# Micro/Nano Structured Materials

## Stabilization of Functional Ingredients

### Pickering Emulsions for Food and Cosmetic Applications

**KEYWORDS:** Pickering emulsions/ Microencapsulation/ NETmix/ Functional ingredients/ Food/ Cosmetics

**Pickering emulsions use solid particles to stabilize the interface between two immiscible fluids. In this work, stable emulsions, with no chemical surfactants, were produced with using nano-hydroxyapatite, chitosan/gum Arabic, and chitosan/collagen peptides as Pickering stabilizers. These Pickering emulsions (PEs) were used to develop innovative alternatives for food and cosmetic applications and to encapsulate active ingredients such as lipophilic vitamins and cannabidiol.**

#### Introduction

Pickering emulsions for food and Cosmetic Applications

Emulsions are widely used in various fields, namely food, pharmaceuticals, cosmetics, and agrochemicals, playing an important role in traditional and novel emulsion-based products. Emulsions are bi-phasic systems where a third compound is needed to stabilize the two immiscible phases. In Pickering emulsions (PEs), the third compound, acting as the stabilizer, are solid particles. These emulsions provide high physical and chemical stability due to their irreversible solid particles' adsorption at the oil-in-water interface. Thus, PE stabilization arose as an alternative to traditional emulsifiers, contributing to the development of clean-label products and promising encapsulation systems for bioactive compounds such as vitamins or natural extracts, receiving high interest, both from academic and industrial perspectives.

#### Current Development

<u>Pickering Emulsions for Food Applications</u>

Vitamin E is essential in human health, avoiding cellular ageing and reducing diseases such as dementia; however, vitamin E has a high lipophilic character and needs to be encapsulated to increase the stability and compatibility with hydrophilic food matrices. Nano-hydroxyapatite is an appropriate material for developing oil-in-water (O/W) PEs to protect and deliver lipophilic compounds and further develop functional foods. Additionally, aiming at a more feasible process, the NETmix technology was tested to produce vitamin E-loaded PEs.

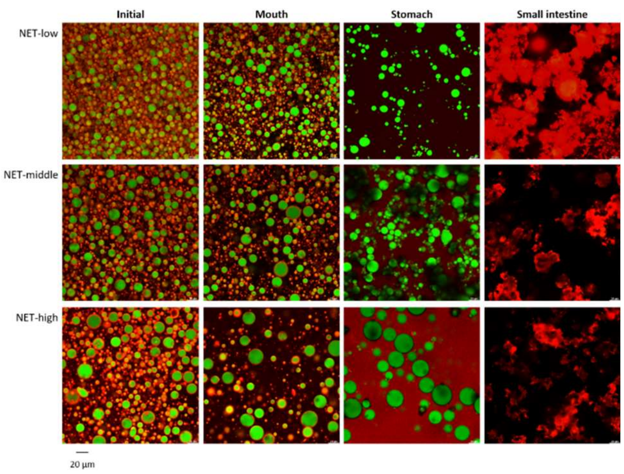


Fig 1. Overlapped CLMS images of the vitamin E loaded Pickering emulsions with different sizes (NET-low = ~7 µm, NET-middle: ~11 µm; NET-high: ~18 µm) after each stage of digestion (mouth, stomach and small intestine), comparatively with the initial emulsion. The n-HAp particles are presented as red and the oil droplets as green colour.

The vitamin E-loaded PEs were produced in NETmix and digested in vitro through a simulated gastrointestinal tract (GIT): mouth, gastric and small intestine (Fig 1).

The vitamin E-loaded PEs presented similar behaviour to thise with no vitamin E, with n-HAp particles being dissolved under gastric conditions with subsequent formation of aggregates under the intestinal environment. When the Vitamin E-loaded PE was incorporated in food matrices (gelatine and milk, Fig. 2 B), vitamin E bioaccessibility increased significantly (10.87±1.04% for gelatine and 18.07±2.90% for milk), putting in evidence the positive effect of the food matrix in the vitamin bioaccessibility (Fig. 2 A). This work was performed in collaboration with Centre of Biological Engineering (https://www.ceb.uminho.pt).

