# Paclitaxel Conjugate Block Copolymer Nanoparticle Formation by Flash NanoPrecipitation

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## ABSTRACT

Block copolymer drug nanoparticles have been explored for their potential in solubilizing hydrophobic drugs, reducing drug toxicity, and extending drug circulation times in vivo. Key factors in such applications involve the control of stability and nanoparticle size. Flash NanoPrecipitation, which was introduced recently, is an easily scalable technique that provides high solute loading, and controlled size nanoparticles using amphiphilic diblock copolymer stabilization. Using this technology, the anti-cancer drug paclitaxel conjugated to vitamin E succinate was formulated into stable and controlled size nanoparticles. Conjugation of the drug to vitamin E succinate to form a paclitaxel conjugate prior to mixing by Flash Nanoprecipitation reduces its solubility, and allows for the drug release rate to be controlled through the linker chemistry used to conjugate the drug to the nanoparticle.

***Keywords***: nanoparticle, paclitaxel, diblock copolymer.

## 1 INTRODUCTION

Nanoparticle formulations of organic actives are of interest for various applications, including printing inks, cosmetics, and drug delivery. In cancer therapy, block copolymer nanoparticles have been explored for their potential in solubilizing hydrophobic drugs, reducing drug toxicity, and extending drug circulation times in vivo. The method used to form nanoparticles greatly affects their size and stability, which are required in most applications. Several techniques used to form nanoparticles, such as slow anti-solvent addition, and emulsification-based methods, have serious limitations, including long processing times, process scale-up, low nanoparticle drug loading, and lack of control of nanoparticle size. Flash NanoPrecipitation, which was introduced recently [1,2,4], is an easily scalable technique that provides high solute loading, and controlled size nanoparticles using amphiphilic diblock copolymer stabilization. This technique is effective with hydrophobic components such as β-carotene. However, other solutes, such as the anti-cancer drug paclitaxel, have resulted in formulations showing considerable Ostwald-Ripening and recrystallization. In order to stabilize the formulation and control the drug release rate from the nanoparticles, paclitaxel was conjugated to a hydrophobic "anchor" to form a paclitaxel conjugate prior to mixing by Flash Nanoprecipitation. Conjugating the drug to a hydrophobic anchor reduces its solubility, and allows for the drug release rate to be controlled through the linker chemistry used to conjugate the drug to the nanoparticle. Flash NanoPrecipitation has been used for the formation of paclitaxel-vitamin E succinate conjugate nanoparticles using methoxy poly (ethylene glycol)-b-poly (ε-caprolactone) (mPEG5-PCL7) block copolymer with molecular weight of 5,000-b-7,000 g/mole, respectively.

## 2 EXPERIMENTAL METHODS

### 2.1 Paclitaxel Conjugation

Paclitaxel was conjugated to vitamin E succinate based on the procedure of Greenwald et al. [3], which used methoxy poly (ethylene glycol) (mPEG) with terminal carboxylic acid to form an mPEG-paclitaxel conjugate. HPLC was used to characterize the reaction components and products including paclitaxel, vitamin E succinate, and paclitaxel-vitamin E succinate conjugate. The resulting conjugate was purified and un-conjugated drug removed using a prep-HPLC setup.

### 2.2 Nanoparticle Formation

Nanoparticles were produced by Flash NanoPrecipitation [2]. Paclitaxel-vitamin E succinate conjugate and mPEG5-PCL7 at a 1/1 ratio (w/w) were dissolved in tetrahydrofuran and mixed at high velocity against water through a tangential flow vortex mixer. The produced nanoparticles were dialyzed against water, and characterized for size by dynamic light scattering (DLS).

## 3 DISCUSSION

The Flash NanoPrecipitation process involves high velocity jets mixing, which provides micromixing of the copolymer and drug, leading to mixing timescales that are shorter than the timescale for nucleation and growth of particles, and allowing for the formation of nanoparticles with size distributions, and drug loading efficiencies not provided by other technologies. The rapid mixing offers a uniform residence time distribution, and creates a high energy dissipation region provided by the turbulence

generated through the jets streams at high velocity in a confined volume. Following formation, nanoparticles are subject to instability due to Ostwald-ripening and recrystallization processes, which limits the range of solutes that can be formulated into stable nanoparticles via NanoPrecipitation. Drug conjugation was introduced to overcome this limitation, and allow for nanoparticle formation regardless of the solute's water solubility.

