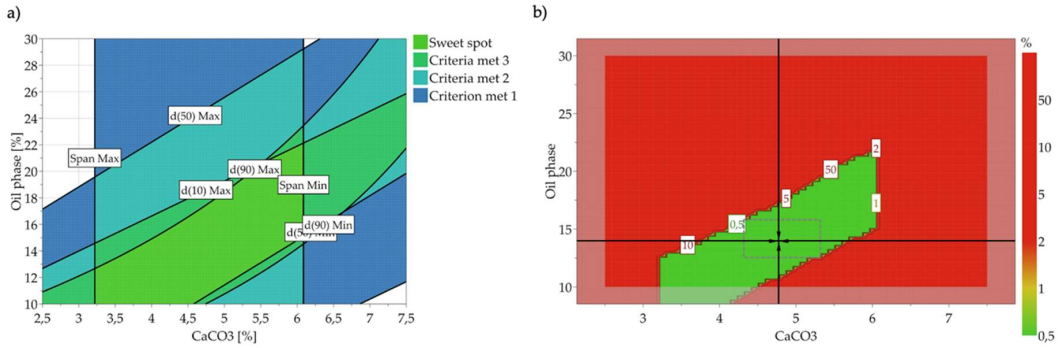

Figure 5. Plot evidence: the (**a**) Sweet Spot and the (**b**) Design Space for the CC-based emulsion.

In these plots, every point corresponds to an arrangement of the studied variables. The green area corresponds to a range of combinations for which the droplet size remains within the pre-defined tolerable limits shown in Figure 4. The shown plots indicate the range of CPP values within which the final response will not be affected (i.e., if the shown variables are kept within this range, the size of the emulsion droplets can be predicted and controlled). The SS and DS values determined for formula optimization are shown in the green regions (Figure 5). The optimal conditions defined by the SS and DS plots were 14% of oil phase and 4.7% of CC (Figure 5b).

3.3. Physicochemical Characterization of the Final Formulation

The pH of the final formulation was 7.92 at room temperature, being suitable and compatible with human skin. The flow curve (Figure 6a) shows a viscosity decrease with the increment of the shear

rate (ramp up). This dependence of the viscosity on the shear rate means that the formulation can be considered as a non-Newtonian fluid [35]. Moreover, the CC-stabilized emulsion also presented a shear thinning behavior, since the apparent viscosity significantly decreased with increasing shear rate (ramp up). When the shear rate decreased again (ramp down), the viscosity increased, but to values lower than those measured initially. For this formulation, the apparent viscosity in the ramp up at 0.1 s^{-1} was 10.89 Pa.s, while in the ramp down, after applying a shear rate of 100 s^{-1}, it was 0.36 Pa.s at 0.1 s^{-1}. Therefore, after the application of a 100 s^{-1} shear rate, the viscosity of the formulation did not recover during the time of the experiment, revealing thixotropy.

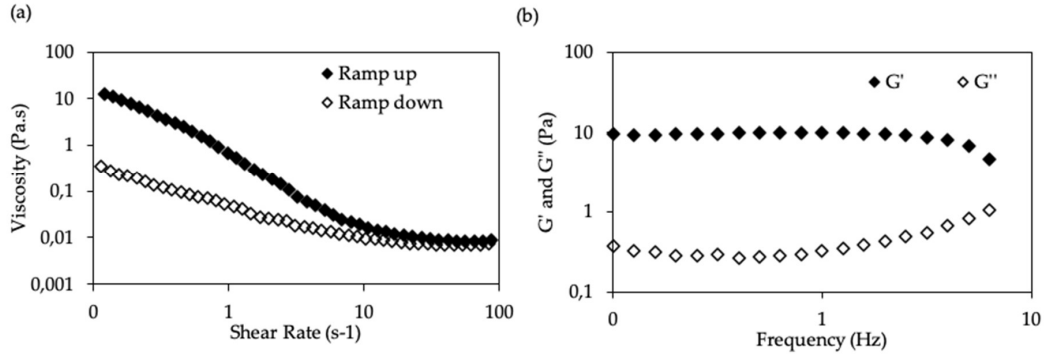

Figure 6. Flow curve (**a**) and oscillatory results obtained from a frequency sweep test (**b**) of the CC-stabilized emulsion.