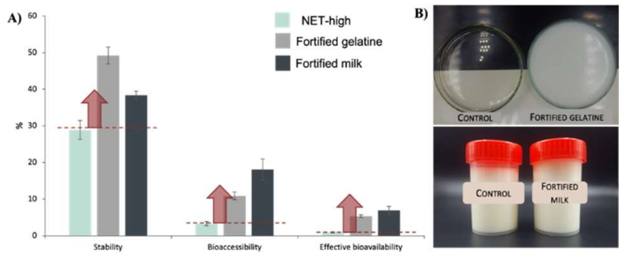


Fig. 2. A) Stability, bioaccessibility, and effective bioavailability of vitamin E-loaded Pickering emulsions (PEs) after in vitro digestion; and B) Photographic of control samples and fortified foods.

Additionally, to cover a wide range of applications, solutions to stabilize water-in-oil (W/O) PEs have started to raise interest. W/O systems are interesting solutions for encapsulating and delivering hydrophilic active compounds, such as phenolic extracts, which can open new opportunities for products based on nano-hydroxyapatite particles. For this purpose, sodium oleate (SO) surface modified nano-hydroxyapatite (n-HAp) particles were produced and tested as W/O stabilizer.

The amount of sodium oleate was estimated considering the number of layers at the particle surface (0.5, 1 and 2; Fig. 3 A). As the number of sodium oleate layers increased, the contact angle of hydroxyapatite particles increased up to 1 layer (92°), decreasing for 2 layers (38°), indicating that the second layer is formed over an existing layer, switching the hydrophobic character. W/O PEs were successfully produced with one layer of the sodium oleate; the confocal images in Fig. 3 B confirm the emulsion type where it is possible to identify the sunflower oil as the continuous phase and the modified particles positioned at the boundary of the droplets.

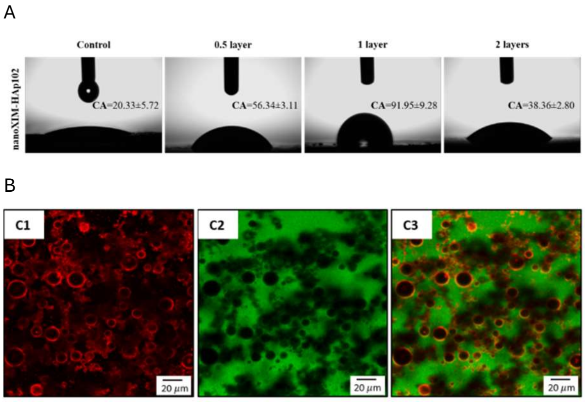


Fig. 3. A) Contact angle measurements of the original n-HAp (control) and SO modified n-HAp at different layers (0.5, 1, and 2). B) Confocal images of the W/O (20/80) Pickering emulsion (PE).

<u>Pickering Emulsions for Cosmetic Applications</u>

PEs stabilized by chitosan/collagen peptides (CH/CP) nanoparticles were assessed as green surfactant-free vehicles for the topical delivery of cannabidiol (CBD), a highly lipophilic unstable drug that is finding an increasing appeal in the cosmetic market (Fig. 4). The influence of the oil phase volume fraction and the oil type on the emulsion properties, stability, rheological properties, as well as on the ex-vivo skin absorption of CBD was evaluated. The PE prepared with olive oil exhibited elastic gel-like properties and showed long-term stability after 5 months of storage, with a CBD content of 99.45% of the initially added amount. Skin absorption studies showed that CBD was retained in high amounts in the stratum corneum, while the CBD skin permeation was extremely low, indicating that the produced formulations are suitable as topical delivery vehicles. ATR-FTIR examination of the treated skin samples confirmed that the produced PEs were able to overcome the stratum corneum barrier. These findings suggest that the PEs stabilized with CH/CP nanoparticles provide an effective surfactant free alternative for the topical delivery of CBD.

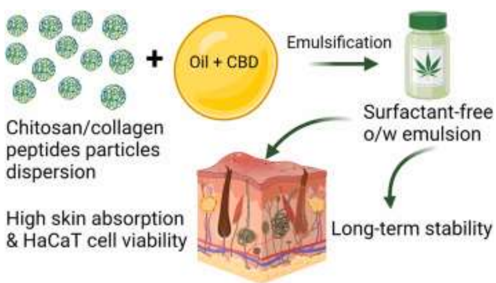


Fig 4. PEs stabilized by chitosan/collagen peptides (CH/CP) nanoparticles for the topical delivery of cannabidiol (CBD).

The effect of the degree of deacetylation (DDA) of chitosan on the properties of chitosan/gum Arabic (CH/GA) particles and PEs was also studied. CH/GA particles with a DDA of 96% (high DDA) and 78% (low DDA) were prepared. Both PE formulations demonstrated shear thinning and elastic gel-like properties. The amount of cannabidiol (CBD) 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.

