

Supporting Information

PEO-b-PCL/DPPC chimeric nanocarriers: self-assembly aspects in aqueous and biological media and drug incorporation.

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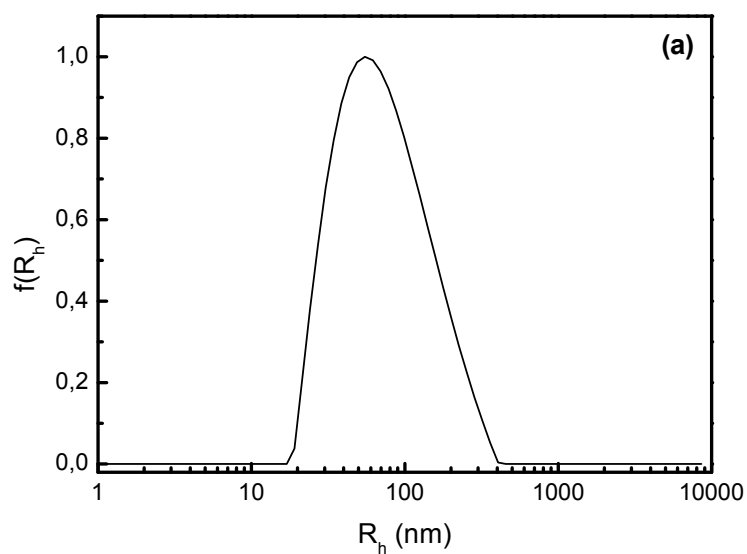
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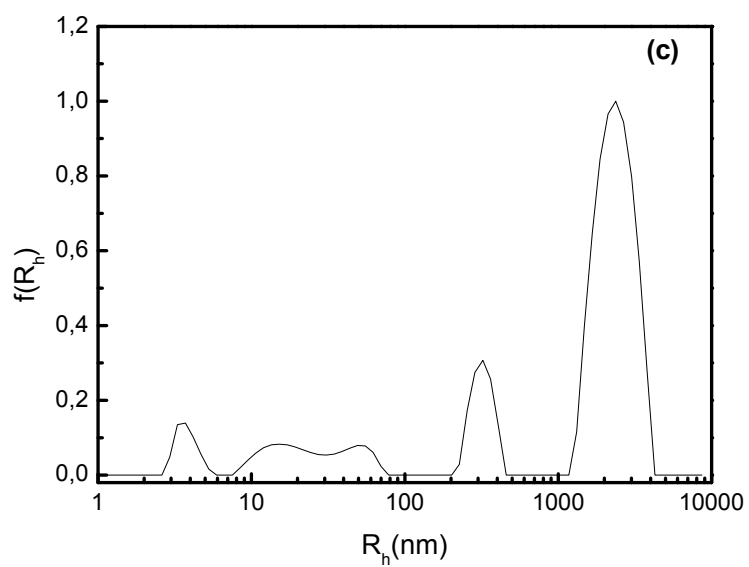
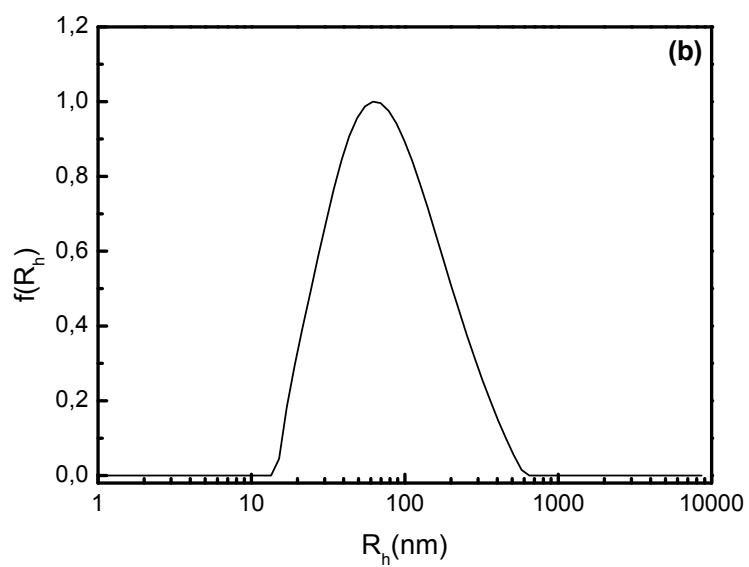
Incorporation Efficiency

IND concentration was estimated with the aid of the following IND calibration curve in chloroform:

$$\text{IND concentration (mg/ml)} = \frac{\text{absorbance} + 0.0567}{0.0667} \quad (R^2 = 0.9985) \quad (1)$$

R_h distributions for DPPC:PEO-b-PCL (9:1 molar ratio) nanoassemblies in different dispersion media





