International Journal of Pharmaceutics 434 (2012) 460–467

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Evaluation of surface deformability of lipid nanocapsules by drop tensiometer technique, and its experimental assessment by dialysis and tangential flow filtration

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ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 4 June 2012
Accepted 5 June 2012
Available online 12 June 2012

Keywords:
Lipid nanocapsules
Drop tensiometer
Dialysis
Tangential flow filtration
Rheology

ABSTRACT

Deformability of nanoparticles might affect their behaviour at biological interfaces. Lipid nanocapsules (LNCs) are semi-solid particles resembling a hybrid of polymer nanoparticles and liposomes. Deformability of LNCs of different sizes was modelled by drop tensiometer technique. Two purification methods, dialysis and tangential flow filtration (TFF), were applied to study experimental behaviour and deformability of LNCs in order to evaluate if these properties contributed to membrane passing. Rheological parameters obtained from the drop tensiometer analysis suggested decreasing surface deformability of LNCs with increase in diameter. Dialysis results showed that up to 10% of LNCs can be lost during the process (*e.g.* membrane accumulation) but no clear evidence of the membrane passing was observed. Instead, LNCs with initial size and size distribution could be found in the TFF filtrate although molecular weight cut-off (MWCO) of the membrane used was smaller than the LNC diameter.

1. Introduction

Extensive studying of nanosized particles during the last decades have resulted in drug delivery systems that protect the active substance, improve solubility and target the drug to specific tissues in the body (Farokhzad and Langer, 2009). Areas such as cancer therapy benefit from these advances (Hirsjärvi et al., 2011). Most of the nanosized drug carrier particles consist of polymer or lipid materials (Kumari et al., 2010; Müller et al., 2011; Torchilin, 2005,2007). Lipid nanocapsules (LNCs) are synthetic particles whose structure can be characterized as a hybrid between polymer nanoparticles and liposomes (Heurtault et al., 2001, 2002a). They consist of low-toxicity materials (PEGylated surfactant, lecithin, triglycerides) and their fabrication, based on low-energy organic solvent-free phase inversion process, can be easily scaled up. Moreover, their size can be tuned within the range of 20–100 nm (Heurtault et al., 2003b). LNCs have been applied *e.g.* in the delivery of cancer therapeutics (Cirpanli et al., 2011; Lacoeuille et al., 2007; Peltier et al., 2006) and other drug molecules (Lamprecht et al., 2004), macromolecules such as siRNA and DNA (Morille et al., 2010, 2011), and in radiotherapy (Allard et al., 2008; Vanpouille-Box et al., 2011a,b).

Apart from size, surface charge and hydrophilicity, elasticity and shape are less studied parameters that can have a profound influence on the nanoparticle fate *in vivo* (Decuzzi et al., 2010; Longmire et al., 2011; Perry et al., 2011). In fact, elasticity is reported to have an effect on the biodistribution and membrane passing capacity of nanoparticles (Arkhangelsky et al., 2011; Christian et al., 2009). For example, renal clearance of flexible dendrimers (~15 nm) was more pronounced compared to "hard" dendrimers of the same size (renal clearance cut-off ~10 nm) (Longmire et al., 2011). Elasticity is also considered as an important factor in the evaluation of toxicology of nanomaterials (Elsaesser and Howard, 2012). Elasticity might also be related to the mobility of the molecular chains on the nanoparticle surface, thus affecting the uptake by the mononuclear phagocyte system (MPS) (Vonarbourg et al., 2006a).

Due to their semi-solid hybrid structure, shell of an LNC can be expected to express dynamic behaviour and elasticity. These properties have been studied by interfacial deposition technique with the help of Langmuir balance (Heurtault et al., 2003a; Minkov et al., 2005a,b). Comportment of Solutol®, the main surfactant on the LNC shell, on an oil–water interface has been studied using drop tensiometer technique (Heurtault et al., 2002b). The results demonstrated, indeed, the dynamic nature of LNCs (elastic behaviour upon compression) but also their structural stability.

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http://dx.doi.org/10.1016/j.ijpharm.2012.06.019

In vitro, regardless their sizes in the range of 20–100 nm, LNCs are rapidly internalized by cells (*e.g.* macrophages, different cancer cells) (Paillard et al., 2010; Vonarbourg et al., 2006b). Smaller LNCs (20 nm) were observed to escape lysosomes more efficiently than the bigger ones (Paillard et al., 2010). Passage of LNCs across a model intestinal barrier has been shown to be size-independent (Roger et al., 2009). Increasing LNC size (20–100 nm) resulted in increased activation of the complement system (Vonarbourg et al., 2006b). However, these previous LNC studies have not elucidated the role of the possible particle deformability on the biological behaviour.

Therefore, in this study, to make next step towards deformability evaluation of LNCs, characterization of rheological properties of oil-in-water drops mimicking LNCs of different sizes was performed by drop tensiometer. Drop tensiometer technique has been used previously to study rheology of different molecules such as lipids (Anton et al., 2007; Li et al., 1999; Wüstneck et al., 1999b), proteins (Benjamins et al., 1996; Liu et al., 2011; Wüstneck et al., 1999a), polyoxyethylene-type and other surfactants (Ramírez et al., 2011; Santini et al., 2007), and biological samples (serum) (Kazakov et al., 2008) on diverse interfaces. Deformability of LNCs was then evaluated experimentally according to their behaviour during purification by dialysis and tangential flow filtration (TFF). Dialysis is a widely used classic method to remove impurities and excess molecules. TFF is a less-used alternative purification method for nanoparticle dispersions (Dalwadi et al., 2005; Dalwadi and Sunderland, 2007, 2008; de Jaeghere et al., 1999; Hirsjärvi et al., 2009, 2010; Limayem et al., 2004; Saez et al., 2000; Sweeney et al., 2006). Particularly, loss of LNCs during these processes was assessed.