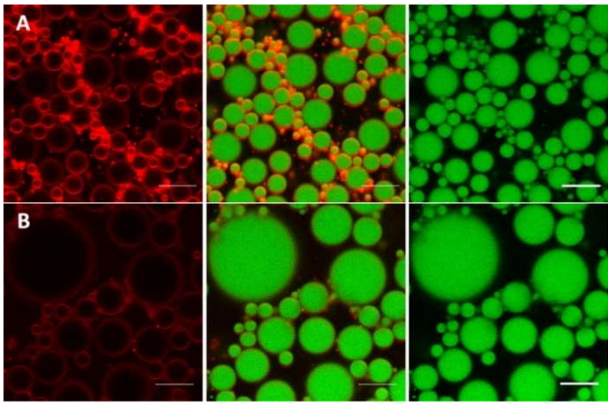


Fig. 5. CLSM images of CBD-loaded chitosan/gum Arabic Pickering emulsions formulated with chitosan of (A) High DDA (96%), and (B) Low DDA (78%). The images on the left show the particles as red rings stained with Nile Blue, whereas the images on the right represent the emulsion oil droplets stained with Nile Red, and the images in the middle are an overlay of these images. Scale bars = 10 μm.

**Future Perspectives**

The PE encapsulation process can be extended to other lipophilic vitamins or active compounds and other Pickering stabilizers towards innovative food development. The obtained results also pointed out the interest in proceeding with further studies to understand the effect of the food matrix composition on the achieved bioaccessibility, highlighting the importance of combining the study of PEs with their final applications to evaluate more accurately the real potential of these solutions. This work will continue as part of the workplan of a FCT Stimulus of Scientific Employment Grant (2022.00798.CEECIND).

Studies using chitosan-based PEs, and materials thereof, in skin applications are very few, and thus represent a valued research opportunity in order to leverage chitosan non-toxic and biodegradable properties, as well as its skin benefits. The sensorial perception of cosmetic PEs stabilized with chitosan particles has never been studied. The type of particles remarkably influences the sensory properties of the developed cosmetic PEs as they determine the level of greasiness, whitening residues, ease of spreading and glossiness. Thus, future efforts should be also directed towards the study of the sensory properties of PEs stabilized by different chitosan particles.

**Related Sustainable Development Goals**

**Outputs**

**PhD Theses**

[1] Andreia Ribeiro, Nanohydroxyapatite Pickering emulsions: From product development to functional ingredients in Food Applications, PDEQB, FEUP, 2021

[2] Asma Sharkawy, Novel chitosan-based Pickering emulsions as green encapsulation systems and delivery vehicles of bioactive agents, PDEQB, FEUP, 2021

**Selected Publications**

[1] A. Ribeiro, J.C.B Lopes, M.M Dias, M.F. Barreiro, Molecules, 28, 131287 (2023)

[2] A. Ribeiro, R.F.S. Gonçalves, A.C. Pinheiro, Y.A. Manrique, M.F. Barreiro, J.C.B. Lopes, M.M. Dias, LWT, 154, 112706 (2022)

[3] A. Ribeiro, Y.A. Manrique, J.C.B. Lopes, M.M.Dias, M.F. Barreiro, Surfaces and Interfaces, 29, 101759 (2022)

[4] A. Sharkawy, F. Barreiro, A.E. Rodrigues. Journal of Molecular Liquids 355 (2022).

[5] A. Sharkawy, M.F. Barreiro, A.E. Rodrigues. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 616, 126327 (2021) DOI: 10.1016/j.colsurfa.2021.126327

[5] A. Sharkawy, A.M. Silva, F. Rodrigues, F. Barreiro, A. Rodrigues. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 631, 127677 (2021) DOI: 10.1016/j.colsurfa.2021.127677

[6] A. Sharkawy, M.F. Barreiro, A.E. Rodrigues. Carbohydrate Polymers, 250, 116885, 2020 DOI: /10.1016/j.carbpol.2020.116885
[7] A. Sharkawy, F.M. Casimiro, M.F. Barreiro, A.E. Rodrigues. International Journal of Biological Macromolecules, 147, 150-159 (2020) DOI: 10.1016/j.ijbiomac.2020.01.057

