Colloids and Surfaces B: Biointerfaces 164 (2018) 201–209

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Pickering emulsion stabilized by cashew gum- poly-L-lactide copolymer nanoparticles: Synthesis, characterization and amphotericin B encapsulation

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ARTICLE INFO

Article history:
Received 18 July 2017
Received in revised form
27 November 2017
Accepted 15 January 2018

Keywords:
Pickering emulsion
Cashew gum
Poly-L-lactide
Copolymer
Nanoparticles
Amphotericin B

ABSTRACT

In this work, we provide proof-of-concept of formation, physical characteristics and potential use as a drug delivery formulation of Pickering emulsions (PE) obtained by a novel method that combines nanoprecipitation with subsequent spontaneous emulsification process. To this end, pre-formed ultra-small (d.~10 nm) nanoprecipitated nanoparticles of hydrophobic derivatives of cashew tree gum grafted with polylactide (CGPLAP), were conceived to stabilize Pickering emulsions obtained by spontaneous emulsification. These were also loaded with Amphotericin B (AmB), a drug of low oral bioavailability used in the therapy of neglected diseases such as leishmaniasis. The graft reaction was performed in two CG/PLA molar ratio conditions (1:1 and 1:10). Emulsions were prepared by adding the organic phase (Miglyol 812®) in the aqueous phase (nanoprecipitated CGPLAP), resulting the immediate emulsion formation. The isolation by centrifugation does not destabilize or separate the nanoparticles from oil droplets of the PE emulsion. Emulsions with CGPLAP 1:1 presented unimodal distributions at different CGPLA concentration, lower values in size and PDI and the best stability over time. The AmB was incorporated in the emulsions with a process efficiency of 21–47%, as determined by UV–vis. AmB in CGPLAP emulsions is in less aggregated state than observed in commercial AmB formulation.

1. Introduction

The first studies documenting the ability of solid particles to stabilize oil droplets in water were reported by Ramsden and Pickering and date back from more than a century ago [1]. Pickering emulsions, either o/w, w/o or multiple, are singled out from classical ones, as they are stabilized by solid particles and by the absence of surfactants [2]. By avoiding the need of use of synthetic surfactants, Pickering emulsions offer several advantages over their classical counterparts, such as better stability, low toxicity and less pollution to the environment. Over recent years, Pickering emulsions stabilized using different type of particles have been reported. Halloysite nanotubes (HNT)($(Al_2Si_2O_5(OH)_4 \cdot nH_2O)$) molecularly imprinted, have been developed to extract herbicides from water [3]. The same research group, has recently published other studies based on Pickering emulsion by interfacial molecular imprinting and Pickering emulsion polymerization to recognize bovine hemoglobin using different strategies, namely HNT and magnetic nanoparticles [4], hydroxyapatite hybridized polydopamine polymers [5-6]. Another study found evidence of the feasibility to obtain Pickering emulsions responsive to pH changes. These particles are based on polysiloxane microsphere bearing phenolphthalein groups, turned from pink to deep red with the augment of pH from 9 to 12. The emulsions also exhibited doubly pH-responsive property: two emulsification/demulsification processes occurred at pH 9 and pH 12, respectively [7].

Despite the great interest focused on Pickering emulsions stabilized by inorganic and synthetic polymeric particles, only recently researchers have started to account for the use of natural edible polysaccharides, proteins and other natural food constituents for this purpose. Therefore, particulate systems comprising alginate [8], modified starch [9,10], chitin nanocrystals [11], chitosan [12],

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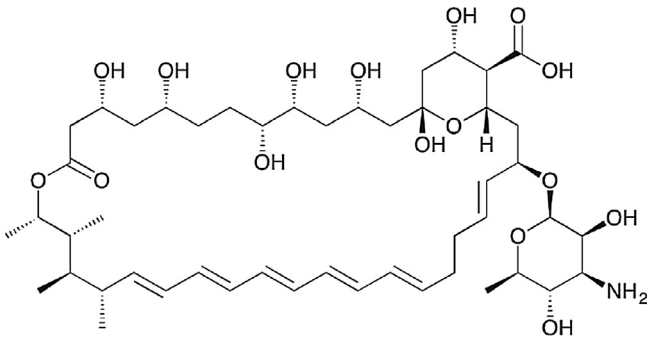
https://doi.org/10.1016/j.colsurfb.2018.01.023
0927-7765/



Scheme 1. Structure of AmB.

cellulose nanocrystals [13–15]; soy protein nanoparticles [16] or whey protein microgels [17] have been reported.

