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The interplay between the rate of release from polymer grafted liposomes and their fractal morphology

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ABSTRACT

The purposes of this study were to investigate the indomethacin (IND) release profile from dipalmytolphosphatidylcholine:poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline (DPPC: MPOx) (in different molar ratios) mixed liposomal nanovectors, to examine the relevance of power law using these experimental release data, and to detect the relationship of the fractal dimension (d_f) of nanovectors with the fraction of the IND release. The d_f of the mixed liposomes was determined by Static Light Scattering during the release of IND from the nanocontainers. It is observed that the in vitro release of the drug from the prepared nanostructures is quite fast especially for the nanovectors prepared with the lower ratio of MPOx. The release kinetics was studied by regression analysis of drug concentrations in fractal matrices with respect to time. A power law, a piece-wise power law functions and Weibull distribution were fitted to the release data and the model parameters were estimated. Good fits were observed in all datasets analyzed, while distinct regions of different release rates corresponding to different d_f values were described. The authors proposed that the fractal morphology of the mixed liposomes affects the drug release and must be taken into account to develop liposomal drug with complete knowledge of their structural properties.

1. Introduction

One of the promising categories in pharmaceutical drug delivery nanocarriers are liposomes which belong to the class of bio-colloidal nanoparticles (Bangham et al., 1965; Gregoriadis et al., 1974). Liposomal formulations improve the therapeutic values of many drugs (Lasic, 1996; Xiong et al., 2005a,b; Eldar-Boock et al., 2013). Liposomes also improve the drug release and pharmacokinetic profile of the encapsulated drug (Allen et al., 2006; Slingerland et al., 2012). Stealth liposomes have advantages such as extend blood-circulation time and low immunogenicity (Moghimi and Szebeni, 2003; Cattel et al., 2004; Immordino et al., 2006; Samad et al., 2007; Mufamadi et al., 2011).

Additionally, the shape and the size of liposomal carriers play a key role for their in vivo behavior (Champion and Mitragotri, 2006; Champion et al., 2007; Canelas et al., 2009; Tomalia, 2009; Longmire et al., 2011). Namely, small Unilamellar Vesicles are believed to release drugs more readily due to their larger curvature and packing of the lipids in the membrane (Düzgüneş et al., 1983; Nagayasu et al., 1995; Yamauchi et al., 2007). The shape of the nanoscale drug vectors plays a significant role in therapeutic delivery processes, such as particle adhesion and biodistribution (Liu et al., 2012). The fractal analysis have been recently used in order to quantify the morphological characteristics of liposomal aggregates and liposomes, as well as mixed liposomal nanoparticles (Sabín et al., 2007a,b; Roldán-Vargas et al., 2008, 2009; Pippa et al., 2012a,b; Hadjidemetriou et al., 2013; Pippa et al., 2013a,b,c).

Mathematical modeling of drug release can be very helpful to speed up development and to better understand the mechanisms controlling drug release from drug delivery systems (Siepmann and Siepmann, 2011, 2012). Several physicochemical factors are controlling the drug release rate from advanced dosage formulations, especially from polymeric systems with controlled release properties (Siepmann et al., 1999; Siepmann and Peppas, 2001; Lee et al., 2012). Furthermore, many articles were published for the modeling of drug stochastic processes; dissolution and drug release using the fractal analysis (Macheras and Dokoumetzidis, 2000; Rinaki et al., 2003; Dokoumetzidis et al., 2001, 2005, 2007, 2010; Pereira, 2010; Dokoumetzidis and Macheras, 2011).

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; MPOx, poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline); d_f, mass fractal; PBS, phosphate buffer saline; IND, indomethacin; NSAIDs, nonsteroidal anti-inflammatory drugs; aDDnSs, advanced drug delivery nano systems; DSC, differential scanning calorimetry.

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The purpose of this study was to investigate the indomethacin (IND) release profile from polymer grafted liposomes composed by, dipalmytolphosphatidylcholine (DPPC) and poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline) (MPOx) at different molar ratios (9:0.1, 9:05, 9:1, 9:2 and 9:3), to examine the relevance of power law using these experimental release data, and to detect the relationship of the fractal dimension (d_f) of mixed nanovectors with the fraction of the IND release. A gamut of light scattering techniques and differential scanning calorimetry (DSC) were used in order to extract information on the physicochemical, structural (via fractal analysis) and thermodymanical characteristics of liposomes. Mathematical models were used to quantitatively describe the release of IND from the liposomes. These models could provide important insights concerning the interplay between the fractal dimensions of polymer grafted liposomes and the rate of drug release.

2. Materials and methods

2.1. Materials

The phospholipid used for liposomal formulations were 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). It was purchased from Avanti Polar Lipids Inc., (Albaster, AL, USA) and used without further purification. The MPOx gradient block copolymer was prepared via cationic polymerization (Milonaki et al., 2012). Chloroform and all other reagents used were of analytical grade and purchased from Sigma–Aldrich Chemical Co. Indomethacin was supplied by Fluka and was used as received.