## Team

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## Funding

LSRE-LCM Base Funding, UIDB/50020/2020, 2020-2023
LSRE-LCM Programmatic Funding, UIDP/50020/2020, 2020-2023
LA LSRE-LCM Funding, UID/EQU/50020/2019, 2019
LA LSRE-LCM Funding, POCI-01-0145-FEDER-006984,2013-2018
PDEQB, NORTE-08-5369-FSE000028, 2016-20020
FCT Grant: 2022.00798.CEECIND (A. Ribeiro)

## Micro/Nano Structured Materials

### Stabilization of Functional Ingredients

#### Extraction, Stabilisation, and Incorporation of Functional Extracts/Compounds

**KEYWORDS:** Functional Ingredients/Extraction/Stabilisation/Emulsions/Incorporation

**This topic aims at the development of methodologies to stabilize and enable incorporation of functional extracts/compounds and achieve sustained release in applications such as agro-food, pharmaceutical, cosmetics or nutraceuticals. Within the period of this reporting period three PhD projects have been underway: study of nanoemulsions based on natural components, with emphasis on saponin, including saponin-rich extracts from different sources; replacing synthetic colorants by natural based counterparts based on the *Spirulina platensis*, a microalgae cianobacterium rich in phycocyanin, a bright blue pigment; development of lignin-based pickering stabilizers for innovative cosmeceutical formulations.**

**Introduction**

This work results from a close colaboration with CIMO, a R&D Unit located at Polytechnic Institute of Bragança, where most of the experimental work has been carried out.

Saponin and Saponin-Rich Extracts Nanoemulsions

Replacing synthetic surfactants by natural alternatives in emulsion formulation has gained attention as a sustainable approach, due to its strong environmental impact. This work aimed to study nanoemulsions based on natural components, with emphasis on saponin, including saponin-rich extracts from different sources, never studied before as surfactants: Tribulus terrestris, Trigonella foenum-graecum, and Ruscus aculeatus. Furthermore, the ability to incorporate lipophilic vitamins (e.g. vitamin E) will add functional properties to the emulsions making them advantageous over conventional formulations, represents an exciting new direction of study.

Phycocyanin and co-bioproducts from Spirulina

One strategy towards the sustainability of algal biomass production is to generate multiple products in a productive value-chain, i.e. use a biorefinery approach. Biomass of microalga cianobacterium Spirulina platensis is rich in nutritional compounds (proteins, lipids, carbohydrates) and natural pigments (phycobiliproteins, carotenoids, chlorophylls). Phycocyanin, the most abundant phycobiliprotein, is a bright blue pigment finding with applications in the food sector. Based on the high market demand for natural-based colorants, intended to substitute synthetic counterparts, and the potential to achieve a sustainable and high volume production, Spirulina biomass will be exploited to produce phycocyanin. This will be based on an innovative process, where Spirulina biomass is immobilized in gel-like particles (as such or after cell disruption), to extract phycocyanin in aqueous medium.

Lignin-based pickering stabilizers

Among the numerous applications of coloidal lignin particles (CLPs), their use as Pickering stabilizers has gained interest, constituting an alternative to conventional synthetic surfactants due to their biodegradability and renewability. This new focus expanded lignin's applications to areas such as cosmetics, pharmaceuticals, and food. In this context, it is important to understand the effect of the productive process variables on CLPs' properties, namely Pickering potential.

**Current Development**

Saponin and Saponin-Rich Extracts Nanoemulsions

Nanoemulsions based on sweet almond oil and stabilized by saponin from Quillaja bark with glycerol as cosurfactant were prepared by the high-pressure homogenization method. The effects of oil/water (O/W) ratio, total surfactant amount, and saponin/glycerol ratio on their stability were analyzed. The formation and stabilization of the oil-in-water nanoemulsions were analyzed through the evaluation of stability over time, pH, zeta potential, and particle size distribution analysis. Moreover, a design of experiments was performed to assess the most suitable composition based on particle size and stability parameters. The prepared nanoemulsions are, in general, highly stable over time, showing zeta potential values lower than −40 mV, a slight acid behavior due to the character of the components, and particle size (in volume) in the range of 1.1 to 4.3 μm. Response surface methodology revealed that formulations using an O/W ratio of 10/90 and 1.5 wt% surfactant resulted in lower particle sizes and zeta potential, presenting higher stability. The use of glycerol did not positively affect the formulations, which reinforces the suitability of preparing highly stable nanoemulsions based on natural surfactants such as saponins.

