Formation of polymeric nano-emulsions by a low-energy method and their use for nanoparticle preparation

Gabriela Calderó [a,b,*], María José García-Celma [c,a], Conxita Solans [b,a]

[a] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Barcelona, Spain
[b] Institut de Química Avançada de Catalunya (IQAC), CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Departament de Farmàcia i Tecnologia Farmacèutica, Univ. de Barcelona, Unitat Associada d'I+D al CSIC, Av Joan XXIII s/n, 08028 Barcelona, Spain

ARTICLE INFO

ABSTRACT

Formation of polymeric O/W nano-emulsions has been studied in the water/polyoxyethylene 4 sorbitan monolaurate/ethylcellulose solution system by the phase inversion composition (PIC) method. These nano-emulsions were used for the preparation of nanoparticles by solvent evaporation. Composition variables such as O/S ratio or final water content as well as emulsification path have been found to play a key role in the formation of stable, nanometer sized emulsions. Nano-emulsions with a constant water content of 90 wt.% and O/S ratios from 50/50 to 70/30 showed an average droplet size of about 200 nm as assessed by dynamic light scattering. Mean nanoparticle diameters, as determined by transmission electron microscopy image analysis, were of the order of 50 nm and showed a slight increase as well as a broader size distribution at increasing O/S ratios. The findings verify that the low-energy emulsification methods are not only valid for aliphatic and semipolar oils, but also for a highly polar solvent such as ethylacetate containing a preformed polymer.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Nanoparticles are solid materials with sizes in the nanometer scale, generally below 500 nm, which are focusing increasing research interest. They can be obtained from a large variety of materials (lipids [1,2], polymers [3–9], metal oxides [10], etc.) and using different methods. The choice of the right materials as well as the appropriate method is essential for ensuring the intended properties and applications. In this context, the demands for size control and the increasing awareness of the need of low-toxicity biocompatible systems, especially for human use (pharmacy, cosmetics, food), as well as for environmentally friendly technologies, points to the nano-emulsion template approach as a challenging alternative for the preparation of nanoparticles [11]. Nano-emulsions are emulsions with droplet sizes typically in the range of 20–500 nm. They are usually prepared by high-energy methods, that is, applying a mechanical energy input. Nevertheless, emulsification can also be achieved by low-energy methods, which take advantage of the physicochemical properties of the system [10–14]. Emulsification by low-energy methods produce generally, smaller and more uniform droplets. They can be achieved by the well known Phase Inversion Temperature (PIT) method (changing temperature at constant composition) [11–20] or by the phase inversion composition (PIC) method (by changing composition at constant temperature) [21–25].

In the nano-emulsion approach, nanoparticles are prepared from the dispersed phase of nano-emulsion systems by polymerization reactions [7–9] or from preformed polymers [3–6,26,27]. The use of the latter is advantageous because it avoids employing reactive substances thus improving the biocompatibility of the system, and reducing purification steps needed to remove undesired by-products or reactants in excess, such as residual monomer or initiator. Among the preformed polymeric materials usually considered for the preparation of nanoparticles, a growing attention has been focused to ethylcellulose (ethyl ether of cellulose). This material is a semisynthetic cellulose derivative: a long chain lipophylic polymer of anhydroglucose units joined by ß(1,4) acetal linkages [28]. The three reactive hydroxyl groups of each anhydroglucose unit may be substituted by an ethoxyl group usually up to a 48.0–49.4% content. Ethylcellulose is insoluble in water, and is listed as GRAS ("Generally Recognised As Safe") by the Food and Drug Administration (FDA). It is approved for topical and oral use, and is being employed in many applications such as food, cosmetics and pharmaceutical products [29]. Ethylcellulose micro and nanoparticles may be prepared by means of several methods, such as solvent evaporation in emulsion media [5,30,31], phase separation - coacervation induced by non-solvent [32,33], electrolytes [34,35], by a temperature change [36–39], spray drying [40,41] or supercritical antisolvent [42]. One interesting feature

* Corresponding author at: Institut de Química Avançada de Catalunya (IQAC), CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain. Fax: +34 932045904.
E-mail address: gabriela.caldero@iqac.csic.es (G. Calderó).

of ethylcellulose is its ability to adsorb at interfaces contributing to lowering interfacial tension [43–45]. However, this lowering is not enough to achieve the formation of droplets in the nanometric size range. Therefore, the use of surfactants as well as high energy mechanical dispersion methods, such as high shear stirrers, ultrasound generators or high pressure homogenizers are generally required [3,30,34].

