Colloids and Surfaces B: Biointerfaces 125 (2015) 190–196

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Eco-friendly aqueous core surface-modified nanocapsules

C. Carbone[a,b,*,1], T. Musumeci[a,b,*,1], M.R. Lauro[c], G. Puglisi[a,b]

[a] Laboratory of Drug Delivery Technology, Department of Drug Sciences, University of Catania, V.le A. Doria 6, 95125 Catania, Italy
[b] NANO-i Research Centre On Ocular Nanotechnology, University of Catania, Catania, Italy
[c] Department of Pharmacy, University of Salerno, via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form
21 November 2014
Accepted 24 November 2014
Available online 2 December 2014

Keywords:
Cationic nanoparticles
Hybrid nanoparticles
Ocular delivery
PLA
Cationic lipid
Turbiscan

ABSTRACT

In this work, positively charged nanocapsules have been developed for potential ocular delivery exploiting the deposition of PLA onto the droplet surface of a W/O nanoemulsion prepared by the reversed procedure of the PIT method. PLA in combination with different amounts of various oils and surfactants have been studied in order to select the best formulation for polymeric nanocapsule preparation. The traditional visual observation together with the Turbiscan® technology were exploited in order to identify the best combination of polymer/oil for nanocapsule preparation. Two different primary surfactants (Span® 60 and Span® 80) have been tested to select their influence on the field of existence of the nanoemulsion by the construction of the pseudoternary phase diagrams. Cationic hybrid NC have been prepared by the addition of a coating layer of DDAB. The physico-chemical and morphological properties of all the prepared nanocapsules have been evaluated and compared by PCS, DSC and AFM. Therefore, positively charged nanocapsules can be easily prepared by a simple eco-friendly technique that exploits biocompatible materials avoiding a large input of mechanical energy as a potential ocular delivery systems for hydrophilic compounds or gene materials.

1. Introduction

The detection and treatment of several diseases occur in the deep understanding of molecular basis for disease initiation, progression and efficacious treatment based on the discovery of unique biomarkers. In this field, and in particular in ocular delivery, genomics and proteomics have rapidly developed during the past few years. At the same time different drug delivery systems are being evaluated for the delivery of gene materials, proteins, enzymes or hydrophilic drugs with the aim of achieving a controlled release, site/cells targeting and to protect the compound against chemical and biological degradation providing an optimal microenvironment. Among drug delivery systems, nanoparticles have received a great attention and rapid development for the delivery of different molecules. The nanometric structure, as reported in literature, allows obtaining more advantages compared to microparticles [1]. The term nanoparticles includes nanospheres and nanocapsules. Above all, different studies have been developed over the past few years concerning the preparation of nanocapsules (NC) due to their potential application in drug delivery, diagnostics, catalysis, separation, chromatography and biomolecular-release systems [1]. In order to allow the encapsulation of hydrophilic drugs, increasing attention is being paid to the development of aqueous-core NC dispersed in water in respect to the oily-core NC. Different methods have been studied for the preparation of aqueous-core NC, such as the interfacial polymerization, water-in-oil microemulsions, layer-by-layer surface modification and in situ polymerization [2–6].

Here, we present a novel experimental eco-friendly technique for NC preparation with a low impact on the environment due to the use of biocompatible materials, less energy in heating and the avoidance of organic solvents. This new approach is based on the deposition of the polymer at the interface of a preformed W/O nanoemulsion, prepared by a reversed phase inversion temperature method (PIT method), already used to prepare lipid

Abbreviations: AFM, atomic force microscopy; BS, back scattering; Brij® 98, Polyoxyethylene (20) oleyl ether, Oleth-20; DDAB, dimethyldioctadecylammonium bromide; DSC, differential scanning calorimetry; ΔT, variation of transmission; HLB, hydrophilic lipophilic balance; IPM, isopropyl myristate; IPP, isopropyl palmitate; IPS, isopropyl stearate; NC, nanocapsules; PCS, photon correlation spectroscopy; PDI, polidispersity index; PIT, phase inversion temperature; PLA, Polylactic acid or polylactide; S_{mix}, surfactant/cosurfactant mixture; Span® 60, Sorbitan monostearate; Span® 80, Sorbitan monooleate; T, transmission; TCT, Caprylic/Capric Triglyceride, Tegosoft® CT; TSI, Turbiscan Stability Index; Tween® 80, Polysorbate 80; Zave, mean particle size; ZP, zeta potential; W/O, water in oil.

