Colloids and Surfaces A 555 (2018) 539–547

Contents lists available at ScienceDirect

Colloids and Surfaces A

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

Studying the colloidal behavior of chimeric liposomes by cryo-TEM, micro-differential scanning calorimetry and high-resolution ultrasound spectroscopy

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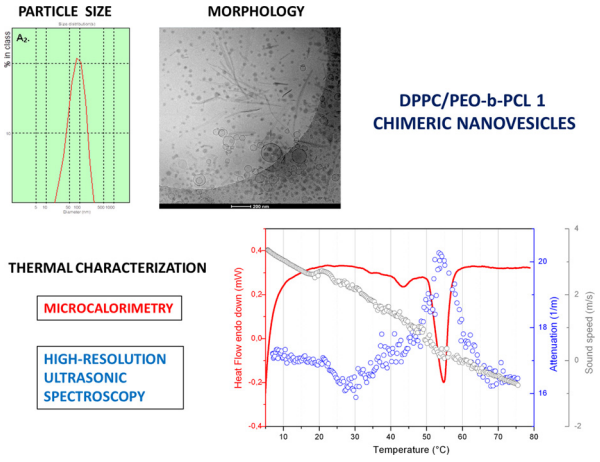
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Nanovesicles
Copolymers
Chimeric systems
Thermal analysis
High-resolution ultrasound spectroscopy
cryo-TEM

ABSTRACT

The investigation of colloidal properties of nanosystems represents a fundamental issue for the development of nanotechnology-based medicines. The aim of this study is to combine various techniques in order to characterize more comprehensively chimeric nanovesicles composed of block or gradient block copolymers with different architectures and compositions. Several chimeric systems were prepared and the impact of the block [poly(ε-caprolactone) – poly(ethylene oxide); PEO-b-PCL] and gradient block [poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline); MPOx] copolymers on the physicochemical and morphological characteristics of conventional 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes was examined. Light scattering techniques and cryo-TEM were used for the physicochemical and morphological characterization of the prepared systems. The size and the morphology were strongly related to the architecture and the composition of the polymeric compounds. Micro differential scanning calorimetry and high-resolution ultrasound spectroscopy were used for investigating the interactions between the DPPC lipids and the polymeric guest. An increase in the

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https://doi.org/10.1016/j.colsurfa.2018.07.025
Received 4 June 2018; Received in revised form 16 July 2018; Accepted 17 July 2018
Available online 20 July 2018
0927-7757/

main transition temperature was observed for the prepared chimeric systems in comparison to DPPC liposomes. In conclusion, a detailed characterization of the colloidal behavior of chimeric liposomes can benefit from the combination of the aforementioned techniques that operate synergistically, giving information on their physicochemical and morphological characteristics as well as on their thermotropic behavior.

1. Introduction

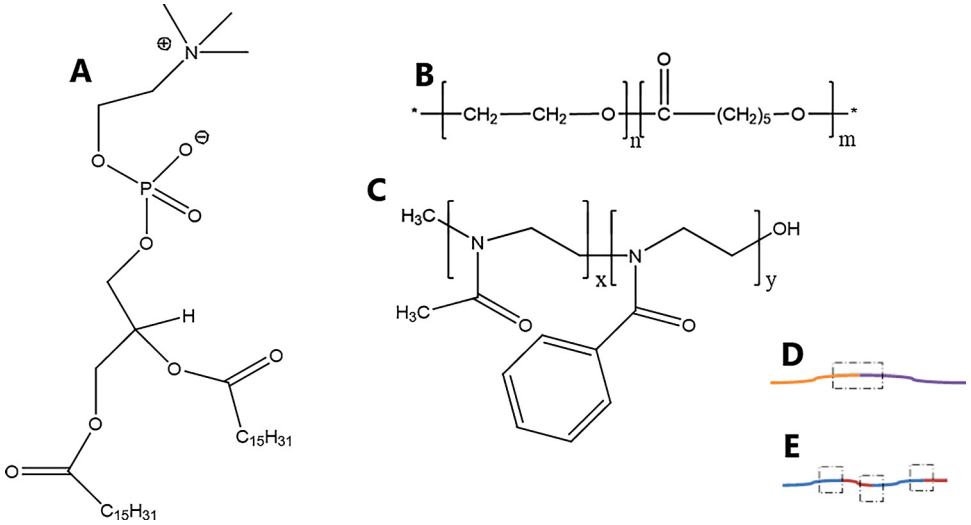
With its accelerated development in the past three decades, pharmaceutical nanotechnology is an innovative field for the design and the preparation of drug delivery systems. These drug delivery systems are nanocarriers which belong to the class of soft colloidal nanomaterials with unique properties e.g. stimuli-responsiveness [1,2]. A new class of smart nanostructured platform with applications in drug delivery and targeting is represented by advanced chimeric drug delivery systems, including polymer-grafted liposomes [3–8]. The interactions of lipids and polymers play a key role on the morphology of the resulting chimeric nanosystems i.e. weak polymer-lipid attraction (Coulombic and hydrogen interactions) and hydrophobic interactions [3,7].

