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Fabrication of doxorubicin nanoparticles by controlled antisolvent precipitation for enhanced intracellular delivery

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ABSTRACT

Over-expression of ATP-binding cassette transporters is one of the most important mechanisms responsible for multidrug resistance. Here, we aimed to develop a stable polymeric nanoparticle system by flash nanoprecipitation (FNP) for enhanced anticancer drug delivery into drug resistant cancer cells. As an antisolvent precipitation process, FNP works best for highly lipophilic solutes ($\log P > 6$). Thus we also aimed to evaluate the applicability of FNP to drugs with relatively low lipophilicity ($\log P = 1–2$). To this end, doxorubicin (DOX), an anthracycline anticancer agent and a P-gp substrate with a $\log P$ of 1.3, was selected as a model drug for the assessment. DOX was successfully incorporated into the amphiphilic diblock copolymer, polyethylene glycol-*b*-polylactic acid (PEG-*b*-PLA), by FNP using a four-stream multi-inlet vortex mixer. Optimization of key processing parameters and co-formulation with the co-stabilizer, polyvinylpyrrolidone, yielded highly stable, roughly spherical DOX-loaded PEG-*b*-PLA nanoparticles (DOX.NP) with mean particle size below 100 nm, drug loading up to 14%, and drug encapsulation efficiency up to 49%. DOX.NP exhibited a pH-dependent drug release profile with higher cumulative release rate at acidic pHs. Surface analysis of DOX.NP by XPS revealed an absence of DOX on the particle surface, indicative of complete drug encapsulation. While there were no significant differences in cytotoxic effect on P-gp over-expressing LCC6/MDR cell line between DOX.NP and free DOX in buffered aqueous media, DOX.NP exhibited a considerably higher cellular uptake and intracellular retention after efflux. The apparent lack of cytotoxicity enhancement with DOX.NP may be attributable to its slow DOX release inside the cells.

1. Introduction

According to the World Cancer Report, cancer still remains the most deadly disease in the world with possibly more than 26 million people being diagnosed with cancer by 2030 and about 17 million people dying from it [1]. Although most chemotherapeutic agents are effective enough to eradicate cancer cells with high therapeutic efficacy, poor or inefficient delivery of the drug to the target sites and the inherent or acquired resistance of tumor cells towards anticancer drugs remain a major stumbling block in the treatment of cancers. Over-expression of ATP-binding cassette (ABC) transporters is one of the most important mechanisms responsible for multidrug resistance (MDR), in which tumor cells exhibit simultaneous resistance to structurally unrelated anticancer drugs. The best known and best characterized of the highly diversified ABC proteins is P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), or ATP-binding cassette sub-family B member 1 (ABCB1) [2]. Overexpression of plasma membrane P-gp is believed to be the main cause of MDR. Membrane bound P-gp can efficiently efflux a broad spectrum of cytotoxic drugs (e.g., paclitaxel and DOX) to the extracellular space [3]. The major drawback of resistance to chemotherapeutic agents is the limited uptake into tumor sites and reduced cytotoxic effect of anti-cancer drugs [4]. Another problem with conventional chemotherapeutic agents is their non-selective distribution in the body which results in

Abbreviations: ABC, ATP-binding cassette; ACT, acetone; AFM, atomic force microscopy; DLS, dynamic light scattering; DMF, dimethylformamide; DOX, doxorubicin; DOX.NP, doxorubicin-loaded polymeric nanoparticle; EE, encapsulation efficiency; EPR, enhanced permeability and retention; FNP, flash nanoprecipitation; MDR, multidrug resistance; MIVM, multi-inlet vortex mixer; PDI, polydispersity index; PEG-*b*-PLA, polyethylene glycol-*b*-poly (DL-lactide); P-gp, P-glycoprotein; PVA, polyvinyl alcohol; PVP, polyvinylpyrrolidone; *Re*, Reynolds number; XPS, X-ray photoelectron spectroscopy.

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unwanted toxicity towards normal healthy tissues and inadequate drug level at the tumor site [5].

Recent advances in nanotechnology have made possible the development of nanocarrier formulations for improving the efficacy and reducing the systemic toxicity of chemotherapy for cancer treatment [6]. It has been shown that polymeric micelles prepared from block copolymers in the size range of 20–50 nm accumulate more readily in tumors by utilizing the unique pathophysiologic characteristics of tumor vasculature which is highly permeable and dilated with numerous pores and gaps as large as 600–800 nm between endothelial junctions [5–8]. The leaky vasculature coupled with poor lymphatic drainage leads to the so-called enhanced permeability and retention (EPR) effect, which accounts for the tendency of macromolecules and nanoparticles to accumulate at the tumor site [9]. Nanoparticles accrued thus can reverse P-gp mediated MDR by cellular internalization through endocytosis, thereby bypassing the plasma membrane P-gp [10]. This mode of delivery has been shown to be capable of attaining sufficiently high intracellular drug concentration for overcoming efflux-pump mediated drug resistance [11].

