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Influence of the ratio of amphiphilic copolymers used as emulsifiers on the microstructure, physical stability and rheology of α-pinene emulsions stabilized with gellan gum

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ABSTRACT

α-Pinene is a terpenic solvent whose use in the formulation of emulsions entails a double benefit from the environmental point of view since it is a green solvent, easily biodegradable, which also has certain antimicrobial properties.

In this work a combination of Atlas™ G-5000 and Atlox™ 4913 amphiphilic copolymers was used to obtain O/W emulsions formulated with α-pinene and gellan gum. These emulsions may find applications related to the design of complex biotechnological systems with different uses.

In order to investigate the microstructure and the physical stability of these emulsions, a combination of different techniques such as rheology, microscopy, laser diffraction and multiple light scattering turn out to be a useful methodology.

The results demonstrated the need to include a minimum amount of Atlas™ G-5000 copolymer in the formulation of these emulsions to improve their stability. These results were supported by the information revealed by optical micrographs, according to which Atlas™ G-5000 is directed to the continuous medium to structure water (this surfactant is particularly effective at forming hydrogen bonds with water). On the other hand Atlox™ 4913 is preferentially adsorbed at the α-pinene–water interface, such that a high Atlox™ 4913/Atlas™ G-5000 mass ratio slows down the kinetics of coalescence as shown by multiple light scattering. However, a very low relative concentration of Atlas™ G-5000 causes creaming to become the dominant destabilization mechanism. Increasing the Atlas™ G-5000/Atlox™ 4913 mass ratio yields emulsions with enhanced viscosity and viscoelasticity.

1. Introduction

An O/W emulsion consists of oil droplets dispersed in an aqueous phase [1–3], which is a thermodynamically unstable system such that given enough aging time the two immiscible phases will undergo phase separation [4]. In order to increase the physical stability of emulsions, the use of surfactants and/or proteins and/or thickeners is essential [5]. The influence of the components of emulsions on the physicochemical properties has been extensively studied [6].

Emulsions are widely used in a large number of industrial applications [7] in, for example, the food industry (e.g. mayonnaise), detergents (e.g. removal of oil deposits), pharmaceuticals (e.g. drug administration), cosmetics (e.g. skin creams) and agricultural products (e.g. pesticides) [8].

In recent years, a large number of research studies have dedicated a great effort to exploring the use of essential oils due to their antimicrobial [9] and antioxidant properties. These properties may lead to increased interest in the utilization of essential oils in different fields such as the food, pharmaceutical and cosmetics industries [10,11] and the agrochemical industry as natural pesticides in organic agriculture and as insecticides [12–14]. For this purpose, the use of α-pinene as the organic phase in the formulation of emulsions has been assessed for different applications. α-Pinene is a terpenic solvent, which can be produced from pine bark or by distillation, is easily degraded in the environment. There exists another isomer, β-pinene, and both pinene isomers can be extracted from the resins of other conifers, and from other plants, such as juniper. The content of both isomers in essential oil derived from juniper can exceed 80 wt%. It is also possible to obtain them from rosemary and eucalyptus essential oil [15]. Recently, the

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antimicrobial properties of essential oils have been discovered and new applications as natural pesticides in organic agriculture and as insecticides are emerging [13,14]. Several authors [16–20] affirm that the pesticidal activity of eucalyptus oils is due to components such as α-pinene. Thus, the use of α-pinene in the formulation of these emulsions entails a double benefit from the environmental point of view since it is a green solvent, easily biodegradable, which also has certain pesticide properties, thus contributing to the overall natural pest control.

In order to emulsify α-pinene, amphiphilic copolymers have been used. These surfactants have an essential role in the formation and stabilization of emulsions [21]. These molecules tend to adsorb at the interface formed between the dispersed droplets and the continuous phase of the emulsions [5]. The presence of these surfactants helps to control the different processes involved in emulsion destabilization. Thus, the surfactant may contribute to the adsorbed electrical charge, either neutralizing or increasing it. Their adsorption at the interface, especially the high molecular weight surfactants, may lead to a steric repulsion effect which depends on the size of the group which is responsible for interactions between droplets and on the interfacial adsorption density. In this way the presence of surfactants influences the steric and electrostatic repulsion and, therefore, the stability of emulsions [22].

In this paper, the use of Atlas™ G-5000 hydrophilic copolymer (AB block (EO-PO) polyalkylene copolymer, HLB = 16.9) and the Atlox™ 4913 amphiphilic copolymer (grafted copolymer, HLB about 11–12) as emulsifiers will be explored. The latter polymeric surfactant consists of a main chain of methyl methacrylate (PMMA) as an anchor fraction and polyethylene glycol chains (PEG) of appropriate length as a stabilizer fraction [23]. Atlas™ G-5000 hydrophobic chains, poly-B block of poly(propylene oxide), act as anchoring groups in the organic phase while the hydrophilic chain, poly-A block of poly(ethylene oxide), ensures the stability of the external aqueous phase. It is characterized by a high HLB, which is appropriate for the stabilization of O/W interfaces. The penetration of the hydrophobic side chains in the organic phase may be several times higher than that of a conventional surfactant such as C18 sorbitan ester [23]. These copolymers can reduce interfacial tension because the hydrophobic parts of the molecules are situated at the interface of the organic phase droplets which are dispersed in the aqueous medium (O/W emulsion). The formation of emulsions is favored by this reduction in interfacial tension. Simultaneously, the hydrophilic parts of the macromolecules have a stabilization function since they may extend into the continuous medium by promoting steric repulsion forces between the solvent droplets. Nevertheless, an additional stabilizer, gellan gum, which is a commercial biopolymer obtained by fermentation, has also been used. In solution, gellan gum gives rise to highly viscous solutions at extremely low concentrations, which favors its applications as a suitable stabilizer [24].

