Colloids and Surfaces B: Biointerfaces 123 (2014) 797–802

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Development of eco-friendly submicron emulsions stabilized by a bio-derived gum

Luis María Pérez-Mosqueda, Pablo Ramírez*, Luis Alfonso Trujillo-Cayado, Jenifer Santos, José Muñoz

Reología Aplicada, Tecnología de Coloides, Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla c/P, García González, 1, E41012 Sevilla, Spain

ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form 6 October 2014
Accepted 10 October 2014
Available online 22 October 2014

Keywords:
Rosin gum
Limonene
Eco-friendly emulsions
Pluronic
Ostwald ripening

ABSTRACT

Many traditional organic solvents are being gradually replaced by ecofriendly alternatives. D-Limonene is a terpenic (bio)-solvent that fulfils the requirements to be considered a green solvent. D-Limonene submicron emulsions suffer from Ostwald ripening destabilization. In this study, we examined the influence of the addition of a natural gum (rosin gum) to D-limonene in order to prevent Ostwald ripening.

This contribution deals with the study of emulsions formulated with a mixture of D-limonene and rosin gum as dispersed phase and Pluronic PE9400 as emulsifier. The procedure followed for the development of these formulations was based on the application of product design principles. This led to the optimum ratio rosin gum/D-limonene and subsequently to the optimum surfactant concentration. The combination of different techniques (rheology, laser diffraction and multiple light scattering) was demonstrated to be a powerful tool to assist in the prediction of the emulsions destabilization process.

Not only did the addition of rosin gum highly increase the stability of these emulsions by inhibiting the Ostwald ripening, but it also reduced the emulsions droplet size. Thus, we found that stable sub-micron D-limonene-in-water emulsions have been obtained in the range 3–6 wt% Pluronic PE-9400 by means of a single-step rotor/stator homogenizing process.

1. Introduction

Emulsions are thermodynamically unstable colloidal dispersions, which are destabilized by several mechanisms such as flocculation, coalescence, gravitational separation (sedimentation or creaming) and Ostwald ripening. Production of stable submicron emulsions is a key achievement in several fields such as food, pharmaceutical and agrochemicals [1–3].

One of the critical parameters that have to be carefully controlled in emulsion formulation is the emulsifier concentration in order to achieve submicron stable emulsion. A minimum surfactant bulk concentration is required to fully cover the available interfacial area created during the emulsification process. However, an excess of surfactant in solution will lead to the formation of a large number of micelles. It is well-known that non-adsorbed surfactant micelles could induce the flocculation of the emulsion droplets due to a depletion mechanism. Although flocculation is a reversible process, it could develop irreversible destabilization processes such as creaming and/or coalescence [4–9].

Submicron emulsions are usually produced by two-step emulsification processes. First a coarse emulsion is usually produced by means of a rotor/stator system. Then high pressure homogenizer or ultrasonication devices are used to decrease the droplet size to a submicron level [10]. Nevertheless, in the present work emulsions with volumetric diameters of ca. 0.5 μm were produced by using a single-step rotor/stator emulsification process.

D-Limonene is an interesting bio-derived solvent which can be obtained from citric peels. This organic compound presents interesting applications in different fields such as cosmetics, food, pesticide applications and pharmaceutical industries [11–15]. Furthermore, D-limonene is a good candidate to be used as a (bio-)solvent in the design of novel agrochemical products, replacing more pollutant chemicals [16].

The formulation of the disperse phase was completed with rosin gum as stabilizer. Rosin gum is a natural gum obtained from pine trees. It is chiefly composed of 90% rosin acids (abietic acid, palustric acid, neoabietic acid, and others) and is 10% non-acidic. There exist several papers which comment on the application of rosin

* Corresponding author. Tel.: +34 954 557180; fax: +34 954 556447.
E-mail address: pramirez@us.es (P. Ramírez).

http://dx.doi.org/10.1016/j.colsurfb.2014.10.022
0927-7765/

gum in biocompatible systems such as drug delivery [17,18] or microencapsulation [19].

The emulsifier function was carried out by an ABA block copolymer, Pluronic PE 9400, where A and B stand for poly(ethylene) oxide and poly(propylene) oxide respectively. These surfactants have found many industrial applications as foam and emulsion stabilizers [20,21]. On the other hand, these polymeric surfactants have been employed recently in more sophisticated technological applications such as drug delivery, gene therapy and the developing of foodstuffs that provide specific physiological responses, such as control of lipid digestion or satiety [22–29].

The main objective of this work was the study of the influence of the addition of a lipophilic gum (rosin gum) to an eco-friendly (bio)-solvent (D-limonene) on the physical stability of concentrated O/W emulsions, as well as the influence of the surfactant concentration. These emulsions may be find applications related to the design of biotechnological complex systems with different uses.