In light of the above advantages using nanotechnology-based drug carrier systems, considerable attempts have been made in developing novel nanoparticle formulations. Broadly, there are two major types of technology for nanoparticle fabrication, namely, top–down and bottom–up approaches. The former is the process of breaking down coarse materials to nano-sized particles by mechanical means, whereas the latter is the process of building up particles from individual building units (e.g., ions, molecules) through nucleation and growth to the desired particle size. The top–down approach employs various milling and high pressure homogenization techniques to produce nanoparticles [12,13]. These techniques have successfully developed several nano drug products which are being marketed or under clinical trials [14,15]. However, the top–down approach tends to generate relatively large particles (500–2000 nm), which render them unsuitable for targeted drug delivery. On the other hand, the bottom–up approach, which utilizes various nanoprecipitation techniques such as emulsification solvent evaporation [16], supercritical fluid processing [17,18], and antisolvent precipitation [19,20], is capable of generating substantially smaller particles (≤200 nm). The bottom–up approach possesses a significant number of advantages over the top–down strategy in nanoparticle production; it is non-destructive to the materials being processed, capable of affording uniform particles with a narrow size distribution, less time- and energy-consuming, and versatile in terms of formulation design and drug delivery options (e.g., sustained release, active drug targeting).

Of the various nanoprecipitation techniques available, the antisolvent precipitation approach appears to be most pragmatic and cost-efficient. There exist different variants of antisolvent precipitation method for nanoparticle preparation. Of particular merits is the flash nanoprecipitation (FNP) technology developed by Johnson and Prud'homme [19]. FNP involves rapid mixing of an organic solution of hydrophobic drug and amphiphilic copolymer with an antisolvent (normally water) in a confined impinging jet (CIJ) mixer [21] or a multi-inlet vortex mixer (MIVM) [20] to create a sufficiently high supersaturation level for triggering nanoprecipitation over an extremely short time scale (i.e., within milliseconds). The amphiphilic copolymer serves as a stabilizer of the generated nanoparticles where it forms an outer protective layer through interactions with both the co-precipitating hydrophobic drug in the core via its hydrophobic groups and the external aqueous medium via its hydrophilic groups. Despite their differences in design and mixing mechanisms, both CIJ mixer and MIVM are capable of producing uniformly sized nanoparticles. Mixing in the CIJ mixer is achieved by continuous frontal collision between two high-velocity linear streams (i.e., drug solution and antisolvent) in the center of a cylindrical mixing chamber. Although the mixing time in the CIJ mixer can be as short as milliseconds, the efficiency of the mixer is limited by the requirement of identical momenta of the drug solution and antisolvent (water) streams, which may not be attainable in such a short time scale, thus leading to poor solvent redistribution. On the other hand, MIVM adopts a vortex mixing principle, which not only provides efficient micromixing but, more importantly, also permits independent contribution of the momentum from each stream to the mixing process. Therefore, it is possible to use different flow rates for the drug solution and antisolvent streams, and deliver up to four formulation components via its inlets into the mixer [20,22]. Both CIJ mixer and MIVM possess a significant number of advantages over other existing nanoparticle production technologies, including higher flexibility in operation, capability of generating consistently small nanoparticles (50–200 nm) with a narrower particle size distribution, and amenability to scale-up for industrial production [23].

As an antisolvent precipitation technique, FNP works best for highly lipophilic solutes. It has been demonstrated that lipophilic compounds or drugs with a log P value above 6 (e.g., β-carotene, bifenthrin) form highly stable nanoparticles with amphiphilic copolymers as stabilizers by FNP [24,25]. The superior nanoparticle stability for such compounds can be attributed to their tight binding to the hydrophobic core of the polymer-encased particles. However for drugs in the typical log P range of 3–5, such binding in the nanoparticle cores is conceivably weaker. Indeed, we have shown that employing curcumin, a bioactive natural product from turmeric having a log P value of ~3 and a wide array of proven beneficial pharmacological activities, as a model solute, the nanoparticles produced by FNP are relatively unstable when formulated with amphiphilic copolymer alone and would require an additional stabilizer or co-stabilizer, e.g., PVP or PVA to achieve the desired stability [26,27]. Curcumin nanoparticles stabilized thus were shown to display elevated curcumin concentration in

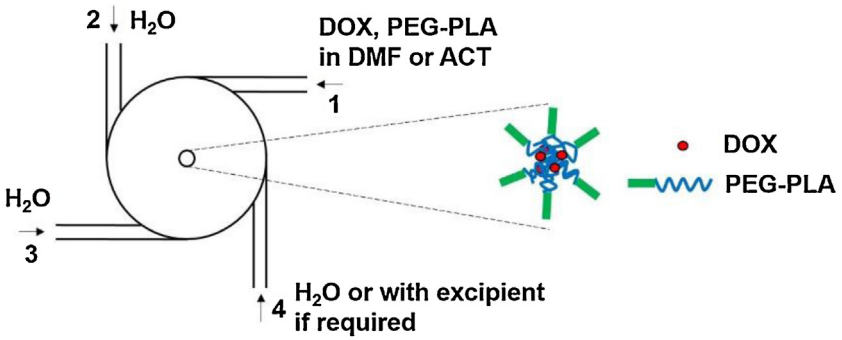

Fig. 1. Preparation of DOX.NP using a four-stream multi-inlet vortex mixer (MIVM).