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Asymmetric lipid–polymer particles (LIPOMER) by modified nanoprecipitation: role of non-solvent composition

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ABSTRACT

Asymmetric lipid polymer nanostructures (LIPOMER) comprising glyceryl monostearate (GMS) as lipid and Gantrez AN 119 (Gantrez) as polymer, revealed enhanced splenic accumulation. In the present paper, we attempt to explain the formation of asymmetric GMS LIPOMER using real time imaging. Particles were prepared by precipitation under static conditions using different non-solvent phase compositions. The process was video recorded and the videos converted to time elapsed images using the FFmpeg 0.10.2 software at 25frames/sec. Non-solvent compositions comprising >30% of IPA/Acetone revealed significant stranding of the solvent phase and slower onset of precipitation(2–6 s). At lower concentrations of IPA and acetone, and in non-solvent compositions comprising ethanol/water the stranding phenomenon was not evident. Further, rapid precipitation(<1 s) was evident. Nanoprecipitation based on the Marangoni effect is a result of diffusion stranding, interfacial turbulence, and mass transfer of solvent and non-solvent resulting in solute precipitation. Enhanced diffusion stranding favored by high interaction of GMS and Gantrez(low ΔPol), and the low solubility parameter ($\Delta\delta_{total}$) and high mixing enthalpy(ΔH_M) of GMS in IPA resulted in droplets with random shapes analogous to an amoeba with pseudopodia, which on precipitation formed asymmetric particles. Asymmetric particles could be readily designed through appropriate selection of solutes and non-solvent phase by modified nanoprecipitation.

1. Introduction

Particle shape as a new design parameter for altered biodistribution and cell uptake *in vivo*, has acquired immense importance. This increasing recognition of the influence of particle shape is evident from the several studies that have demonstrated the effect of shape on the *in vitro* and *in vivo* effects of nanoparticles. Champion et. al. reported that particle shape significantly influenced the phagocytosis of non-spherical polystyrene particles by alveolar macrophages (Champion and Mitragotri, 2006; Champion and Mitragotri, 2009). Cubic and cylindrical particles of 3–5 μm diameters revealed insignificant internalization by HeLa cells (Gratton et al., 2008), while oblate particles exhibited enhanced adhesion to biological substrates (DecuzzI and Ferrari, 2006). Bypass of kupffer cells of the liver by intravenously injected silica particles, was seen to be greater with discoidal, followed by cylindrical and hemispherical shapes respectively (Decuzzi et al., 2010). The aspect ratio of non spherical mesoporous silica nanoparticles was an important determinant in cell uptake, and also cited to influence uptake mechanism. Rod like particles escaped phagocytosis when attacked by the macrophages on the major axis. Spherical particles up to 15 μm in diameter exhibited rapid macrophage uptake, however spherical particles with irregular edges showed decreased cell uptake (Hao et al., 2010). Recently, elongated polymeric particles revealed significantly reduced cell uptake by J774.A1 macrophages as compared to spherical particles (Mathaes et al., 2014). Particle shape has also exerted a significant influence on tumor uptake of nanoparticles. In one study, rod shaped gold nanoparticles had shown longer circulation time and enhanced concentration of nanoparticles within tumor tissue as compared to spherical gold nanoparticles. In the same study, PEGylated nanorods revealed less uptake in murine like macrophages as compared to spherical nanoparticles (Arnida et al., 2011) filamantous micelles have shown higher anticancer drug loading and apoptotic efficiency as well as optimum therapeutic effect against solid tumor (Geng et al., 2007; Oltra et al., 2013). Recently, worm like micelles have shown higher cell uptake and lower inhibitory dose to MCF-7 cell lines as compared to spherical micelles, vesicles and free drug (Karagoz et al., 2014). In another study, nanorods made from PLGA revealed 10-fold decrease in dose of camptothecin as compared to the spherical bovine serum albumin-coated camptothecin (Chauhan

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et al., 2011). In addition to micelles and polymeric nanoparticles, gold nanorods have also been used for *in vivo* diagnostic and plasmonic photothermal therapy to inhibit malignant tumors (Huang et al., 2006). Cylindrical-shape PLGA nanoparticles have been used for the delivery of docetaxel to mice bearing a human ovarian carcinoma SKOV-3 flank xenograft (Chu et al., 2013). Needle shaped nanoparticles has been used for the delivery of siRNA in the cytoplasm (Kolhar et al., 2011).

A number of specialized techniques were investigated for the design of nanoparticles of varying geometric shapes. Rod disk and ellipsoidal shaped particles in the size range of 20–1000 nm were generated using a microfluidic device (Xu et al., 2005). PRINT technology was adapted for the design of trapezoidal polymeric particles (Rolland et al., 2005). Champion et al. prepared particles of more than twenty different shapes including rod, oblate ellipse, elliptical disks and circular disks in the size range of 60 nm–30 μm using a film stretching method (Champion et al., 2007). Nevertheless using these approaches, drug loading could pose significant challenges.

