Impact of short-chain alcohols on the formation and stability of nano-emulsions prepared by the spontaneous emulsification method

Liya Zeng, Yalin Zhang*

Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China, China

HIGHLIGHTS

- Spontaneous emulsification method was used to fabricate nano-emulsions stabilized by Cremophor EL with short-chain alcohols.
- The droplet size of nano-emulsions was increased with increasing Log P.
- The main mechanism for drop formation was local supersaturation and the liquid crystal/bicontinuous phase had significant effect on it.
- Ostwald ripening rate was reduced by the ethanol addition.

GRAPHICAL ABSTRACT
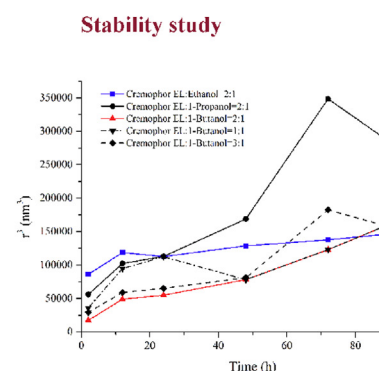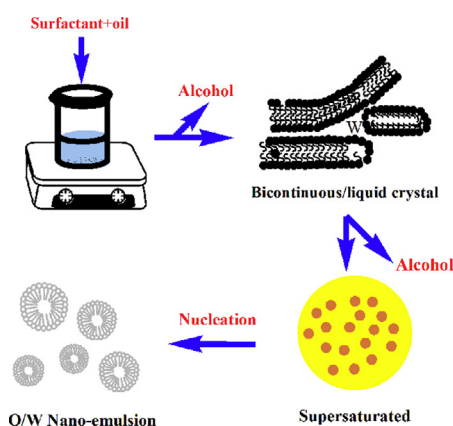
ARTICLE INFO

ABSTRACT

The principles of formation and stability of nano-emulsions have been extensively investigated in previous research. In this study, we focus on the impact of short-chain alcohols (C < 5) on the formation and stability of oil-in-water nano-emulsions prepared by the spontaneous emulsification method. Due to its smaller droplet size and excellent optical transparency, the nano-emulsion stabilized by Cremophor EL was selected for further study. Addition of alcohol facilitated the nano-emulsion formation, moreover, the droplet size decreased with increasing alcohol chain length (C > 2) and hydroxyl groups. Nevertheless, the droplet size then increased further with the addition of alcohols, which we ascribed to the effect of alcohols on droplet flocculation. An optimum Cremophor EL-to-1-butanol ratio of 2:1 was employed to construct the ternary phase diagram. It was found that the fine droplets (r < 100 nm, PDI < 0.2) were obtained only when converting the initial oil drops completely into the lamellar liquid crystalline phase and/or bicontinuous phase and then this phase became supersaturated in oil, so that the nucleation of oil drops occurred spontaneously. According to the dynamic light scattering results, coalescence and Ostwald ripening were the main destabilization mechanisms in our systems. Notably, nano-emulsion stability was greatly improved with ethanol in comparison to 1-propanol and 1-butanol.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Emulsions are dispersions of two or more immiscible liquids [1]. Many previous publications have focused on the commer-

* Corresponding author.
   E-mail address: yalinzh@nwsuaf.edu.cn (Y. Zhang).

cial application of emulsions-based delivery systems in pharmacy [2–4], food [5,6], cosmetic [7,8], agrochemistry [9,10] and polymer synthesis [11,12]. However, emulsions are thermodynamically unstable systems and tend to breakdown over time due to a variety of destabilization mechanisms [13,14] such as gravitational separation, flocculation, coalescence, Ostwald ripening, and phase inversion. Nano-emulsions are a class of emulsions with droplet sizes ranging from 20 nm to 200 nm [14,15], which are also referred to in the literature as mini-emulsions [16,17], ultrafine emulsions [18], submicron emulsions [19,20] and unstable microemulsions [21]. Due to their characteristic size, nano-emulsions are optically transparent or translucent and stable against sedimentation or creaming. Unlike microemulsions, which are thermodynamically stable, nano-emulsions are only kinetically stable, i.e. they are metastable and their formation requires external energy [21–23]. Moreover, a high surfactant concentration (usually about 20% and higher) is required to produce microemulsions, whereas nano-emulsions can be fabricated at a lower surfactant level (3–10%) [13]. The small droplet size of nano-emulsions implies that Brownian motion is enough to overcome the gravitational separation force so as to prevent sedimentation or creaming, and consequently improves the stability over time. Hence, the main breakdown processes controlling the shelf life of nano-emulsions are Ostwald ripening (mass transport of dispersed phase through the continuous phase driven by Laplace pressure differences) [24] and Brownian-induced coalescence [25].