The oscillatory frequency results for the final formulation, showed that the storage modulus—elastic component, G′ was higher than the loss modulus—viscous component, G″ (Figure 6b). Therefore, this formulation has a strong network structure ("solid-like"), with a suitable spreadability on the skin. Furthermore, because viscosity values decrease with increasing shear rate, the formulation can also easily be rubbed into the skin, allowing an easy application. Since the viscosity increases after ceasing the shear rate, the product will also easily stay on the application area. The value of the loss tangent, Tan δ (G″/G′), for this formulation is 0.04 at a frequency of 0.1 Hz, which is in accordance with the results obtained, since G′ > G″. Loss tangent, Tan δ, values must be smaller than 1.

3.4. In Vitro Cytotoxicity Assays

The hypothetical cytotoxicity of the CC-stabilized emulsion was evaluated in vitro by determining the viability of HaCaT cells using the MTT assay. The viabilities of cells exposed to the CC-stabilized emulsion and CC particles were 102 ± 18% and 69 ± 12%, respectively. This means that the CC particles were more cytotoxic than the CC-stabilized emulsion. This difference may be related to the surface roughness of the CC particles, which can minimize the repulsive forces between CC particles and the plasma membrane, inducing membrane damage or cellular uptake [36]. This effect was decreased in the stabilized emulsion, where the CC particles were inside the emulsion structure.

To predict the safety and the irritant potential of the CC-stabilized emulsion and the CC particles, the Organisation for Economic Co-operation and Development (OECD) guideline was considered. This guideline defines a substance as an irritant and unsafe if the mean relative tissue viability is lower than 50% of the mean viability of the negative controls for a 15–60 min exposure time [24]. Therefore, considering the results obtained, it is possible to state that both the CC-stabilized emulsion and the CC particles by themselves can be considered as safe and a non-irritant based on in vitro studies. These results can only be confirmed by in vivo studies.

4. Conclusions

In the present work, a QbD methodology was used to optimize a formulation of a CC-stabilized Pickering emulsion for topical application. A novel and innovative final Pickering emulsion using

biocompatible inorganic CC particles as a stabilizer was obtained and showed high skin compatibility, meeting the consumer demands for eco-friendly and natural products.

The results showed that the CC-stabilized emulsion had a pH compatible with human skin. Furthermore, the formulation presented a strong network structure with shear thinning behavior and an elastic component higher than the viscous component, allowing an easy application.

Finally, the in vitro cytotoxicity results obtained with a human keratinocyte cell line exposed to the CC-stabilized emulsion suggest that the formulation can possibly be considered as safe and a non-irritant.

Taken together, the results suggest that the formulation is a suitable vehicle for topical delivery systems. Moreover, the findings obtained herein contribute to the increasing trusted use of renewable and eco-friendly resources such as CC, modified starch, chitosan, and cyclodextrins as stabilizers of Pickering emulsions [26,37]. These features, alongside the versatility of these emulsions, are very promising for the pharmaceutical and cosmetic industries.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-9284/7/3/62/s1, Table S1: Design of Experiments (CC-stabilized emulsion optimization).

Author Contributions: Conceptualization, J.M.; Methodology, J.M., A.N., L.G., J.C., and P.P.; Formal analysis, J.M., A.N., A.M.M., L.G., J.C., and P.P; Resources, A.M. and A.L.; Writing—original draft preparation, J.M., A.N., and A.M.M.; Writing—review and editing, H.M.R.; Supervision, H.M.R.; Project administration, J.M., H.M.R., A.M., and A.L.; Funding acquisition, A.M. and A.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fundação para a Ciência e a Tecnologia, Portugal (UID/DTP/04138/2019, PTDC/MEC-DER/30198/2017, and CEECINST/00145/2018) and by Carbomin.

Conflicts of Interest: The authors state no conflicts of interest and have received no payment in preparation of this manuscript.