Adapting conventional methods for the preparation of drug loaded nanoparticles which include nanoprecipitation, emulsion solvent evaporation, emulsion solvent diffusion could provide a strategic advantage (Vauthier and Bouchemal, 2009). Spheroidal (rod shaped) microparticles by an oil in water emulsification solvent evaporation method for drug delivery application are reported (Heslinga et al., 2009). Our group has reported non spherical, asymmetric lipid polymer nanoparticles (LIPOMER) by a modified nanoprecipitation method, wherein the LIPOMER comprised glyceryl monostearate (GMS) as lipid and poly vinyl methyl maleic anhydride (Gantrez AN 119) as polymer and doxycycline hydrochloride as the drug (Patil et al., 2008; Devarajan et al., 2010). Nanoprecipitation involves addition of a water miscible organic solvent phase comprising drug, lipid, polymer to an aqueous non solvent phase. As the entrapment efficiency of doxycycline hydrochloride was poor we modified the solvent phase by addition of 50% isopropanol (IPA). A high entrapment efficiency of doxycycline hydrochloride as desired was obtained. Surprisingly we found that with IPA influenced the LIPOMER shape. While nanoprecipitation with water revealed spherical nanoparticles, 50% IPA as non solvent resulted in asymmetric nanoparticles. Further the irregular shape favored kupffer cell bypass with splenotropic behavior (Patil et al., 2008; Devarajan et al., 2010). However as lipomer with another lipid glyceryl tristearate revealed spherical shape even with 50% IPA, we were motivated to explore the anomalous behavior seen with GMS. In the present study we evaluate the role of non-solvent composition, during modified nanoprecipitation in an attempt to explain the asymmetric shape of GMS LIPOMER.

2. Materials and methods

2.1. Materials

Doxycycline hydrochloride was a gift sample from M/s Alembic, India. (Gantrez AN 119) (poly(methyl vinyl ether co-maleic anhydride)) (ISP) was gifted by Anshul Agencies India. Geleol (glyceryl monostearate) of Gatefosse was gifted by Colorcon Asia and Dynasan 118 (glyceryl tristearate) of Sasol was a gift sample from S. Zhaveri Pharmakem, India. All other chemicals and solvents were either spectroscopic or analytical grade.

2.2. Solubility studies

Equilibrium solubility of GMS and Gantrez AN119 was determined in acetone/water, ethanol/water and IPA/water in the ratio of 1:9, 3:7 and 1:1. Solubility of glyceryl tristearate was determined in IPA/water 1:1. Briefly, excess solute was added to the solvent and sonicated for 5 mins and the solution allowed to equilibrate for 24 h. After centrifugation at 20,000 rpm for 10 mins, supernatant (100 μl) was pipetted out into pre-weighed (A_1) eppendorf tubes. The tubes were heated in a water bath till complete evaporation of solvent and weighed (A_2). The difference in the weight (A_2–A_1) indicated the amount of lipid or polymer dissolved in the solvent. Solubility was expressed as mg polymer or lipid /mL of solvent.

2.3. LIPOMER preparation

The solvent phase was prepared by dissolving Gantrez AN 119 (200 mg), DH (100 mg), and lipid (100 mg) in 10 ml tetrahydrofuran (THF). The non-solvent phase (30 mL) comprised acetone/water, ethanol/water and IPA/water in the ratios of 1:1, 3:7 and 1:9 and water. A glass cylinder of 150 mm height and 25 mm internal diameter served as container for the non-solvent phase. The solvent was added drop wise to the non-solvent phase, through a syringe fixed at a constant height (30 cm) to the non-solvent phase, maintained under static conditions. The changes occurring in the non-solvent phase were video recorded using a digital camera (Olympus stylus 7010, 12 megapixel), placed in front at distance of 50 cm while a 30 lumen light source was placed in a lateral position at a height of 30 cm. Videos were recorded during addition of the solvent to the non solvent phase, for about 20 s. The videos were converted to images using the FFmpeg, a command line tool. Conversion was optimized at 25 frames per sec. (Jayakumar et al., 2010) Fig. 1 is a schematic depiction of the experimental setup for the real time imaging study.

2.4. Calculation of total solubility parameter, mixing enthalpy and polarity

Total solubility parameter (δ_{total}) was calculated using the method of group contributions (Van Krevelen, 1990). Group contributions taken from literature (Van Krevelen, 1990) are reported in Table 1.

2.4.1. Total solubility parameter

Total solubility parameter (δ_{total}) was calculated from the Eq. (1)

$$(\delta_{total}) = [(\delta_d)^2 + (\delta_p)^2 + (\delta_h)^2]^{1/2} \quad (1)$$

wherein δ_d,δ_p and δ_h are partial solubility parameters and calculated from equations given below.

The partial solubility parameter associated with dispersion forces δ_d, is expressed as

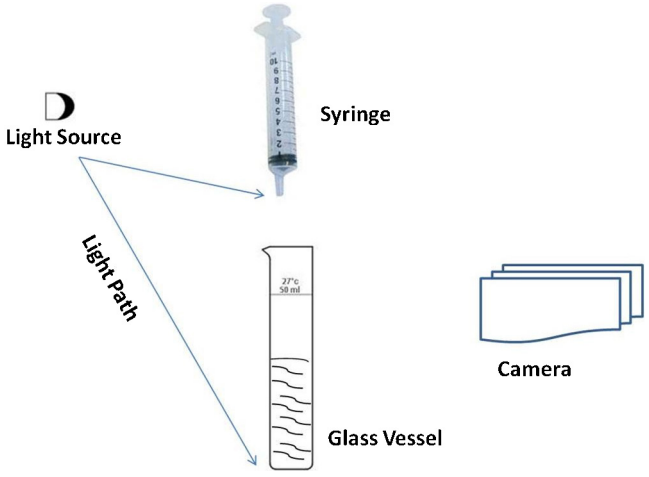

Fig. 1. Experimental set up for real time imaging study.