Although the preparation of ethylcellulose microparticles has been extensively reported, few reports have been published on the preparation of nanoparticles, and to our knowledge, only two of them make use of low-energy methods [46,47]. It should be noted that most low-energy emulsification methods commonly reported, are based on low polarity oils mainly of the hydrocarbon type such as cyclohexane [15], decane [21,23,24], hexadecane [19], mineral oil [18], toluene [46,47], etc. Studies with oils of higher polarity are limited to triglycerides [20,53], esters such as cetearyl isononanoate [16] or isopropyl myristate [25] or mixtures of hydrocarbons with esters like decyl oleate [17]. Attempts made to further increase polarity of the oil by using mixtures of triglycerides and partially water-miscible solvents by using low-energy methods resulted in a notable mean droplet size increase (above 500 nm) [61]. Another important issue, is that oils reported for nanoparticle preparation by the solvent evaporation method are based on the use of organic solvents which are not acceptable due to their toxicity (e.g. methylene chloride [48], benzene [49,50], toluene [46], tetrahydrofuran [35], etc.) Moreover, a considerable amount of proposed procedures are performed at high temperature [46,51] which may contribute to degradation of thermolabile actives and/or using ionic surfactants [51], which may interact with ionic species present in the system. This study aims to contribute new knowledge on low-energy emulsification in systems based on high polarity solvents (ethyl acetate) and a preformed polymer, employing low toxicity, non-ionic components at 25 °C and the use of these nano-emulsions for the obtention of ethylcellulose nanoparticles. These nanoparticles are interesting from a basic viewpoint and also for pharmaceutical applications where safety and environmental concerns are important issues.

2. Experimental

2.1. Materials

Ethylcellulose (ETHOCEL Std 10 Premium), in the following abbreviated as EC10 (MW ∼ 78,000 g/mol; ethoxyl content of 48.7%) was a generous gift from DOW CHEMICALS. Ethyl acetate (Ph Eur grade) used as the organic solvent, was purchased from Panreac. This solvent has a boiling point of 77 °C. It is soluble up to 8.2 wt.% in water and can dissolve up to 3.3 wt.% water at 25 °C. Polyoxyethylene 4 sorbitan monolaurate, also known as polysorbate 21 (Tween 21®), was kindly supplied by Croda. This surfactant is a yellow oily liquid at room temperature, and has an HLB value of 13.3 [52]. Water was MilliQ filtered.

2.2. Methods

2.2.1. Phase inversion determination

Samples of about 4 g were prepared by addition of water to oil/surfactant mixtures at intervals of 5–10 wt.% of water up to 95 wt.%. Electrical conductivity of samples was measured in each composition under continuous magnetic stirring at 25 °C, by means of a Crison-GLP 31 conductimeter with a Pt/platinised electrode. Measurements were performed with no addition of electrolytes to the aqueous phase.

2.2.2. Formation of O/W nano-emulsions

Nano-emulsions were prepared by stepwise addition of water to oil (10% EC10 in ethyl acetate)/surfactant mixtures at 25 °C. The region of formation of O/W nano-emulsions in the water/surfactant/oil system was assessed visually. Compositions were considered as nano-emulsions when regardless of their viscosity, they were transparent, translucent or opaque, showing a bluish or reddish shine when observed through a spotlight. It was confirmed that they were not microemulsions as their properties depended on the preparation method. Nano-emulsions intended for nanoparticle obtention were prepared by the same procedure up to a water content of 90 wt.%, at 25 °C.

2.2.3. Nanoparticle preparation

Nanoparticles were prepared from selected nano-emulsions by overnight evaporation of the solvent present in the nano-emulsion under continuous stirring at room temperature.

2.2.4. Dynamic light scattering

The mean droplet size and size distribution of the nano-emulsions were determined by dynamic light scattering (DLS) using a Malvern 4700 photon correlation spectrometer (Malvern Instruments, Malvern, UK) equipped with an argon laser (λ = 488 nm) with variable intensity. Measurements were carried out at a scattering angle of 90°. The samples were diluted 1/100 to avoid multiple scattering.

2.2.5. Nano-emulsion stability

Nano-emulsion stability was assessed by light backscattering by means of a Turbiscan Lab Expert at constant temperature (25 °C). For this purpose 15 g of sample were prepared and filled in a glass measurement cell tightly stoppered to avoid solvent evaporation. The light source is a pulsed near infrared light-emitting diode LED (λ = 880 nm). The detector consists on two synchronous optical sensors. The transmission sensor receives light transmitted through the sample at 180° from the incident beam while the backscattering detector receives light backscattered by the sample at an angle of 45° from the incident beam. Transmission and backscattering data were acquired for 24 h in intervals of 1 h.

2.2.6. Transmission electron microscopy (TEM)

One drop of sample was placed on a copper grid, stained with 2 wt.% phosphotungstic acid and observed on a Jeol JEM1010 transmission electron microscope. The size of a minimum of 1000 randomly selected particles was measured from about 40 TEM micrographs taken at different magnifications, by means of an image analysis software package (Analysis®) in order to obtain the average size and size distribution.

3. Results and discussion

3.1. Nano-emulsion formation and characterization

O/W nano-emulsion formation was studied in the water/polyoxyethylene 4 sorbitan monolaurate/[10% EC10 in ethylacetate] system at 25 °C by the phase inversion composition method (PIC). The region of nano-emulsion formation, assessed as described in Section 2.2.2., is shown in Fig. 1a. O/W nano-emulsions were formed at oil/surfactant (O/S) ratios comprised between 30/70 and 70/30 and water contents above 40 wt.%. It should be noted that transparent dispersions were not obtained if the order of addition of the components was changed, a clear indication that they were not microemulsions but nano-emulsions. Nano-emulsions became more transparent, with a growing intense bluish to reddish shine, the higher the water content. It is worth noting that