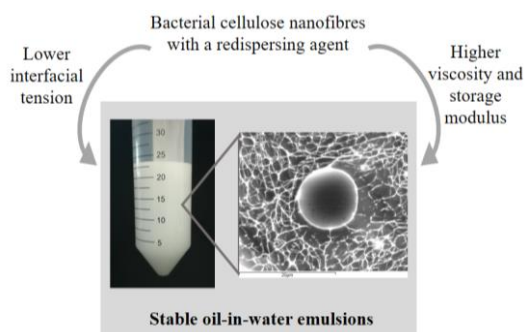# Bacterial cellulose as a stabilizer for oil-in-water emulsions

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Cellulose is the most abundant biopolymer on earth, being plant sources the most significant. This material has been showing increasing applications in many fields: biomedicine, food, cosmetics, electronics, composite materials, among others. Cellulose from bacterial sources is a prominent alternative to the already marketed celluloses, being more pure, crystalline, having nanoscale fibres and excellent mechanical properties. One of the many uses of cellulose, in its various forms, is the stabilization of heterogeneous systems – more specifically, the stabilization of oil-in-water emulsions. In this work, bacterial cellulose's potential as a stabilizer for Pickering emulsions was assessed. A model system of an isohexadecane-in-water emulsion was studied in detail. A dry bacterial cellulose formulation was able to lower the interfacial tension between the immiscible phases. Emulsions prepared with this formulation showed good stability over time and desired rheological behavior.

Emulsions are obtained from at least two immiscible liquids, when they are mixed together and one of the phases arranges in small droplets (internal phase) dispersed in the other continuous outer phase. In an oil-in-water (o/w) emulsion, the oil is the dispersed/internal phase, and the water is the continuous phase. This sort of mixture is unstable without the addition of an emulsifying agent of some kind. Conventionally, to produce and stabilize an emulsion, surfactant molecules are added in order to lower the interfacial tension (IFT) between the immiscible liquid phases – in other words, to reduce the energy of the liquid-liquid interface. Surfactants arrange in the oil-water interface because they are amphiphilic molecules [1, 2]. Particle-stabilized emulsions - known as Pickering emulsions - have gained increasing interest, including for food applications. Particles suitable for Pickering stabilization of emulsions strongly and irreversibly adsorb at the oil-water interface, depending on their relative affinity for both phases. Organic solid particles, or more precisely biopolymers (polysaccharides, aromatic macromolecules and polypeptides), have been shown to be capable of stabilizing emulsions: they exhibit surface activity at liquid–liquid interfaces [1]. The solid particle coating at the droplets surface acts as a mechanical barrier, preventing coagulation and coalescence phenomena [3, 4, 5].

Besides the stabilization attained by adsorption to the interface of two distinct fluids, solid particles have yet another mechanism of stabilization: structuring the continuous phase. When the interfacial area of a biphasic system is completely covered, and an excess amount of particles remains in the continuous phase, they interact with each other and can form a three-dimensional structure. The viscosity of the continuous phase increases, providing higher support to the disperse phase droplets, reducing coalescence and creaming. Thickening of the emulsions and foams slows down the processes that lead to phase separation, improving long-time storage, an interesting outcome for some applications [1, 3, 4].

Solid cellulosic particles, in the form of nano- or micro- fibres or crystals, also show capability to form Pickering emulsions. Despite the overall hydrophilic nature of cellulose, the less significant hydrophobic domains within the crystal structure greatly contribute for the stabilization of Pickering oil-in-water emulsions, by adsorbing to the interface [6].

Hydrocolloidal microcrystalline cellulose (MCC), from plant sources, is already widely used in food industry to regulate the stability, texture, rheology and organoleptic properties of many food and cosmetic formulations [7].

Bacterial cellulose (BC) is a sophisticated material produced biotechnologically by different microorganisms, but most efficiently by acetic acid bacteria from the genera _Gluconacetobacter_ [8, 9]. While chemically identical to plant cellulose, BC is chemically pure. Each BC nanofiber is a bundle of cellulose nanofibrils. Due to their nano-size, these aggregates of extended cellulose chains have a rather large surface area. The unique properties of BC account for extraordinary physico-chemical and mechanical behavior [7]. This biomaterial has also been used for the stabilization of o/w emulsions [6, 10], but in its hydrated form. For the sake of storage, economy and practicality, additives for industries are preferentially provided in a dry or powder form. After drying, most cellulose products do not re-hydrate properly. Co-drying with water soluble polysaccharides helps redispersion of the dried fibres or crystals, while maintaining the rheologic and structuring properties. Thus, it is very common to find i.e. carboxymethyl cellulose (CMC) in commercial MCC formulations, for the most diversified applications.

The main objective of this study was to assess the stabilizing properties of BC in o/w emulsions, for use as a novel hydrocolloid. For this, an equimassic formulation of BC and 90 kDa CMC (BC:CMC) was prepared and spray dried. Isohexadecane-in-water emulsions (10:90) were prepared in the presence of 0.1%, 0.25% and 0.5% of the BC:CMC formulation, previously dispersed in the water phase. Samples were mixed with a homogenizer for 4 minutes at 20 000 rpm, and stored at room temperature. Visual and microscopic aspect of the emulsions was registered over time. Samples were also visualized in Cryo-SEM the day after preparation. Rheological tests were performed to assess the emulsion's viscosity profile, storage and loss moduli.

Interfacial tension between the immiscible phases was measured both with the pendant drop method and with the Du Noüy ring method. BC:CMC dispersions density was assumed the same as of the distilled water (1.004 g/mL, determined at room temperature, approximately 22 °C). Isohexadecane's density at the same temperature was 0.783 g/mL.

Microscopic analyses showed large oil droplets, but stable over time. Despite a visible creaming in the emulsions with lower BC:CMC concentration (0.1% and 0.25%), there was no evident separation of the oil phase. This reaffirms that the particle adsorption to the oil-water interface is strong, and that the amount of particles was sufficient to cover all the interfacial area. The emulsions showed a viscoelastic and shear-thinning behavior, with a yield stress between 0.01 $s^{-1}$ and 0.1 $s^{-1}$. An increase in BC:CMC concentration results in higher emulsion viscosity, along with progressively less significant creaming phenomenon. The storage and loss moduli also increase with BC:CMC concentration, and the elastic behavior becomes more prominent.

IFT measurements (Table 1) showed that the presence of the BC:CMC formulation actually diminishes the interfacial energy between the immiscible phases.

At a concentration of 0.5%, the dry BC:CMC formulation was able to effectively stabilize the o/w emulsions against coalescence or creaming for up to 60 days without the need to add any other emulsifying agents, thus confirming the potential of BC as a stabilizing agent for Pickering emulsions.

Table 1. Interfacial tension values between aqueous dispersions of BC:CMC and isohexadecane, determined by the du Noüy Ring method and by the Pendand Drop method

| Method | | $H_2O$ | 0.1% BC:CMC | 0.25% BC:CMC | 0.5% BC:CMC | 0.5% CMC |
|---|---|---|---|---|---|---|
| Du Noüy Ring | IFT (mN/m) | 45.9 ± 1.3 | 44.8 ± 1.6 | 40.6 ± 1.2 | 36.6 ± 1.7 | - |
| Pendant Drop | IFT (mN/m) | 48.5 ± 1.7 | 45.9 ± 1.1 | 42.1 ± 0.8 | 35.2 ± 1.2 | 48.9 ± 0.2 |

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