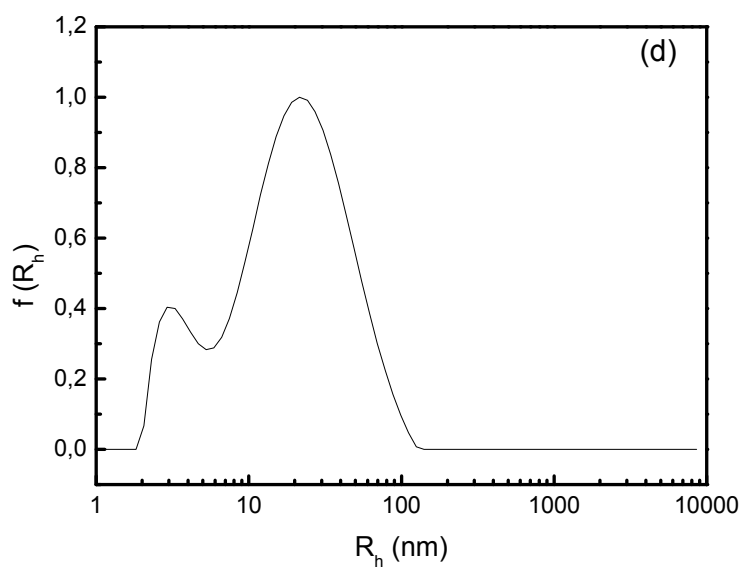


Figure S1. Distributions of hydrodynamic radius for DPPC:PEO-b-PCL (9:1 molar ratio) nanoassemblies in (a) PBS (b) HPLC-grade water (c) FBS at 25°C, at a scattering angle $\theta=90^\circ$ and at concentration $c=5 \times 10^{-3}$ mg/mL and for (d) FBS components. Distributions of R_h were analyzed by the CONTIN software.

Stability assessment of chimeric nanocarriers prepared by a different protocol

In this part of the study, we investigated the stability assessment of the chimeric nanosystem obtained by following a different preparation protocol. As mentioned in the main body of the manuscript, the hydrodynamic radii (R_h) of chimeric systems in HPLC-grade water decreased in the process of heating up to 50°C and the population of chimeric formulation becomes more homogeneous (Fig. S2(a) and (b)). This observation indicates that the thermal history of the chimeric nanostructures, as this is determined from the preparation protocol, may influence their physicochemical characteristics and behavior. In order to get additional insights on the effects of the preparation scheme, and the thermal history in particular, we prepared chimeric nanocarriers using a different preparation protocol.

The preparation protocol differed from that described in the experimental section in the following: after the annealing of the resultant small unilamellar vesicles (SUVs) by sonication, the chimeric nanocarrier dispersion was heated for 3 hours at 50°C. With this protocol, the prepared chimeric nanocarriers were approximately 5nm smaller in size and showed an analogous physicochemical behavior and stability in comparison to those discussed so far (Figures 2(a) and S2(a)). However, the population of the newly prepared chimeric nanocarriers became more heterogeneous during the stability study. This observation is a first indication that the new chimeric nanocarrier dispersion is quite different, especially regarding its colloidal stability and size changes over time. Therefore, the preparation protocol may influence the properties of the chimeric nanostructures to a significant extend.

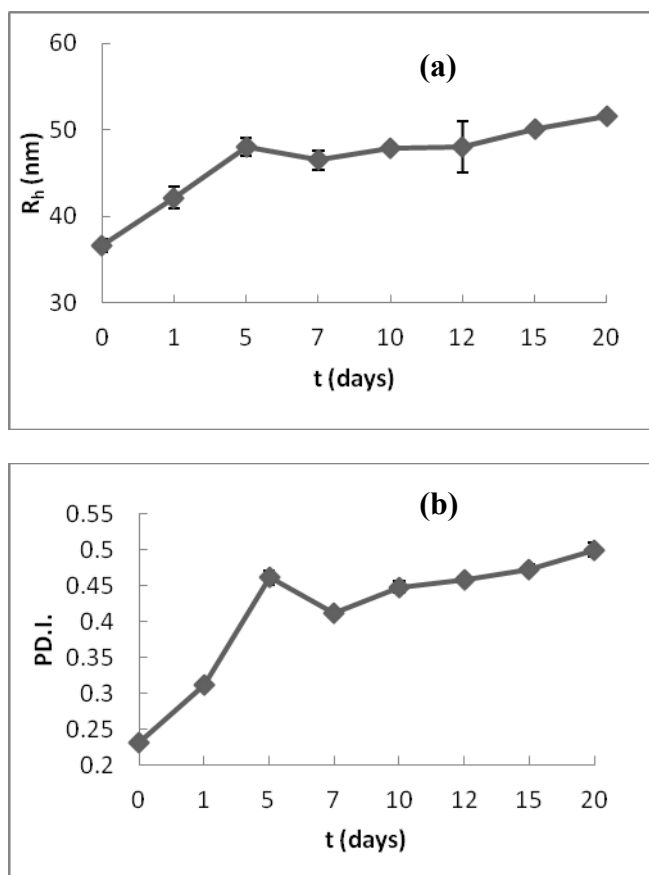


Figure S2. Stability assessment of DPPC:PEO-b-PCL (9:1 molar ratio) chimeric liposomal formulations in HPLC-grade water prepared by a different protocol (a) R_h and (b) PDI of chimeric liposomal dispersion vs time.