Three saponin-rich extracts (Fig. 1) from different sources (Tribulus terrestris (TT), Trigonella foenum-graecum (FG), and Ruscus aculeatus (RA)) were tested as emulsifiers, and their performance compared with Quillaja Bark saponin (QS). Characterisation comprised FTIR, solubility studies, CMC assays, and emulsifying properties (emulsifying capacity (EC) and foaming capacity (FC). For all samples, solubility assays indicated high solubility in water and low in apolar solvents (e.g., n-hexane), compatible with their O/W emulsifier character. The saponin content ruled extracts' performance (PS >TT >FG >RA). EC values (without pH adjustment) were found to be 82.5, 55.0, 47.5, 36.3%, respectively. When pH changed for 7 and 9, a shift in FG and RA order was observed. The pseudo-ternary diagrams, constructed to map emulsion's composition zones, indicate the formation of single-phase systems in the region of low oil and high extract content. Except for RA extract, gel samples were formed, which are interesting, technological solutions for several applications. Among the studied samples, and in alternative to QS, TT extract showed the best performance.

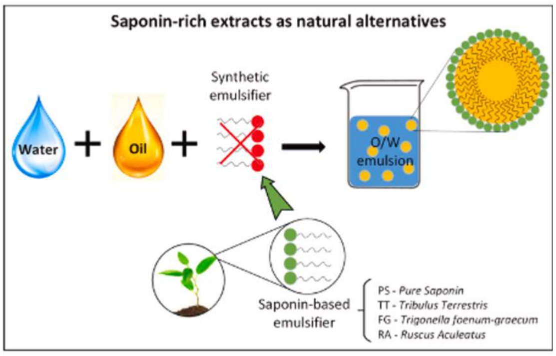


Fig 1. Saponin-rich extracts as emulsifiers natural alternatives.

Lipophilic compounds of the vitamin E (VE) family (tocopherols and tocotrienols) are well-known for their high antioxidant activity and capacity to protect the skin from oxidative stress. In this context, oil-in-water (O/W) nanoemulsions with and without α-tocopherol were formulated with two emulsifier alternatives, Quillaja saponin (QS), and a combination of QS with Tribulus terrestris (QSTT) (50/50, w/w). The emulsions were evaluated concerning stability, microstructure, droplet size, colour attributes, encapsulation efficiency, UV photostability, antioxidant activity, and in vitro permeation studies to assess the delivery potential. Results showed highly stable systems, with round-shape droplets of 80–121 nm size. QS and QSTT samples' colours were close to white and light brownish, respectively. The topical nano cream had the capacity to entrap VE, producing a protective effect from UV degradation, and very significant antioxidant activity, with IC50 values around 0.01 %wt. The skin permeation profiles showed the efficiency of the formulations in the delivery of VE, with permeabilities between 64 and 74 μg/cm2, while the control sample showed no VE permeation.

Phycocyanin and co-bioproducts from Spirulina

Spirulina (Arthrospira platensis) proteins have been proven to present emulsifying properties. A Spirulina protein-rich extract obtained by ultrasound extraction (SpE) was tested to stabilize oil-in-water (O/W) emulsions (Fig, 2). A sequential experimental design strategy (Fractional Factorial Design (FFD) 24–1 followed by a Central Composite Rotatable Design (CCRD $2^2$) was applied. Four variables (SpE concentration, O/W weight ratio, pH and storage time) on emulsions' zeta potential and number-mean droplet diameter was considered for the FFD $2^{4-1}$, indicating SpE concentration and storage time as the relevant variables for CCRD $2^2$. According to zeta potential and number-mean droplet diameter evaluation, for the studied SpE concentration range (2–5 wt%), quite stable emulsions were obtained along the tested 30-days period. However, for 5%, visual inspection revealed extract segregation after 20-days. The optimal solution comprised 4 wt% of SpE, for an O/W weight ratio of 30/70 and a pH of 7.0 (number-mean droplet diameter of 55.66 nm and zeta potential of − 43.83 mV). Overall, SpE has proven to be an excellent emulsifier, offering the potential to substitute animal-based proteins and synthetic emulsifiers. In addition, no signs of contamination by microorganisms were observed, suggesting that the SpE may also act as an antimicrobial agent.


Fig 2. Spirulina protein extraction and emulsification process.