References

1. Marto, J.; Gouveia, L.; Jorge, I.M.; Duarte, A.; Goncalves, L.M.; Silva, S.M.; Antunes, F.; Pais, A.A.; Oliveira, E.; Almeida, A.J.; et al. Starch-Based Pickering emulsions for topical drug delivery: A QbD approach. *Colloids Surf. B Biointerfaces* **2015**, *135*, 183–192. [CrossRef]
2. Ribeiro, H.M.; Morais, J.A.; Eccleston, G.M. Structure and rheology of semisolid o/w creams containing cetyl alcohol/non-ionic surfactant mixed emulsifier and different polymers. *Int. J. Cosmet. Sci.* **2004**, *26*, 47–59. [CrossRef]
3. Marto, J.; Ascenso, A.; Simoes, S.; Almeida, A.J.; Ribeiro, H.M. Pickering emulsions: Challenges and opportunities in topical delivery. *Expert Opin. Drug Deliv.* **2016**, *13*, 1093–1107. [CrossRef]
4. Bouyer, E.; Mekhloufi, G.; Rosilio, V.; Grossiord, J.L.; Agnely, F. Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field? *Int. J. Pharm.* **2012**, *436*, 359–378. [CrossRef]
5. Wang, Y.; Zhang, Y.; Li, X.; Sun, M.; Wei, Z.; Wang, Y.; Gao, A.; Chen, D.; Zhao, X.; Feng, X. Exploring the effects of different types of surfactants on zebrafish embryos and larvae. *Sci. Rep.* **2015**, *5*, 10107. [CrossRef] [PubMed]
6. Pickering, S.U. CXCVI.—Emulsions. *J. Chem. Soc. Trans.* **1907**, *91*, 2001–2021. [CrossRef]
7. Chevalier, Y.; Bolzinger, M.-A. Emulsions stabilized with solid nanoparticles: Pickering emulsions. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *439*, 23–34. [CrossRef]
8. Marto, J.; Gouveia, L.F.; Goncalves, L.; Chiari-Andreo, B.G.; Isaac, V.; Pinto, P.; Oliveira, E.; Almeida, A.J.; Ribeiro, H.M. Design of novel starch-based Pickering emulsions as platforms for skin photoprotection. *J. Photochem. Photobiol. B* **2016**, *162*, 56–64. [CrossRef]
9. Matos, M.; Timgren, A.; Sjöö, M.; Dejmek, P.; Rayner, M. Preparation and encapsulation properties of double Pickering emulsions stabilized by quinoa starch granules. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *423*, 147–153. [CrossRef]
10. Yang, Y.; Fang, Z.; Chen, X.; Zhang, W.; Xie, Y.; Chen, Y.; Liu, Z.; Yuan, W. An overview of Pickering emulsions: Solid-Particle materials, classification, morphology, and applications. *Front. Pharmacol.* **2017**, *8*, 287. [CrossRef]
11. Leclercq, L.; Nardello-Rataj, V. Pickering emulsions based on cyclodextrins: A smart solution for antifungal azole derivatives topical delivery. *Eur. J. Pharm. Sci.* **2016**, *82*, 126–137. [CrossRef]