The main goal of this work has been to study the influence of the amphiphilic copolymer combination (Atlas G-5000 and Atlox 4913) as emulsifier on the microstructure, rheology and physical stability of emulsions formulated with an organic phase composed of a "green" solvent (α-pinene) and gellan gum as thickener.

2. Materials and methods

2.1. Materials

Commercial low-acyl gellan gum, Kelcogel F type was used as stabilizer as supplied by CP Kelco Company (San Diego, USA). The total concentration of gellan gum in the emulsions was 0.4 wt%.

In order to prevent the growth of microorganisms, final emulsions contained 0.1 wt% sodium azide.

The organic solvent used was rectified α-pinene Leavo 95, an extremely non-polar solvent, which was supplied by Destilaciones Bordas-Chinchurreta Company (Sevilla, Spain). Its density (20 °C) is 898 kg/m^3, its refractive index is 1.464 and its boiling point is around 67.5 °C. The α-pinene concentration was fixed at 30 wt%.

Two types of amphiphilic copolymers, which may be considered to be polymeric surfactants [25], were used as emulsifiers.

Atlas G-5000™ (hydrophilic AB block copolymer) and Atlox 4913™ (grafted copolymer) surfactants were supplied by Croda International plc (Snaith, UK).

The total concentration of copolymers was 3.0 wt%, although the ratio Atlas™ G-5000 to Atlox™ 4913 was varied: 3/0, 2.5/0.5, 2/1,1.5/1.5, 0.5/2.5 and 0/3.

In addition, a defoaming agent (Dow Corning® MD 10) supplied by Dow Corning was used (0.05 wt%).

The nomenclature used to denote the composition of emulsions is as follows: E30/X/Y stands for 30 wt% oil (α-pinene) emulsions containing *X* wt% Atlas™ G-5000 and *Y* wt% Atlox™ 4913.

2.2. Methods

First the gellan gum solutions (250 g per batch) were prepared by slowly dispersing 0.8 wt% in de-ionized water at room temperature. Afterwards, they were submitted to mechanical treatment under 700 rpm by using an IKA-Visc MR-D1 mixer equipped with a saw-tooth type rotor in a bath at 80 °C. Subsequently, the emulsions were prepared by a protocol which had proved useful when dealing with hydrocolloids [26]. This protocol consists of the following consecutive steps. Both copolymers were first dispersed into the corresponding amount of water by using a magnetic stirring plate (SB162-3, Stuart, Scientific Laboratory Supplies) before emulsification. Afterwards, the oil phase, α-pinene, was added to the aqueous phase and homogenized with an Ultraturrax T-50/G45 rotor-stator device at a rotational speed of 4000 rpm for 150 s. The pre-emulsion obtained with the rotor-stator (250 g) was then passed through a high-pressure valve homogenizer three times at 14,104 kPa (Avestin EmulsiFlex-C5 type). Finally, this emulsion was mixed with an equal amount of 0.8 wt% gellan gum solution, again using the Ultra-Turrax T-50 for 30 s at 700 rpm.

2.2.1. Oscillatory shear tests

Stress sweeps were carried out in a range of 0.05–3 Pa at three different frequencies: 0.1, 1 and 3 Hz.

Frequency sweep tests were performed by selecting a stress within the linear range for the three previously studied frequencies.

A controlled-stress rheometer AR2000 (TA Instruments) with a serrated plate-plate sensor (40 mm diameter, gap: 1 mm) was used. Equilibration time prior to rheological tests was 10 min. These rheological measurements were performed at 20 ± 0.1 °C, using a Peltier system for sample temperature control.

The results represent the mean of four measurements.

2.2.2. Steady shear flow tests

The flow tests were carried out using a rheometer from Haake Thermo Scientific (Karlsruhe, Germany), a Mars controlled-stress rheometer with a Vane FL40 sensor system (R_i = 20 mm, R_a = 21.70 mm, H = 55 mm). Three different segments were used to provide a greater number of points along the flow curve: (1) from 0.1 to 1 Pa; (2) from 1.1 to 10 Pa; (3) from 12.5 to 70 Pa.

These rheological measurements were performed at 20 ± 0.1 °C, using a C5P Phoenix circulator (Thermo-Scientific, Germany) for sample temperature control.

The results represent the mean of three measurements.