Nano-emulsions can be prepared by a number of different approaches which are generally divided into two classes: high-energy [26,27] and low-energy [28,29] emulsification methods. High-energy methods utilize mechanical devices to produce intense disruptive forces in order to intermingle and disrupt the oil phase, such as high pressure homogenizers, high-shear stirrers, microfluidizers and ultrasound generators. In general, the higher the energy inputs the smaller the droplet size is obtained. Nevertheless, nano-size droplet formation requires large amount of energy input and is therefore cost-inefficient. In contrast, low-energy emulsification methods, taking advantage of the internal chemical energy of components by changing the spontaneous curvature of the surfactant, are more efficient since only simple stirring is needed to produce nano-emulsions. The low-energy methods include phase inversion temperature (PIT) [30], phase inversion composition (PIC) [31] and spontaneous emulsification method [32]. During the past years, much attention has been paid to spontaneous emulsification due to its simplicity of implementation, low cost and high energy efficiency. In this study, spontaneous emulsification method was employed to study the impact of different non-ionic surfactants and small alcohols on the droplet size and size distribution of nano-emulsions.

Many former studies have found that the addition of alcohol had appreciable effect on the formation, properties and stability of nano-emulsions through reducing the oil-water interfacial tension [6,33]. In addition, the mechanisms of spontaneous emulsification (for example, interfacial turbulence, interfacial expansion due to negative interfacial tension and local supersaturation) have been reviewed [23,32]. Rang and Miller [34,35] reported that in order to completely emulsify the oil phase to form small oil droplets, it is necessary to convert the initial oil drop into the lamellar liquid crystalline phase (LC) and/or a microemulsion which is supersaturated with oil. Sole et al. [36] found that the smaller droplet size and lower Ostwald ripening rate were obtained only when using hexanol as co-surfactant in the water/SDS/co-surfactant/dodecane system rather than pentanol. However, both of them only discuss spontaneous emulsification systems with medium- and long-chain alcohols. To our knowledge, although the short chain alcohols (e.g. ethanol and *n*-propanol) have been extensively used as co-surfactant for drug delivery, there have been few papers addressing

their spontaneous mechanisms [37]. Recently, Zeeb et al. [38] studied the impact of alcohols (ethanol, 1-propanol, 1-butanol) on the formation and stability of protein-stabilized nano-emulsions prepared by high-pressure homogenization. They found that the type of alcohol used influenced the reduction of the droplet size in the following order: 1-butanol > 1-propanol > ethanol. In current study, similar results were discovered in the spontaneously emulsified nano-emulsions containing environment-friendly non-ionic emulsifiers (Cremophor EL) and fatty acid esters (ethyl oleate). Furthermore, we also observed that the droplet size decreased with increasing hydroxyl groups and decreasing Log P (the logarithm of the partition-coefficient).

The aim of the current study was to investigate the influence of various short-chain alcohols (ethanol, ethylene alcohol, *n*-propanol, and *n*-butanol) on the formation and stability of nano-emulsions prepared by the spontaneous emulsification method. Due to the rapid diffusion of the surfactant and/or co-surfactant from the oil phase into the aqueous phase, the emulsions form spontaneously when the oil phase (containing oil, surfactant and/or co-surfactant) is injected into the aqueous phase. Tween 20, Tween 80, Cremophor EL and Cremophor RH 40 were selected as surfactants since they are widely used in practical applications. Phase behavior of the ethyl oleate/Cremophor EL-1-butanol (2:1)/water system at 25 °C was described to establish the relationship between the emulsification paths (surfactant-to-oil ratio (SOR)) and droplet size to illustrate the spontaneous emulsification mechanism. To identify the main breakdown mechanism accurately, a new approach [39] based on the time for evolution of particle size distribution was employed in our study. In opposite to previous studies [36,38,40], we observed that the Ostwald ripening rate was hindered by the ethanol addition. This study may extend the application range of spontaneous nano-emulsions and further recognize the nano-emulsions formation and stability.

2. Materias and methods

2.1. Materials

Norcantharidin (NCTD ≥ purity 98%) was purchased from Alfa Aesar Chemical Co. Ltd. (Massachusetts, USA). Ethyl oleate (purity ≥ 98%), ethanol (purity ≥ 99.8%), ethylene glycol (purity ≥ 99%), 1-propanol (purity ≥ 99.5%), propylene glycol (purity ≥ 99%), isopropanol (purity ≥ 99.5%), 1-butanol (purity ≥ 99.7%), glycerol (purity ≥ 99.5%), PEG-400, Tween 80 and Tween 20 were obtained from Aladdin® (Shanghai, China). Cremophor RH 40 and Cremophor EL were obtained from the BASF Corporation (Germany). All other chemicals and solvents were of analytical grade and used as supplied without any further purification.

Deionized water was generated from a Milli-Q gradient system of Millipore (Synergy, Millipore SAS, Molsheim, France).

2.2. Nano-emulsion preparation

Nano-emulsion formation was carried out by the spontaneous emulsification procedure described in a previous study [41] with some slight modifications. Briefly, spontaneous emulsification was performed by addition of an organic phase into the aqueous phase with mild stirring at 25 °C. In these samples, the oil, surfactant, and co-surfactant were first mixed together to form an organic phase, then injected into the aqueous phase under continuous stirring. Each sample was further stirred for 15 min. Unless otherwise stated, the water content was always 98 wt.%.

In some experiments, the effect of surfactant and co-surfactant concentration on droplet size was investigated using the following