2. Materials and methods

2.1. Materials

D-Limonene (4-isopropenyl-1-methylciclohexane) is an organic compound widely used as an industrial solvent. It is obtained from the peel of citrus fruit, mainly from oranges and lemons. D-Limonene (97%) was purchased from Sigma–Aldrich® and purified with Florisil® resins (Fluka, 60–10 mesh) prior to use by following the procedure used elsewhere [30,31]. Namely, a mixture of oil and Florisil® in proportion 2:1 (w/w) was shaken gently for 3 h and then separated by decanting.

Rosin gum (Sigma–Aldrich®) is an exudate gum which is solid at room temperature. It is a mixture of different acids such as abiatic acid, palaustric acid, neoabetic acid. Its molecular weight is 302.45 g mol^{-1} and its density at 20 °C is 1.06 g mL^{-1}.

The triblock copolymer Pluronic PE9400 (PEO_{21}–PPO_{50}–PEO_{21}, Mw = 4600 g mol^{-1} and HLB = 12–18) was kindly provided by BASF and used as received.

Ultrapure water, cleaned using a Milli-Q water purification system was used. All glassware was washed with 10% Micro-90 cleaning solution and exhaustively rinsed with tap water, isopropanol, deionized water, and ultrapure water in this sequence. All measurements were carried out at 20 °C.

2.2. Emulsification methods

First, an excess of the required amount of rosin gum was dissolved in purified D-limonene by gently stirring overnight. This solution was filtered in order to remove the insoluble fraction of the rosin gum and then D-limonene was added to the filtered solution, reaching a final rosin gum concentration of 30 wt%.

The continuous phase was produced by dissolving PE9400 surfactant using a magnetic stirrer for 15 min at 300 r.p.m.

The emulsion was then formed by using a batch process; the oil phase (70 wt% D-limonene and 30 wt% rosin gum) was added over the continuous phase and homogenized for 60 s at 17,500 r.p.m. using a rotor/stator system Ultraturrax T-25/KV11. The mass disperse fraction ϕ = 50 wt% and the final emulsion weight was 50 g. The influence of Pluronic PE9400 concentration on the stability and physicochemical properties of the emulsions was studied in the range from 1 to 8 wt%.

2.3. Emulsion droplet size analysis

Droplet size distribution (DSD) was determined by a laser diffraction technique using a Mastersizer X (Malvern Instruments). These measurements were carried out after 1, 7, 15, 30, 45 and 75 days ageing time to analyse the time evolution of the DSD. All measurements were carried out in triplicate.

The mean droplet diameter was expressed as the Sauter diameter ($d_{3,2}$) and volume mean diameter ($d_{4,3}$).

$$d_{m,n} = \frac{\sum_i N_i D_i^m}{\sum_i N_i D_i^n} \quad (1)$$

where N_i is the number of droplets with a diameter, D_i. To determine the distribution width of droplet sizes, "span" was used, calculated by the following formula:

$$\text{Span} = \frac{D(v, 0.9) - D(v, 0.1)}{D(v, 0.5)} \quad (2)$$

where $D(v,0.9)$, $D(v,0.5)$, $D(v,0.1)$ are diameters at 90%, 50% and 10% cumulative volume, respectively.

2.4. Rheology of emulsions

Flow curves were obtained by using a Mars rheometer from Haake Thermo Scientific (Germany). This is a controlled stress rheometer with a sandblasted Z20 coaxial cylinder sensor system (Ri = 1 cm, Re/Ri = 1.085). Flow curves were carried out at 1, 30 and 75 days of emulsion storage.

All rheological measurements were performed at 20 ± 0.1 °C, using a C5P Phoenix circulator (Thermo-Scientific) for sample temperature control. Samples were taken at about 2 cm from the upper part of the container. Sampling from the top part of the container in contact with air was avoided. The results represent the mean of three measurements. Equilibration time prior to rheological tests was 180 s.

2.5. Viscosity of the continuous phase

The viscosity values for the continuous phase have been calculated using a Falling Ball Viscometer C (Haake). Viscosity is obtained from the following equation:

$$\eta = K(\rho_{ball} - \rho_l)t \quad (3)$$

where η is the viscosity of the continuous phase, ρ_{ball} is the density of the ball (2.2 g/cm^3), ρ_l is the density of the continuous phase, K is a constant that depends on the ball (0.007 mPa s cm^3 g^{-1} s^{-1}) and t is the time.

All the measurements were performed at 20 ± 0.1 °C and the result is the average of five measurements.

2.6. Stability

Multiple light scattering measurements with a Turbiscan Lab Expert were used in order to study the destabilization of the emulsions. Measurements were carried out during 75 days at 30 °C to determine the predominant mechanism of destabilization in each emulsion.

2.7. Interfacial tension measurements

A drop profile analysis tensiometer (CAM200, KSV, Finland) was used in this study. A droplet of the lightest phase is formed at the tip of a hooked capillary, which is immersed in the denser phase and thermostatized at 20 °C. The surface pressure is defined as $\Pi = \gamma_0 - \gamma$, where γ is the interfacial tension of the rosin gum solution against limonene, and γ_0 = 44.0 ± 0.5 m Nm^{-1} is the interfacial tension of the limonene–water interface at 20 °C.