2.2. Methods

2.2.1. Liposome preparation

Polymer grafted liposomes incorporated IND were prepared by thin-film hydration method, as described elsewhere (Pippa et al., 2013b). Briefly, appropriate amounts of DPPC:MPOx mixtures (9:0.1, 9:0.5, 9:1, 9:2 and 9:3 molar ratios) were dissolved in chloroform/methanol (9:1, v/v) and then transferred into a round flask connected to a rotary evaporator (Rotavapor R-114, Buchi, Switzerland). Vacuum was applied and the phospholipids/copolymer thin film was formed by slow removal of the solvent at 50 °C. The mixed film was maintained under vacuum for at least 24 h in a desiccator to remove traces of solvent and subsequently it was hydrated in phosphate buffer saline (PBS), by slowly stirring for 1 h in a water bath above the phase transition of lipids (41 °C for DPPC). The resultant structures (i.e. multilamellar vesicles, MLVs) were subjected to two, 3 min and 2 min sonication cycles (amplitude 70, cycle 0.7) interrupted by a 3 min resting period, in water bath, using a probe sonicator (UP 200S, dr. Hielsher GmbH, Berlin, Germany). The resultant nanostructures were allowed to anneal for 30 min. The lipid/copolymer formulations containing the drug indomethacin (IND) were prepared by dissolving IND in the initial lipid/copolymer mixture resulting in the following molar ratios: DPPC:MPOx:IND 9:0.1:1, DPPC:MPOx:IND 9:0.5:1, DPPC:MPOx:IND 9:1:1, DPPC:MPOx:IND 9:2:1 and DPPC:MPOx:IND 9:3:1. The effect of IND incorporation in the chimeric preparations was evaluated by measuring the size, size distribution, fractal dimension and ζ-potential of the resulting nanostructures.

2.2.2. IND incorporation efficiency and in vitro release

Mixed nanostructures incorporating IND were frozen at −80 °C overnight and were subjected to lyophilization in order to be reconstituted by chloroform and calculate the incorporation efficiency. The lyophilization was achieved using a freeze drier (TELESTAR[Q7] Cryodos-50, Spain) under the following conditions: condenser temperature from −50 °C, vacuum 8.2×10^{-2} mb). The lyophilized liposomal suspensions were stored at 4 °C.

Freeze-dried nanostructures were reconstituted by chloroform to the original volume of the preparation under gentle agitation. Each sample was allowed to anneal for 30 min followed by vortexing, and a relaxation period of 15 min.

The percentage of IND incorporated into nanocarriers was estimated by spectrophotometry (Stat Fax® 4200, Microplate Reader, NEOGEN® Corporation). The absorbance was measured at 492 nm. Non incorporated IND was separated from formulations on a Sephadex G75column. Incorporation efficiency (IE) was calculated by using the following equation:

$$\%\mathrm{IE} = \frac{\mathrm{IND(after\ column)}}{\mathrm{IND(initial)}} \times 100 \quad (1)$$

The release profile of IND from DPPC:MPOx (9:0.1:1, 9:0.5:1, 9:1:1, 9:2:1 and 9:3:1 molar ratio) nanovectors was studied in PBS at 37 °C. Mixed nanovectors incorporating IND (1 ml of each sample) were placed in dialysis sacks (molecular weight cut off 12,000; Sigma–Aldrich). Dialysis sacks were inserted in 10 mL (PBS) in shaking water bath set at 37 °C. Aliquots of samples were taken from the external solution at specific time intervals and that volume was replaced with fresh release medium in order to maintain sink conditions. The amount of IND released at various times, up to 3 h, was determined using spectrophotometry (Stat Fax® 4200, Microplate Reader, NEOGEN® Corporation) at λ_{max} = 492 nm with the aid of the calibration curve of the equation:

$$\mathrm{IND\ concentration}\left(\frac{\mathrm{mg}}{\mathrm{ml}}\right) = \frac{\mathrm{absorbance} + 0.0567}{0.0667} (R^2 = 0.9985) \quad (2)$$

2.2.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) experiments, were performed on an 822[e] Mettler-Toledo (Schwerzenbach, Switzerland) calorimeter calibrated with pure indium (T_m = 156.6 °C). Sealed aluminum 40 μl crucibles were used as sample holders. The samples investigated were DPPC:MPOx liposomes at molar ratios 9:0, 9:0.1, 9:0.5, 9:1 and 9:3 with a 30 mg/ml concentration (with reference to the whole dispersion) for the overall lipid content. An empty aluminum was used as reference. Prior to measurements the crucibles were subjected to a temperature over the transition of DPPC to ensure equilibration. All samples were scanned repeatedly until identical thermograms were obtained. Two cooling-heating cycles were performed; 10–60 °C at 20 °C/min and 2 °C/min scanning rate respectively. The second heating run was taken into account. Enthalpy changes and characteristic transition temperature were calculated with Mettler-Toledo STAR[e] software.

2.2.4. Physicochemical characterization of the mixed liposomal formulations

A gamut of light scattering (Dynamic, Static and Electrophoretic) techniques was used in order to extract information on the morphological characteristics of mixed liposomal IND nanocarriers, as described in our previous work (Pippa et al., 2013b).

2.2.5. Mathematical modeling

Three mathematical models were used to describe the different datasets, namely the Weibull equations (Weibull, 1951), the power law (Peppas, 1985; Ritger and Peppas, 1987) and a variation of the latter a piecewise power law equation. All three equations included an additive term C_0 accounting for the initial rapid drug release (<2 min). The Weibull equations used was written as follows:

$$F = C_0 + C_1(1 - e^{-b_1 t^{a_1}}) \quad (3)$$