Lignin-based pickering stabilizers

The effect of the initial lignin concentration, antisolvent pH, final ethanol concentration, and antisolvent rate addition was studied using a screening design (FFD 24-1) to determine the most significant process variables impacting CLPs properties (particle size, zeta potential, color, contact angle, and emulsifying potential) using the antisolvent precipitation method. Under the tested ranges, the initial lignin concentration had a positive and significant effect on the particle size being also observed that the particles with the larger sizes had higher stability (higher negative zeta potential) if produced using a high antisolvent pH, showing better performance as Pickering stabilizers. Interestingly, these particles were also the ones showing a lighter color, which is an interesting attribute for cosmetic applications. The final ethanol concentration was only significant for the particle size and the antisolvent addition rate was not significant for any of the responses. Under the tested conditions, the most favorable conditions for preparing high concentrated and stable CLPs with suitable characteristics to act as Pickering stabilizers comprised an initial lignin concentration of 50 g/L, antisolvent pH of 8, a final ethanol concentration of 45% (volume), and an antisolvent addition rate of 14 mL/min.

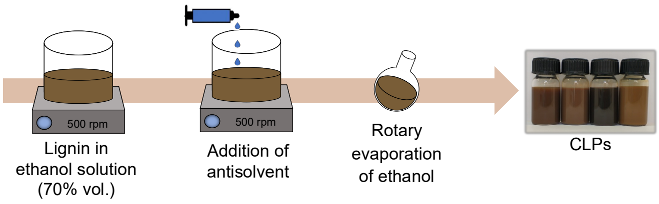


Fig 3. Schematic representation of the antisolvent precipitation method to produce colloidal lignin particles (CLPs)

**Related Sustainable Development Goals**

**Outputs**

**PhD Theses**

[1] Tatiana La Banca Schreiner. Saponin and Saponin-rich Extracts as Innovative Solutions for Nanoemulsions Production, PDEQB, FEUP, 2023

[2] Samara Cristina da Silva. Development of an integrated approach to produce phycocyanin and valorize co-bioproducts from Spirulina (Arthrospira platensis), PDEQB, FEUP (on going).

[3] Giovana Colucci. Lignin-based pickering stabilizers for innovative cosmeceutical formulations, PDEQB, FEUP (on going).

**Selected Publications**

[1] T.B. Schreiner et al., Molecules, 27, 1538 (2020)
[2] T.B. Schreiner et al., Colloids and Surfaces A: Physicochemical and Engineering Aspects, 623, 126748 (2021)
[3] T.B. Schreiner et al., Journal of Agricultural and Food Chemistry, 70, 6573-6590 (2020)
[4] T.B. Schreiner et al., Journal of Molecular Liquids, 391, 123371 (2023)
[5] T.B. Schreiner et al., Journal of Surfactants and Detergents, in press, 2023
[6] Silva et al., Molecules, 25, 3406 (2020)
[7] Silva et al., Colloids and Surfaces A: Physicochemical and Engineering Aspects, 648, 129264 (2022)
[8] Colucci et al., Colloids and Surfaces A: Physicochemical and Engineering Aspects, 666, 131287 (2023)
[9] Colucci et al., Molecules, 25, 2150, 2020

**Future Perspectives**

Incorporation of α-tocopherol on saponin emulsions have shown that the proposed formualtions are great candidates for the delivery of VE, presenting nanodroplet size, photoprotective capacity, high antioxidant activity, efficient entrapment, and good penetration and skin retention of VE. This can lead to the development of green-labelled cosmetical products.

The work on exploiting protein extracts from Spirulina (Artrhospira platensis) as natural emulsifiers will continue focusing on using these extracts to produce microgels, which can be applied in Pickering emulsions. In a complementary application, their use in functional films (e.g., for packaging materials) is also under study.

The screening design allowed to identify the significant process variables of the lignin particle production, enabling the obtainment of tailored CLPs with high potential to be applied as Pickering emulsions stabilizers targeting cosmetic applications.

**Team**

**Madalena M. Dias**, Professor; **Alírio E. Rodrigues**, Professor; M. Filomena Barreiro, Professor (CIMO/IPB); Tatiana Schreiner, PhD Student; **Samara Silva**, PhD Student; **Giovana Colucci**, PhD Student

**Funding**

LSRE-LCM Base Funding, UIDB/50020/2020, 2020-2023
LSRE-LCM Programmatic Funding, UIDP/50020/2020, 2020-2023
LA LSRE-LCM Funding, UID/EQU/50020/2019, 2019
LA LSRE-LCM Funding, POCI-01-0145-FEDER-006984,2013-2018
FCT Scholarships: 2020.05564.BD (T. Screiner);
SFRH/BD/148281/2019 (S. Silva); 2021.05215.BD (G. Colucci)