Most approaches to prodrug formation focused on enhancing water solubility of hydrophobic drugs by conjugation to a hydrophilic anchor, such as poly (ethylene glycol). In contrast, the strategy outlined here aims at further reducing water solubility of the solute. This will limit recrystallization of the solute and Ostwald ripening following nanoparticle formation, and retain the solute in the nanoparticle core, where water and enzymatic activities are limited. The nanoparticle formulations show improved stability, and are expected to prolong the drug half-life in vivo, since cleavage of the drug now depends on the nature of the linker used to conjugate the drug to the hydrophobic anchor, which can be designed to achieve desired release rates. The particle size for paclitaxel-vitamin E succinate conjugate formulated by Flash NanoPrecipitation using mPEG5-PCL7 is shown in Figure 1, one and eleven days after nanoparticle formation and storage in DI water at 4 ºC. HPLC analysis over the same period and storage conditions showed no cleavage of paclitaxel from the conjugate.

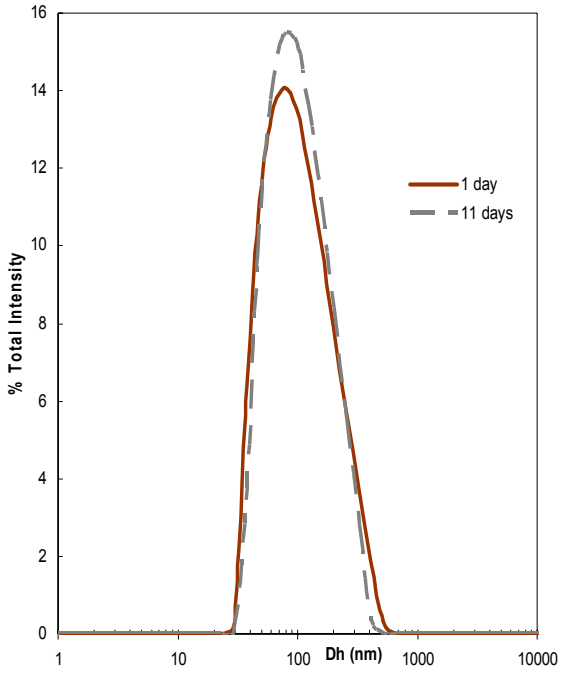


Figure 1: Paclitaxel-vitamin E succinate nanoparticles made by Flash NanoPrecipitation. Nanoparticle size is 86 nm as determined by DLS.

Varying supersaturation levels in the Flash NanoPrecipitation process can be used to control nanoparticle size. One means of changing supersaturation is through solute concentrations. For instance, changing the total initial concentrations of a mixture of paclitaxel-vitamin E conjugate and vitamin E succinate resulted in nanoparticles with different sizes. The results are shown in Figure 2.

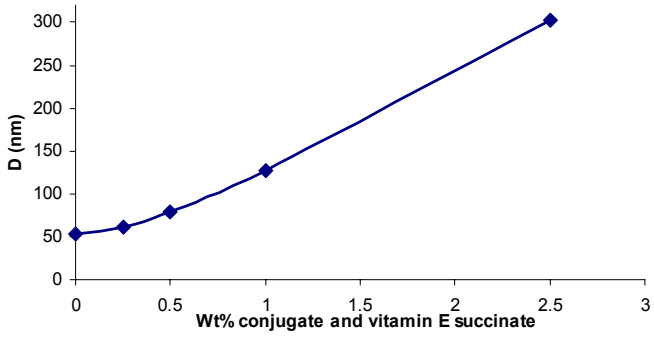


Figure 2: Nanoparticle size can be tuned by changing the concentration of the conjugate and vitamin E succinate. Nanoparticle size determined by DLS.

In addition to providing control of nanoparticle size, this technique allows for multiple solutes to be incorporated into the same nanoparticle formulation. In the example shown in Fig. 2, vitamin E succinate and paclitaxel-vitamin E succinate are incorporated into the same nanoparticle.

## 4 CONCLUSION

Flash NanoPrecipitation is an easily scalable technique to produce nanoparticles with controlled size and narrow polydispersity. For solutes with moderately low water solubility which do not show stability when formulated in nanoparticles using this technique, a solute-hydrophobic anchor conjugate was synthesized prior to mixing by Flash NanoPrecipitation. This approach provides both stability and control of release of the solute from the nanoparticles.

Current focus is on determining the *in vivo* behavior of paclitaxel from paclitaxel-vitamin E succinate conjugate formulated into nanoparticles using Flash NanoPrecipitation and mPEG-PCL block copolymers with various molecular weights.

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