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A novel drug-polyethylene glycol liquid compound method to prepare 10-hydroxycamptothecin loaded human serum albumin nanoparticle

Zhenbo Yang, Wei Gong[1], Zhiyuan Wang, Bingsheng Li[2], Mingyuan Li[2], Xiangyang Xie, Hui Zhang, Yang Yang, Zhiping Li, Ying Li, Fanglin Yu, Xingguo Mei*

Department of pharmaceutics, Beijing Institute of Pharmacology and Toxicology, Beijing, China

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ABSTRACT

Drug loading strategies and the methods derived for implementing those strategies are crucially important to the preparation of drug loaded human serum albumin nanoparticles (HSA-NPs), because each of them is focused on wrapping up specific types of drugs *via* certain physical and chemical properties. However, poor adaptability still exists to load drugs like model substance 10-hydroxycamptothecin (HCPT) by conventional methods. Because it typically represents a large class of water-insoluble drugs, who also structurally possess a certain number of hydrophilic groups. So even though they majorly have lipophilicity but they are of low liposolubility. This article presents a new concept of a loading strategy that takes a drug polymer liquid compound as a loading medium. The drug polymer liquid compound was made from low weight polyethylene glycol (l-PEG) and HCPT. Consequently, this strategy has managed to fabricate HCPT-loaded HSA-NPs through an unconventional approach that overcomes drawbacks of current loading means and better results have been obtained, like high entrapment efficiency (over 99%) and less toxicity involvement. Afterward, *in vitro* and *in vivo* evaluations and characterizations were performed to help with the in-depth interpretation of the loading mechanism in order to reveal and further investigate the possible far-reaching applications of this method.

1. Introduction

Human serum albumin (HSA) is an endogenous material. Due to the nontoxic, nonantigenic, biocompatible and biodegradable properties as well as the ability to bind with various drugs (Peters, 1985), it is rendered a desirable drug-loading carrier for fabricating nanoparticles (Elzoghby et al., 2012). Among all of the raw material candidates, HSA is always favored for its abundant source, robust physical and chemical properties, biocompatibility and low toxicity before or after degradation (Kratz, 2008). Thus, a vast array of essays have intended to incorporate therapeutic drugs onto albumin-based nanoparticulates (HSA-NPs) due to the essential benefits of this material and the nano-effect, such as the release pattern and biodistribution of fabricated NPs (Torchilin, 2008).

Meanwhile, appropriate loading strategies have been developed in order to meet different preparation demands. For these preparations, desolvation (coacervation) and emulsification are classic sequential methods of HSA-NPs formation then followed by fixation *via* chemicals (usually glutaraldehyde) or thermal treatment (Meziani and Sun., 2003; Jahanshahi and Babaei, 2008). Meanwhile, nanospray drying and nab-technology are more suitable to achieve a high efficiency preparation (Lee et al., 2011; Cortes and Saura , 2010). Each aforementioned method has its own adaptivity (advantage) for loading either water-soluble or water-insoluble drugs. The loading of drugs onto human serum albumin nanoparticles (HSA-NPs) can be summarized into three major mechanisms: (1) Drugs undergo noncovalent reversible binding with sites from HSA homologous domains or attachment through electrostatic adsorption (Tajmir-Riahi, 2007; Fasano et al., 2005). This mechanism is extensively applied in the desolvation incubation for water soluble drugs. (2) Multiple HSA molecules physically entrap the drugs during the formation of the NPs when using methods such as emulsification, nanospray drying and nab-technology (Müller et al., 1996; Lee et al., 2011; Cortes and Saura, 2010). (3) Covalent bonds are constructed between HSA and the drug molecule by working with functional groups. It usually applies in self-assembly or desolvation approaches (Xu et al., 2011).

* Corresponding author at: 27 Taiping Road, Haidian District, Beijing 100850, P.R. China, Beijing Institute of Pharmacology and Toxicology. Tel.: +86 10 68211656; fax: +86 10 68211656.
E-mail address: ddsamms606@163.com (X. Mei).
[1] Joint first author.
[2] These authors contributed equally.

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In spite of these current loading trends, some unsatisfactory physicochemical properties of certain drugs still obstruct the generic application of these techniques. And furthermore, low entrapment efficiency (EE), organic solvent consumption and toxicity introduction are also prevalent issues in solvent media preparation for all kinds of HSA-NPs. An example is the loading of 10-hydroxycamptothecin (HCPT), which is water insoluble (functional lactone form). More ill ideally, it slightly soluble in dichloromethane or chloroform and has a solubility just shy of approximately 40 μg/mL in soybean oil, which are common two-phase solvents applied in nab-technology and emulsification for packing lipophilic drugs (Desai, 1995). 10-hydroxycamptothecin is also sensitive to pH changes. And all of these above properties make it difficult to balance preparation procedures and favorable results in characterizations to make the desired NPs. Besides this typical substance, there are many drugs that are similar to HCPT, because they are structurally possessing both hydrophilic and hydrophobic groups. Thus, they all face a dilemma that has water insolubility and low liposolubility simultaneously, that are the least desirable properties for packing drugs to NPs.