* Corresponding authors at: Department of Drug Sciences, University of Catania, V.le A. Doria 6, 95125 Catania, Italy.
E-mail addresses: ccarbone@unict.it (C. Carbone), teresa.musumeci@unict.it (T. Musumeci).

[1] These authors contributed equally to this work and should be considered co-first authors.

http://dx.doi.org/10.1016/j.colsurfb.2014.11.038
0927-7765/

nanoparticulate systems [7]. Polylactic acid (PLA) was selected as preformed polymer due to its non-toxic low-molecular mass that meets the mandatory requirements of degradability and ease of excretion from the organism [8]. In 2004 the Food and Drug Administation (F.D.A.) approved the use of PLA polymers for human use. The nanoparticles surface is an important property that influences degradation, biodistribution and elimination of the system, and should be optimized based on the administration route. Surface nanoparticles modification with cationic materials (lipids and polymers) is the most common way to modulate the surface properties of a colloidal carrier. So, in order to obtain a suitable carrier for the ocular delivery of hydrophilic drugs or gene materials, we prepared cationic hybrid aqueous core NC adding a coating layer of dimethyldioctadecylammonium bromide (DDAB), a cationic lipid which has been demonstrated to play a critical role for the physico-chemical and technological properties of nanoparticulate systems [9].

2. Materials and methods

2.1. Materials

Polylactic acid or polylactide (PLA, Mw 10,000–18,000), Polysorbate 80 (Tween® 80), Sorbitan monostearate (Span® 60), Sorbitan monooleate (Span® 80) and Dimethyldioctadecylammonium bromide (DDAB) were bought from Sigma (Milan, Italy). Polyoxyethylene (20) oleyl ether (Brij® 98, Oleth-20), isopropyl myristate (IPM), isopropyl palmitate (IPP) and isopropyl stearate (IPS) were purchased from ACEF (Piacenza, Italy) while Caprylic/Capric Triglyceride (Tegosoft® CT, TCT) was obtained from Farmalabor (Bari, Italy).

2.2. Turbiscan® AGS and visual observation for solubility studies

The solubility of different PLA ratio in different oils, selected on the basis of their increasing lipophilicity (IPM log P = 7.43 < IPP log P = 8.16 < IPS log P = 9.3 < TCT log P = 10.85), was determined by dissolving different amount of PLA (5, 10 or 20 mg) in increasing amount of each oil (1 ml/h until 5 ml). The mixtures were kept under agitation at 37 °C for 24 h and analyzed at fixed time intervals (1, 2, 3, 4, 24 h) by visual observation. In the visual observation experiments, a number value was used to describe the different behaviors: the presence of visible particles in suspension (values between 1 and 2); the presence of a cloudy solution (values in the range 3–4); formation of a one-phase clear solution (value of 5). Furthermore, in order to found the optimal solubility of PLA in respect to the different oils, we exploited a new application of the Turbiscan® technology that allows achieving an objective, fast and detailed evaluation of the solubility of PLA in each oil. We used the optical analyzer Turbiscan® AGS (Formulaction, L'Union, France), consisting in the Turbiscan® Lab Expert based on the analysis of the multiple dispersion of the light by concentrated suspensions, provided with an Aging Station, constituted by a robot with three thermoregulated blocks for the storage of the samples. The main advantage of Turbiscan® is the ability to detect destabilization phenomena much earlier than the naked eye's operator, enabling faster and more relevant characterization of suspensions compared to common methods such as visual observation, which is time-consuming and, sometimes, non-realistic. The detection head was composed of a pulsed near-infrared light source (λ = 850 nm) and two synchronous transmission (T) and back scattering (BS) detectors. The T detector receives the light, which crosses the sample (at 180° from the incident beam), while the BS detector receives the light scattered backwards by the sample (at 45° from the incident beam). The detection head scanned the entire height of the sample cell (65 mm longitude), acquiring T and BS each 40 μm (1625 acquisitions in each scan). The measuring principle is based on the variation of the particle volume fraction (migration) or diameter (coalescence), resulting in a variation of BS and T signals [10,11]. In our experiment, 1 mg/ml of PLA/oil was placed in a cylindrical glass cell and stored for 24 h at 37 °C, under stirring. Every hour, each batch was placed and analyzed in the Turbiscan®. In order to characterize the physical state of the sample and evaluate the solubility of PLA in terms of particles disaggregation or sedimentation, we exploited the variation of transmission (ΔT). Furthermore, all batches were compared using the Turbiscan Stability Index (TSI) computation that provides a key number related to the general behavior of the formulation and is useful to compare various samples [11].