***In vitro* IND release from the chimeric nanocarriers**

The release profile of IND from DPPC:PEO-b-PCL (9:0.5:1 and 9:1:1 molar ratio) chimeric nanovectors was studied in PBS at 37°C. Chimeric nanovectors incorporating IND (1ml 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 $\lambda_{\text{max}} = 492$ nm with the aid of the calibration curve of the equation (1).

The *in vitro* release of the IND from the chimeric nanovectors is presented in Fig. S4. It is observed that the *in vitro* release of the drug from the prepared chimeric nanostructures is quite fast especially for the mixed nanovectors prepared with the lower ratio of gradient block copolymer (Fig. S3). This phenomenology could serve as a control factor for the preparation and development of chimeric formulations with the desired release profile, modulating the release rate of the IND via the ratio of the components, improving its therapeutic index and finally decreasing any unwanted side effects. The combination of block copolymers with liposomes for the development of a novel chimeric nanovector appears very promising, mostly due to the fact that the PEO-b-PCL acts as a modulator for the release rate of the IND. Though the exact mechanism of release modulation is still under investigation, the initial results presented here show promise for developing advanced chimeric nanocarrier systems based on amphiphilic block copolymers and lipids.

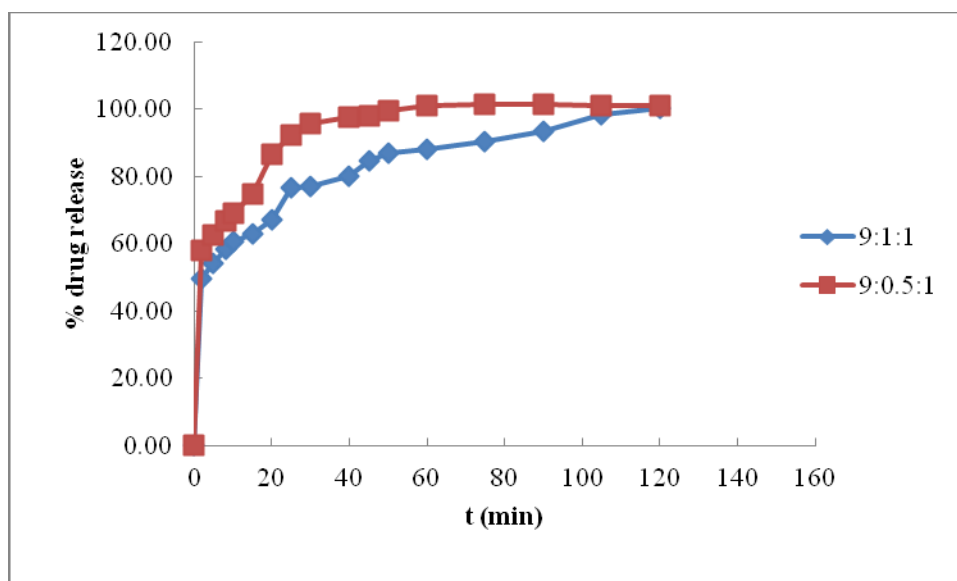


Figure S3. Cumulative drug release from DPPC:PEO-b-PCL: IND 9:0.5:1 and 9:1:1 molar ratio (each value represents the mean \pm S.D. of $n = 3$ independent experiments).

PEO-b-PCL micelles in aqueous media

Briefly, appropriate amounts of PEO-b-PCL copolymer 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 thin films were formed by slow removal of the solvent at 50°C. The films were maintained under vacuum for at least 24h in a desiccator to remove traces of solvent and subsequently it was hydrated in HPLC-grade water and in Phosphate Buffer Saline (PBS), respectively, by slowly stirring for 1h in a water bath at 45°C. The resultant vesicles were subjected to two, 3min and 2min sonication cycles (amplitude 70, cycle 0.7) interrupted by a 3min resting period, in water bath, using a probe sonicator (UP 200S, dr. Hielsher GmbH, Berlin, Germany). The resultant nanostructures were allowed to anneal for 30min. The mean hydrodynamic radius was used for the characterization of the nanoassemblies immediately after preparation ($t = 0d$), as well as the ζ -potential.

The mean hydrodynamic radius was found near to 20nm for PEO-b-PCL micelles in the two dispersion media and the population of micelles is rather polydisperse (Table S1). The ζ -potential values of PEO-b-PCL nanoassemblies were found near zero, because of the absence of net charges on the nanostructure surface. Data indicate the formation of spherical micelles in both media. Increase of solution temperature up to 50°C had no effect on the structural parameters of the block copolymer micelles.

Table S1. The physicochemical and morphological characteristics of PEO-b-PCL nanocarriers in aqueous dispersion media.

Sample	Dispersion Medium	R_h(nm)	PD.I.	ζ-potential (mV)
PEO-b-PCL	HPLC-grade water	19.9	0.20	-2.0
PEO-b-PCL	PBS	18.6	0.23	-3.9