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Synthesis, characterization and evaluation of retinoic acid-polyethylene glycol nanoassembly as efficient drug delivery system

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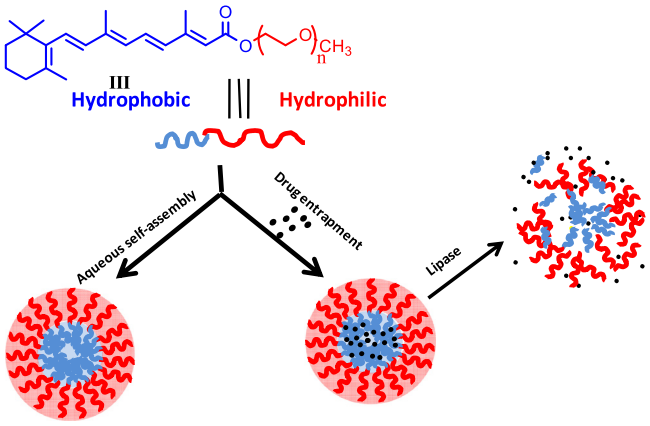
HIGHLIGHTS

- Self-assembly of amphiphilic Ret-PEG conjugate results in formation of nanoassemblies.
- The nanoassemblies efficiently entrapped hydrophobic drugs.
- Biodegradability of the nanoassemblies was evaluated by lipase treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Micelle-based drug delivery systems are gaining attention of the researchers due to their unique properties such as easy preparation, small size, low toxicity and low cost. Here, the main objective of the present work was to design and synthesize an amphiphilic molecule, Ret-PEG, by condensing retinoic acid (Ret), a natural derivative of vitamin A required for growth and development, with polyethylene glycol methyl ether ($PEG_{750}OMe$) in a single step in the presence of a condensing reagent, DCC/DMAP. Self-assembly of this molecule into nanoassemblies in an aqueous system was monitored by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The average hydrodynamic diameter of these structures was found to be ~102 nm bearing spherical shape. Further, these nanoassemblies provided the platform to load hydrophobic drugs such as ornidazole and curcumin into the hydrophobic core. Their release pattern from these drug-entrapped nanostructures showed a sustained release of drugs over a period of time. MTT assay was also carried out to demonstrate their non-toxic nature on mammalian cells, HEK293 cells. Further, enzyme-responsiveness of these assemblies was also investigated by DLS and TEM. All these results demonstrate the potential of the projected formulations to be used as effective drug carrier system for future drug delivery applications.