Cashew gum (CG) is an heteropolysaccharide comprised by β-D-galactose (72–73%), α-D-glucose (11–14%), L-arabinose (4,6%), L-ramnose (3–4%), -rhamnose D-glucuronic acid (4–7%) and a small fraction (5–8%) of protein [18]. The solubility and biodegradability in physiological conditions of CG, anticipates its amenability and potential use to develop matrices to associate and release low molar mass drugs, biologics and cells [18]. In this paper, we report for the first time, the use of self-assembled nanoparticles of CG grafted with polylactic acid (PLA) obtained by nanoprecipitation, and subsequently its use to obtain stable Pickering emulsions by spontaneous emulsification. Synthesis of CG – poly-L-lactide derivatives were previous described by Richter [19]. These type of hybrid materials offer improved functional properties in the development of drug delivery formulations [20] including their enhanced biodegradability [21]. We have selected amphotericin B (AmB) (Scheme 1) as the drug to load into the Pickering emulsions. AmB is a potent fungistatic and fungicide drug produced by the actinomycetes *Streptomycetes nodosus* [22] that was approved for clinical use by FDA in 1959 [23]. AmB is also prescribed in the treatment of visceral leishmaniasis. It is a lipophilic drug that binds to lipids and intercalates into lipid bilayers that then associate to form transmembrane pores [24]. Its selectivity for fungi is associated with its greater affinity for ergosterol than to cholesterol. However, non-selective toxicity towards human erythrocytes is mediated by its state of aggregation [25]. AmB was introduced in the market as a micellar suspension with sodium deoxychlolate (Fungizon®) for intravenous administration. Later, other formulations were introduced, including: a liposomal formulation (Ambisome®), whereby AmB is present in a high state of aggregation, as well as in the lipid complex, Abelcet®; a colloidal dispersion, Amphocil®; and in an emulsion product in association with Intralipid®. These and other type of lipid-based formulations have been known to reduce the systemic toxicity without compromising the therapeutic efficacy of AmB [26,27]. It has been proposed that emulsion-based formulations that preserve and favor the release of the monomeric form of AmB below the critical concentration for self-association are less toxic than micellar suspensions [28]. Recently, it has also been shown that a heating treatment of AmB (20 min at 70 °C) combined with the formulation of a microemulsion leads to a new state of aggregation of AmB that exhibits lower toxicity and increases the *in vitro* and *in vivo* efficacy [29]. AmB also shows very low oral bioavailability due to its structural features that violate Lipinsky's rule (*e.g.*, low Log P, high Mw, large polar surface area). Hence, novel pharmaceutical formulations of AmB are of great interest with a view to contribute to increase its pharmacological bioavailability for oral and other routes of administration, while exerting control on its drug release.

2. Experimental section

2.1. Materials

Cashew (*Anacardium occidentale*) gum exudate (CG) was kindly donated by EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária, Fortaleza City, Brazil). It was isolated and purified according with the protocol previously developed by our Group [30]. CG was grafted with poly-L-lactide in two different CG:PLA molar ratio (1:1 and 1:10) as detailed by Richter [19]. All chemical reagents were from Sigma-Aldrich (São Paulo, Brazil) and used without further purification. Amphotericin B was supplied by Ethycal (Fortaleza, Brazil). Dimethyl sulfoxide (DMSO) and acetone were from Synth (São Paulo, Brazil) and Miglyol 812® (coconut triglycerides of caprylic and capric fatty acids) was from Cremer Oleo (Witten, Germany).

2.2. Synthesis of Pickering emulsions

The Pickering emulsions were prepared using the general principle of spontaneous emulsification, which is the fundamental principle for the preparation protocol of chitosan-based nanocapsules that have been extensively used in previous studies [31,32], though with modifications. Briefly, CGPLAP of the two different CG/PLA molar ratios (1:1 and 1:10) were initially fully dissolved in DMSO at 10 mg/mL. An aliquot of this solution poured into distilled water to a final volume of 20 mL and final three concentrations (0.5, 1.0 and 2.0 mg/mL). This led to the formation of nanoprecipitated particles of CGPLAP, thus comprising the aqueous phase. The organic phase consisted of 0.5 mL of ethanol, 125 μL of Miglyol and 9.5 mL of acetone. The organic phase (~10 mL) was immediately poured into the aqueous phase containing the CGPLAP self-assembled nanoparticles under quiescent conditions and the solution immediately turned milky. The solvents were subsequently evaporated in a rotavapor at 45 °C. The thus obtained Pickering emulsion was isolated by centrifugation for 1 h at 25 °C and at 15,000 × G. The resulting milky cream on the solution was removed with a micropipette and stored under refrigeration until subsequent use. The emulsion type was determined by the drop test [33]. Briefly, a drop of emulsion was added to either water or Miglyol and the ability of the sample to disperse was observed.

2.3. Characterization of physical properties

The particle size distributions of the CGPLAP nanoparticles and Pickering emulsions obtained from them were characterized by dynamic light scattering with non-invasive back scattering (DLS-NIBS) at 25 °C upon irradiation of the sample with a 4 mW helium/neon red laser (λ = 633 nm) and detection was at an angle of 173°. The zeta potential of the Pickering emulsions was measured by mixed laser Doppler velocimetry and phase analysis light scattering (M3–PALS). A Nanosizer ZS 3600 (Malvern Instruments Ltd., Worcestershire, UK) was used for both determinations. The samples were diluted 1:50 in water for size measurements and for zeta potential measurements.

2.4. Storage stability

The storage stability of Pickering emulsions was determined in isolated formulations by measuring the particle size and polydispersity index using DLS-NIBS as described above. To this end, the emulsions were kept in refrigeration (~4 °C) and measurements were registered weekly [34].