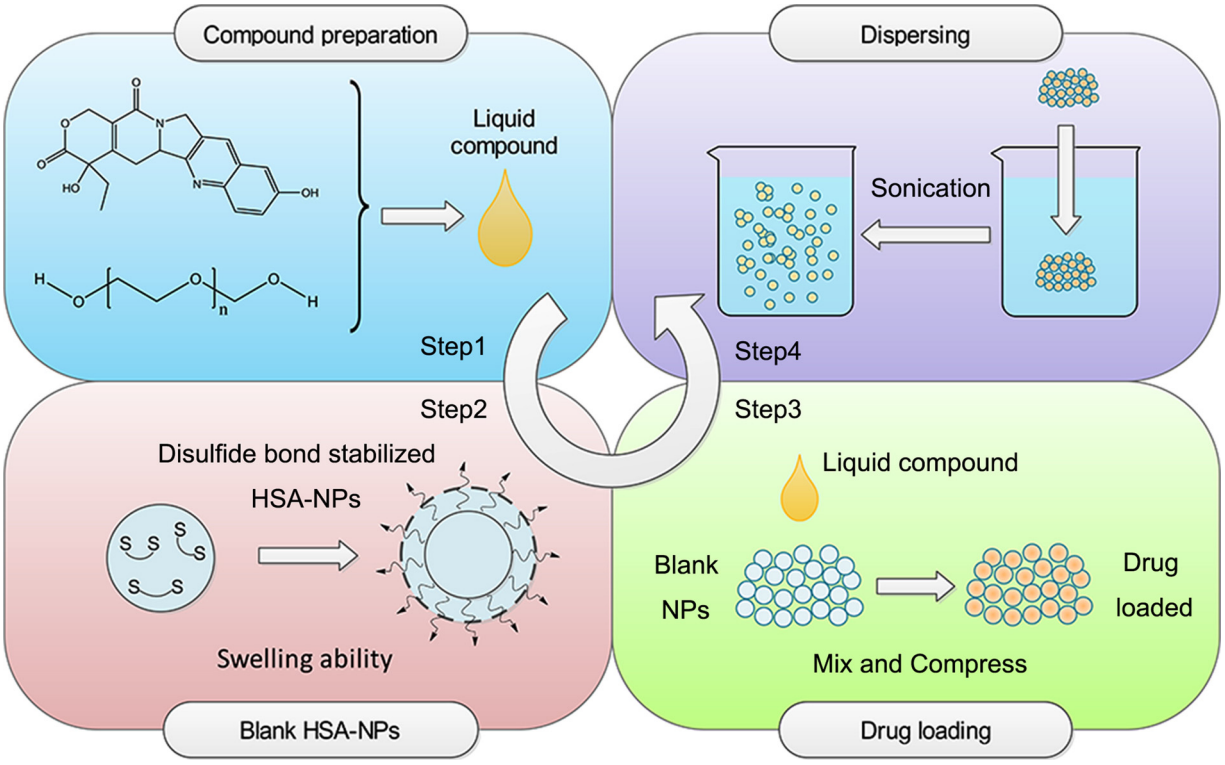
One of the unfavorable results is low entrapment efficiency for HCPT loaded NP formulations. The purpose of entrapment efficiency (encapsulating) is to evaluate the free drug ratio by the equation (1). Since the ultimate drug distribution state is a dynamic balance between the NPs and the solution. That means the entrapment efficiency reflects the drug amount that will perform *via* designed particles in the product. So it is one decisive standard for evaluating the craft of preparation.

$$\text{Entrapment efficiency}(\%) = \frac{\text{Drug}_{\text{total}} - \text{Drug}_{\text{free}}}{\text{Drug}_{\text{total}}} \times 100\% \quad (1)$$

Though, many have reported successful preparation of HCPT loaded albumin-based NPs (Zu et al., 2013; Li et al., 2011; Yang et al., 2007), besides the non-ideal EE, two major issues were prone to be ignored. One is batch feeding (organic solvent consumption) and the other is toxic reagent involved. Due to the low dissolubility in organic solvent, large amount will be used. For instance, one batch of 30 mg HCPT contented HCPT-HSA-NPs preparation needs about 100 mL dichloromethane/chloroform and 700 mL soybean oil by any two-phase method. The elimination of organic solvent will be much difficult. And widely application of dichloromethane/chloroform is somehow not advantageous.

Wang et al. (2013) have developed disulfide bond stabilized HSA-NPs without using toxic regents. The HSA-NPs showed the interesting phenomenon that they swell to about twice their size in low polarity aqueous solutions (*e.g.*, 10–50% ethanol solutions), which proved to be reversible. It was assumed that the hydrophobic association was weakened by the appearance of ethanol. However, their excellent work still faced the problems of uncontrollable NP sizes (110–220 nm) and drug loading problems due to the fact that premade unloaded NPs are insufficient for incubation (model A from M. Merodio's classification Merodio et al., 2001) or other conventional drug loading processes. Nevertheless, it is still a promising method that uses endogenous cross-linker instead of chemical regents to avoid high toxicity.

Considering the drawbacks of loading HCPT by the aforementioned methods, such as the unsatisfactory drug physicochemical properties to fabricate drug loaded HSA-NPs, entrapment efficiency and batch feeding (organic solvent consumption)as well as the use of toxic regents, we managed to develop a new approach to ameliorate. Chiefly, the scheme was inspired by taking note of the low molecular weight polyethylene glycol (l-PEG) could form a liquid compound with HCPT by ethanol distillation. And the compound was at a high content which possesses low polarity and is extremely unstable in water. Thus, a tiny volume of drug compound mixed with dry HSA-NPs could meet both the demands of an adequate drug amount and the volume capacity of the NP



Scheme 1. Title: Preparation flow chart.
Description: Step 1 and step 2 are the preparation of the liquid compound and the blank dry HSA-NPs, respectively. Then, these two substances are mixed sufficiently under dry conditions in step 3 and disperse into pure acidic water (pH 4) *via* a slight sonication treatment in step 4.