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1. Introduction

Nanoparticles with organic and/or inorganic cores are finding widespread applications in the area of environmental and biomedical sciences [1–6]. Recently, nanoparticle-based drug delivery systems have been aimed at making a drug available in right concentrations at the right site of action. This technology has shown the potential in biomedical applications to cure different debilitating and fatal diseases among humans and other animals/organisms. It is more advantageous since the method is more efficient, requires lesser dosage of drug, exhibits lesser side effects, enhances bioavailability and acts faster on target cells/tissues [7,8]. Moreover, nanocarriers improve the aqueous solubility of hydrophobic therapeutic agents by encapsulating them in the hydrophobic pockets of the nanostructures. To develop such efficient drug delivery nanocarriers, various polymeric materials have been explored [9–11]. Some of the polymers that have been widely reported by researchers for this purpose are polyethylene glycol, poly (lactic acid), poly (organophosphazene), poly (D,L-lactic-co-glycolic acid), polycaprolactone (synthetic polymers), chitosan, dextran, cellulose, alginate (natural polymers), etc. Recently, micelle-forming amphiphilic polymers have also been reported as nanocarriers in drug delivery applications [12–15]. These polymers, containing a highly hydrophilic chain and hydrophobic domains, offer the convenience to fabricate and manipulate them according to the need without much difficulty. Besides, these polymers are biocompatible, bioeliminable and biodegradable so that they are accepted to the body and eliminated out of body once the aim has been achieved [16–21]. Generally, the hydrophilic chain consists of either PEG, its derivatives or polysaccharides and hydrophobic domain comprises of a lipid, hydrophobic molecule or a hydrophobic block copolymer [22–26]. PEG is a hydrophilic polymer which has been widely used in the development of drug carrying polymers mainly because of the advantage that it is non-toxic, bio-inert, less immunogenic [27]. The polymeric nanoparticles comprising of hydrophilic PEG fragment are not only more stable in aqueous solutions as compared to micelles made up of conventional surfactants but also helps in increasing the blood circulation i.e. half-lives of the carriers [22–26]. Currently, some of the drug carriers based on PEG moieties are in phase II and III stages suggesting safety of such carriers in vitro and in vivo [28,29]. These PEG-conjugated amphiphilic molecules in aqueous medium self-assemble into micellar nanostructures with hydrophilic outer surface and hydrophobic inner core, which can effectively encapsulate hydrophobic therapeutic molecules [30–37]. The outer shell accounts for pharmacokinetic behavior while the inner core provides stability, drug loading efficiency and drug release behavior. In addition, PEG containing drug carriers escape opsonization during systemic delivery, i.e. recognition by the immune system of the body. The immune system of the body does so by recognizing opsonins and complement proteins bound to surface of the particles/polymers. PEG, being hydrophilic, inhibits binding of such protein molecules thereby makes them unrecognizable by mononuclear phagocyte system (MPS) [38]. Similarly, pegylation of liposomal formulations also prolongs their clearance from the system. These formulations are known as stealth formulations. Recently, it has been observed that some stealth formulations work efficiently in vivo while others do not. This behavior of such formulations has been studied in detail by Papi et al. [39,40] by exploring the bio-nano-interactions between human plasma and Onivyde, an FDA approved pegylated liposomal formulation for pancreatic cancer. They have reported that upon interaction, a protein corona is formed around the surface of the formulation, which enhances the cellular uptake of the Onivyde in PANC-1 cells, i.e. protein corona changes the bio-identity of the formulation. This study contradicts the earlier findings that pegylation (or PEG chains) inhibits the non-specific interactions with the proteins. However, this needs to be further studied in more detail with other formulations to arrive at some conclusion. For hydrophobic fragment in the amphiphilic polymers, many hydrophobic molecules (cholesterol, squalene, cholic acid, fullerenes, palmitoyl, etc.) have been explored according to the requirement. Here, we have selected a hydrophobic molecule, retinoic acid, a natural derivative of vitamin A, which regulates cell behavior during growth and development of embryo, and plays an important role in various activities such as regulation of cell differentiation, inhibition of cell proliferation, migration of tumor cells, etc. Thus, it exhibits anticancer activities in number of cancer cells and tissues. It is an essential molecule throughout an individual's lifetime.

Research has also being undertaken on the development of stimuli-responsive such as thermosensitive, pH sensitive, enzyme-sensitive and light-dependent drug releasing polymers [12–14]. These stimuli-responsive polymers unload their contents or drugs only when they reach their site of action and are triggered to do so by the changes in their immediate environment.

Taking into consideration the benefits of both the constituents, we have conjugated retinoic acid with PEG monomethyl ether (750 Da) via biodegradable ester linkage. π-π interactions between the unsaturated chains of retinoic acid augmented the process of self-assembly of the projected conjugate. The conjugate nanoassemblies, so formed, were studied for their size, morphology, drug loading and entrapment efficiency and in vitro drug release studies. The drug release behavior was analyzed at different pH and temperature. These particles were also tested for the enzymatic degradation by the action of lipase enzyme which established the biodegradability of the nanoassemblies in body so that the degraded products could be cleared out of the body system. Finally, the projected nanoassembles were evaluated for their cytotoxicity on HEK293 cells.

2. Experimental section

2.1. Materials and methods

Polyethylene glycol monomethylether, M.Wt. 750 Da (PEG_{750}-OMe), was purchased from Alfa Aesar, USA. Retinoic acid, ornidazole, Lipase from *Pseudomonas cepacia*, 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), N,N′-dicyclohexyl carbodiimide (DCC), 4-dimethylaminopyridine (DMAP), Trypsin, Dulbecco's modified Eagle's medium (DMEM) and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich chemical Co., USA. The dialysis membrane was obtained from Spectrum labs, USA and other reagents and chemicals were used as purchased locally.

The compound synthesized in the present study was characterized by its ^{1}H-NMR by dissolving in deuterated solvents, methanol-*d4* and D_2O, on a Bruker Avance 400 MHz spectrometer operating at 400 MHz with chemical shifts reported in ppm.

Particle size and zeta potential measurements were carried out on Zetasizer Nano-ZS (Malvern Instruments, UK). For TEM imaging, grids were prepared by depositing 10 μL solution of samples on carbon-coated copper grids with 1% (w/v) uranyl acetate staining and images were observed at an accelerating voltage of 200 kV on HR-TEM (Tecnai G2 30U-twin 200 kV electron microscope). UV absorption spectra were recorded on a UV–VIS spectrophotometer (Cary 60, Agilent Inc., USA). Elisa plate reader (μQuant, Biotek Instruments, USA) was used to read 96-well plates for toxicity assay.