Poly-ε-caprolactone (PCL) is a biodegradable polymer with several applications in drug delivery and tissue engineering [9]. Poly(ethylene glycol) (PEG) is a biocompatible and hydrophilic polymer, well-known for its low immunogenicity and "stealth" properties. The synthesis of PEO-b-PCL copolymers is generally performed via ring opening polymerization as described in the literature [10]. The nanoparticles based on PEO-b-PCL have been used as systems for vaccines, genes, water-insoluble active ingredients, cancer passive and active targeting, stimuli-responsive applications e.g. controlled drug release. In all cases, due to their biocompatible and biodegradable nature, they improve the therapeutic index and the effectiveness of the encapsulated agent [9–11].

Additionally, poly(2-oxazolines) are water soluble and biocompatible polymers. Several investigations showed the wide variety of their applications in pharmaceutical nanotechnology. Drug and protein conjugates of poly(2-oxazoline) increase the half-life and the biological stability of the active substance. A very important class of them is the thermoresponsive poly(2-oxazoline)s with many applications in cancer chemotherapy characterized them as smart bio-inspired polymers due to their solution properties and thermo-responsiveness [12,13]. Synthesis of poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline) copolymers (MPOx) is achieved via cationic polymerization [14]. Recently, systems composed of lipids and polymers have been described in the literature as controlled released drug nanocarriers. These systems are generally referred as mixed, hybrid or chimeric and can be characterized as core-shell structures comprising polymer cores and lipid/lipid-PEG shells [4,7,8,15]. They exhibit unique properties such as colloidal stability, biocompatibility, high loading efficiency, different thermotropic behavior in comparison to pure liposomal membrane, vesicular morphology, etc. [4,7,8,16]. They also have been used as model of cellular membranes due to the macromolecular sculpture of the polymer-grafted liposomal membrane [17].

Furthermore, a gamut of techniques has been already utilized in order to investigate in depth the structure and the solution behavior of the aforementioned colloidal systems. Light scattering techniques are used for the elucidation of size and zeta potential of the colloidal particles [4,18]. Static light scattering offers also the possibility of studying the morphology of colloidal particles using fractal geometry [5]. Thermal analysis techniques (i.e. differential scanning calorimetry, microcalorimetry etc.) are also used for studying the microstructure and the loading capacity of PEGylated and polymer grafted liposomes [18,19]. Additionally, cryo-TEM is a very useful technique to visualize the morphology of colloidal systems [20]. Acoustic spectroscopy is useful to analyze the highly structured colloidal dispersion and gives information about the materials during pre-formulation and formulation studies [21].

The aim of this study is to combine different techniques in order to study chimeric liposomes composed of block or gradient block copolymers with different architectures and compositions. We prepared different chimeric systems in order to examine the impact of the block and gradient block copolymers on the physicochemical and morphological characteristics of conventional 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes. The block copolymers PEO-b-PCL consist of two blocks, one hydrophilic (PEO) and one hydrophobic part (PCL). In the MPOx copolymers, the gradual compositional change along the length of the polymer, which is related to the hydrophilic and hydrophobic units, is presented in Scheme 1. The investigated block copolymers were selected for different reasons including their difference in structure and architecture, high biocompatibility, the ability to entry in lipid membranes (as shown in our previous investigations) and the steric stabilizing effects in DPPC liposomes [4–6]. Light scattering techniques and cryo-TEM were used for the physicochemical and morphological characterization of the prepared systems. Micro



Scheme 1. Chemical structures of (a) DPPC lipid, (b) the block copolymer PEO-b-PCL, (c) the gradient block copolymer MPOx. Macromolecular architecture of (d) PEO-b-PCL (hydrophilic component - PEO: purple line and hydrophobic component- PCL: orange line) and (e) MPOx (hydrophilic component (MeOxz): blue line and hydrophobic component (PhOxz): red line) employed in this study. The boxes with dashed lines show the entry/exit points into/from the liposomal membrane (For interpretation of the references to colour in this Scheme legend, the reader is referred to the web version of this article).