12. Leclercq, L.; Tessier, J.; Douyère, G.; Nardello-Rataj, V.; Schmitzer, A.R. Phytochemical- and Cyclodextrin-based pickering emulsions: Natural potentiators of antibacterial, antifungal, and antibiofilm activity. *Langmuir* **2020**, *36*, 4317–4323. [CrossRef]
13. Taguchi, H.; Tanaka, H.; Hashizaki, K.; Saito, Y.; Fujii, M. Application of Pickering emulsion with cyclodextrin as an emulsifier to a transdermal drug delivery vehicle. *Biol. Pharm. Bull.* **2019**, *42*, 116–122. [CrossRef]
14. Inoue, M.; Hashizaki, K.; Taguchi, H.; Saito, Y. Emulsifying ability of β-cyclodextrins for common oils. *J. Dispers. Sci. Technol.* **2010**, *31*, 1648–1651. [CrossRef]
15. Binks, B.P. Particles as surfactants—Similarities and differences. *Curr. Opin. Colloid Interface Sci.* **2002**, *7*, 21–41. [CrossRef]
16. Laredj-Bourezg, F.; Chevalier, Y.; Boyron, O.; Bolzinger, M.-A. Emulsions stabilized with organic solid particles. *Colloids Surf. A Physicochem. Eng. Asp.* **2012**, *413*, 252–259. [CrossRef]
17. Rayner, M.; Marku, D.; Eriksson, M.; Sjöö, M.; Dejmek, P.; Wahlgren, M. Biomass-Based particles for the formulation of Pickering type emulsions in food and topical applications. *Colloids Surf. A Physicochem. Eng. Asp.* **2014**, *458*, 48–62. [CrossRef]
18. CosIng—European Commission Database for Information on Cosmetic Substances and Ingredients. Available online: https://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.details_v2&id=30534 (accessed on 29 July 2020).
19. Tambe, D.E.; Sharma, M.M. Factors controlling the stability of colloid-stabilized emulsions: I. An experimental investigation. *J. Colloid Interface Sci.* **1993**, *157*, 244–253. [CrossRef]
20. Cui, Z.G.; Cui, C.F.; Zhu, Y.; Binks, B.P. Multiple phase inversion of emulsions stabilized by in situ surface activation of CaCO3 nanoparticles via adsorption of fatty acids. *Langmuir* **2012**, *28*, 314–320. [CrossRef]
21. Cui, Z.G.; Shi, K.Z.; Cui, Y.Z.; Binks, B.P. Double phase inversion of emulsions stabilized by a mixture of CaCO3 nanoparticles and sodium dodecyl sulphate. *Colloids Surf. A Physicochem. Eng. Asp.* **2008**, *329*, 67–74. [CrossRef]
22. Zhu, Y.; Lu, L.H.; Gao, J.; Cui, Z.G.; Binks, B.P. Effect of trace impurities in triglyceride oils on phase inversion of Pickering emulsions stabilized by $CaCO_3$ nanoparticles. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *417*, 126–132. [CrossRef]
23. Huang, F.; Liang, Y.; He, Y. On the Pickering emulsions stabilized by calcium carbonate particles with various morphologies. *Colloids Surf. A Physicochem. Eng. Asp.* **2019**, *580*, 123722. [CrossRef]
24. Carriço, C.; Pinto, P.; Graça, A.; Gonçalves, L.M.; Ribeiro, H.M.; Marto, J. Design and characterization of a new quercus suber-based pickering emulsion for topical application. *Pharmaceutics* **2019**, *11*, 131. [CrossRef]
25. Naves, L.B.; Almeida, L.; Marques, M.J.; Soares, G.; Ramakrishna, S. Emulsions stabilization for topical application. *Biomater. Med. Appl.* **2017**, *1*. [CrossRef]
26. Marto, J.; Pinto, P.; Fitas, M.; Goncalves, L.M.; Almeida, A.J.; Ribeiro, H.M. Safety assessment of starch-based personal care products: Nanocapsules and pickering emulsions. *Toxicol. Appl. Pharmacol.* **2018**, *342*, 14–21. [CrossRef]
27. BSI. BS 1993: Methods for determination of particle size distribution. Part 4, Guide to microscope and image analysis methods. In *British Standards*; BSI: London, UK, 1993.
28. EC (Ed.) *Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products*; Official Journal of the European Union; European Commission (EC): Brussels, Belgium, 2009. Available online: https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_233.pdf (accessed on 30 July 2020).
29. Molina, J.M.; Narciso, J.; Weber, L.; Mortensen, A.; Louis, E. Thermal conductivity of Al–SiC composites with monomodal and bimodal particle size distribution. *Mater. Sci. Eng. A* **2008**, *480*, 483–488. [CrossRef]
30. Ruckenstein, E. Microemulsions, macroemulsions, and the bancroft rule. *Langmuir* **1996**, *12*, 6351–6353. [CrossRef]
31. Wu, J.; Ma, G.-H. Recent studies of pickering emulsions: Particles make the difference. *Small* **2016**, *12*, 4633–4648. [CrossRef]
32. Maestro, A.; Guzmán, E.; Ortega, F.; Rubio, R.G. Contact angle of micro- and nanoparticles at fluid interfaces. *Curr. Opin. Colloid Interface Sci.* **2014**, *19*, 355–367. [CrossRef]
33. Lionberger, R.A.; Lee, S.L.; Lee, L.; Raw, A.; Yu, L.X. Quality by design: Concepts for ANDAs. *AAPS J.* **2008**, *10*, 268–276. [CrossRef]

34. Lam, S.; Velikov, K.P.; Velev, O.D. Pickering stabilization of foams and emulsions with particles of biological origin. *Curr. Opin. Colloid Interface Sci.* **2014**, *19*, 490–500. [CrossRef]
35. De Souza Mendes, P.R. Modeling the thixotropic behavior of structured fluids. *J. Non Newton. Fluid* **2009**, *164*, 66–75. [CrossRef]
36. Kim, M.K.; Lee, J.A.; Jo, M.R.; Kim, M.K.; Kim, H.M.; Oh, J.M.; Song, N.W.; Choi, S.J. Cytotoxicity, uptake behaviors, and oral absorption of food grade calcium carbonate nanomaterials. *Nanomaterials* **2015**, *5*, 1938–1954. [CrossRef]
37. Marku, D.; Wahlgren, M.; Rayner, M.; Sjöö, M.; Timgren, A. Characterization of starch Pickering emulsions for potential applications in topical formulations. *Int. J. Pharm.* **2012**, *428*, 1–7. [CrossRef]