2.3. Pseudoternary phase diagrams

Span® 60 and Span® 80, selected on the basis of their different physico-chemical properties (supplementary Table 1), were studied in combination with Brij® 98 as cosurfactant and the selected oil, to verify the feasibility to obtain W/O nanoemulsion by a reversed phase inversion temperature method (PIT), as described below. Different weight ratios of surfactant/cosurfactant (S_{mix}) have been evaluated: 3:1, 2:1, 1:1, 1:0, 1:2, 1:3. These surfactants mixtures have been chosen in decreasing concentration of surfactant with respect to the cosurfactant and vice versa, to achieve a detailed study of the phase diagrams. For both Span® 60 or Span® 80 ten different weight ratios of S_{mix} and water were studied (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 9:1). The construction of the pseudoternary phase diagrams involved stepwise addition of oil phase to each weight ratio of water and surfactant, and then mixing the components with the help of low energy in heating. The nanoemulsion phase was identified as the region in the phase diagram where clear and transparent formulations were obtained based on the visual observation [12]. The axis of the pseudo-three-component phase diagram represented the aqueous phase, the oil phase and a mixture of surfactant and cosurfactant at a fixed weight ratio (S_{mix}), respectively.

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.11.038.

2.4. Nanocapsules preparation

NC were prepared with a novel experimental eco-friendly technique using polymer deposition onto microemulsion droplets, exploiting a modification of the PIT method previously used [7,13]. Two different aqueous NCs have been prepared using PLA (1 mg/ml) with Span® 60 (4.56%, w/w) (NC-A) or Span® 80 (6.55%, w/w) (NC-B) as primary surfactant, in combination with Brij® 98 (1.52 or 2.18%, w/w, respectively). For the preparation of the NCs, we exploited the deposition of the polymer at the interface of a W/O nanoemulsion, prepared by the reversed PIT method, that is a not very common procedure to prepare W/O emulsions [14]. The oily phase PLA/TCT (1 mg/ml), and the aqueous phase containing the surfactants, were separately heated (85 °C) under continuous stirring (1000 rpm). The aqueous phase was added to the oily phase at constant temperature and under agitation. The mixture was cooled to 60 °C, successively subjected to three heating cycling (85–60 °C) and then cooled to room temperature under slow and continuous stirring for 24 h [7]. In order to purify the oily phase from the excess of surfactants, all samples were washed with water and Tween® 80 or Span® 80 and ultracentrifugation (10,000 rpm, 60 min, 12–22 °C, Beckman model J2-21 Centrifuge). Based on the energy by heating involved in the PIT method, that could improve the solubility of the polymer into the oily phase, NC-A and NC-B have also been prepared with increasing amount of PLA (10, 15 or 20 mg). For the preparation of the cationic NCs we evaluated the effect of the addition of