2. Materials and methods

2.1. Materials

Solutol® HS15 (PEG 660 12-hydroxystearate, $M_W \sim 870$ Da) (BASF, Ludwigshafen, Germany), Labrafac® WL 1349 (caprylic/capric acid triglycerides) (Gattefossé S.A., Saint-Priest, France), Lipoid® S75-3 ($M_W \sim 780$ Da) (Lipoid GmbH, Ludwigshafen, Germany), NaCl (Prolabo VWR International, Fontenay-sous-Bois, France) and MilliQ185 water (Waters, Saint-Quentin-en-Yveline, France) were used in LNC formulation. 3,3-Dioctadecyloxacarbocyanine perchlorate (DiO) was from Invitrogen (Cergy-Pontoise, France). All other used reagents were of analytical grade.

2.2. Methods

2.2.1. Lipid nanocapsule (LNC) preparation and characterization

LNCs were prepared by the phase inversion temperature method described by Heurtault et al. (2003b). A mixture of Solutol®, Lipoid®, Labrafac®, NaCl and water was heated to 85 °C at a rate of 5 °C/min followed by cooling at the same rate to 65 °C. This cycle was repeated twice. During the last decrease of temperature, at 78 °C (during the phase inversion zone), the system was diluted with cold (4 °C) water leading to formation of stable LNCs. Size of LNCs (25, 50, 100 nm) was adjusted by changing the proportions of the components (Table 1). Fluorescent dye (DiO) was dissolved in acetone and added in the LNC preparation vial. Acetone was evaporated before addition of the components of the LNCs. Final concentration of DiO was 3 mmol/L/total Labrafac® amount (LNC core).

Size distributions and zeta (ζ) potentials of LNCs were determined with a Zetasizer ZS (Malvern, Worcestershire, UK). Particle sizing was based on photon correlation spectroscopy (PCS); the results were analysed by CONTIN algorithm and the sizes were presented based on the volume distributions together with polydispersity indices (*PdIs*). Electrophoretic mobilities were converted to ζ-potentials using Smoluchowski's equation.

2.2.2. Evaluation of the LNC composition

Quantities of LNCs (of different sizes) were estimated according to following equation:

$$x = \frac{3m}{\rho\pi 4r^3} \quad (1)$$

where m and ρ are mass (Table 1) and density (945 g/L) of Labrafac®, respectively, and x is the quantity of LNCs. Molar-% proportions of Lipoid® and Solutol® in an LNC formulation were calculated from the total quantity of these components (Table 1) used. Quantity in moles of the components on the surface of a single LNC was

$$\frac{n(\text{total})}{\text{LNC}} = \frac{n(\text{Solutol}^{®}) + n(\text{Lipoid}^{®})}{x} \quad (2)$$

$$\frac{n(\text{total})}{A} = \frac{n(\text{total})/\text{LNC}}{4\pi r^2} \quad (3)$$

where x is the quantity of LNCs, and A and r the surface area and radius of a single LNC, respectively. Previously, the totality of Solutol® and Lipoid® are shown to participate in the particle formation (unpublished data). n(total)/A was used to determine the amounts of Lipoid® and Solutol® on the surface of a model Labrafac® drop that was used in the drop tensiometer studies. Volume of the Labrafac® drop was 5 μL and the surface area of such a drop was evaluated to be about 12.5 mm^2 (the area is smaller than that of a 5 μL drop because of space occupied by the gauge).

2.2.3. Rheology by drop tensiometer technique

Rheological behaviour of an oil–water interface (Labrafac®-water) containing different quantities of Lipoid® and Solutol®, respectively, was measured by a drop tensiometer (Tracker, ITConcept, Longessaigne, France). Lipoid® and Solutol® concentrations were calculated according to their mol% in the surface of LNCs (Table 2). The tested concentrations varied from 100% Solutol® (0% Lipoid®) to 100% Lipoid® (0% Solutol®). *n/A* concentration of LNC 50 nm (Table 2) was used for the calculation of the total Lipoid® + Solutol® concentration (except the model drops for LNC 25 and 100 nm). A 5 μL (~12.5 mm^2) rising drop of Labrafrac® was formed using an Exmire microsyringe and a gauge (Prolabo, Paris, France) into a glass vial filled with the aqueous phase. The axial symmetric shape (Laplacian profile) of the drop was analysed using a camera connected to a computer. From the analysis of digital image with Laplace equation integration of the drop profile, the interfacial tension and surface area could be simultaneously calculated and recorded in real time. Volume was controlled by the motor operating the microsyringe. In order to let Lipoid® and Solutol® adsorb on the interface, the drop was equilibrated for 16 h (at 25 °C) keeping the surface area constant with the help of the syringe motor. At the end of the equilibration time, equilibrium surface tension was registered. To obtain the rheological parameters, the drop was then subjected to harmonic (sinusoidal) surface area alterations. Amplitude of the sinusoids was 0.3 μL (sinusoids were performed by the volume change because of higher precision) corresponding to about 5% change in the surface area. Magnitude of such surface area change enabled retaining linear conditions. Periods (pulsations, ω) of the sinusoids ranged from 3 to 300 s corresponding to a rad/s range 2.09–0.02. Observed alterations in surface tension for each pulsation were treated by a harmonic analysis (Windrop software, ITConcept, Longessaigne, France) that allowed calculation of the parameters G' (elasticity real part) and G'' (elasticity imaginary part), characteristic for the rheological comportment of the interface (Saulnier et al., 2001).