Hematoporphyrin encapsulated polymeric nanomicelles for photodynamically treatment of cancer

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ABSTRACT

Nowadays, developements in nanotechnology have leeds an increased application of this newborn technology in cancer treatment. In this study, a stabilized micelle was developed to load *HpD* in order to be used in photodynamic treatment of cancer in an animal model. To construct micelles, Pluronic P-105 was used and stabilized to encapsulate *HpD*. This process includes a 24 *h* polymerization in which the first 3.5 *h* was in the presence of N_2 purge and was continued at 65°*C*. In this reaction, *NNDEA* and benzoil peroxide (*BP*) were used for stabilization of micelles and initiation of reaction, respectively. DLS analysis of micelles revealed that the size of them before and after drug encapsulation was 14 *nm* and 23.5 *nm*, respectively. To assess drug loading, drug standard curve was obtained and its loading was obtained as 2 *mg/ml*. To extract free drug from complex, it was dialyzed against water and its stability profile was measured up to one month which was more than 80%. According to obtained results, this complex could be used to reduce side effects in photodynamic therapy. Besides, according to tumor characteristics and physical properties of micelles, it is possible to enhance drug release and uptake at tumor site.

Key Words: Nanomicelle; *HpD*; Drug Delivery; Photodynamic therapy

INTRODUCTION

Application of nanotechnology and nanoparticles in medicine is continuously being improved especially in cancer diagnosis and treatment [1-6]. Although significant advances in the definition of cancer, the problem of targeting cancer cells still exists. Nanotechnology has the capability to target cancer cells and reduce side effects to normal tissues [7-9]. Among several modalities in treatment of cancer, one with targeting potential is photodynamic therapy in which it is possible to enhance treatment efficiency using nanotechnology [10]. In this method, a photosensitizer drug is injected systemically or locally to patient and due to drug biokinetics and tumor physiology, drug is accumulated in tumor tissue and then tumor tissue is exposed to laser light which leads to production of cytotoxic agents and destruction of tumor tissue [11, 12] which is being used in a wide range of cancers [11, 13, 14]. The main problem in this method, is hydrophobicity of photosensitizer drugs which after injection and before reaching tumor tissue, disassemble and exits body via several pathways [12, 15, 16]; this causes a reducion in drug uptake by tumor and reduced treatment efficiency.

To overcome this problem and increase drug retention *in vivo*, one way is to use nanocarriers such as liposomes, micelles and polymeric nanoparticles [6, 17-22] which depend on drug type and its chemical properties [1, 2, 5, 23-26].
In this work, due to wide range of *HpD* applications in photodynamic therapy, *HpD* loaded nanomicelles were synthesized and their size and stability were assessed. This complex has the merit of its hydrophobic-hydrophilic structure which enables an enhanced circulation time [16, 18, 27, 28] and has the capability to be used in treatment of a wide range of cancers.

MATERIALS AND METHODS

Chemicals

Hematoporphyrin (*HpD*) was obtained from Merck (*Merck KGaA, Darmstadt, Germany*). Pluronic P-105 was provided by the BASF Corp.

(*Mount Olive, NJ, USA*). N-NDiethylacrylamide (*NNDEA*) was obtained from Polysciences (*Warrington, USA*). N,N'-Bis(acryloyl)cystamine (*BAC*) was obtained from Fluka (*Sigma–Aldrich, UK*), and benzoyl peroxide (*BP*) was obtained from Merck (*Merck KGaA, Darmstadt, Germany*).

Methods

Polymeric micelles were prepared using *Pluronic P-105*, which is a triblock copolymer consisting of blocks of poly propylene oxide (*PPO*) and poly ethylene oxide (*PEO*) in the form PEO_{37}–PPO_{56}–PEO_{37}. A solution of *NaCl* and 10 *wt.% Pluronic P-105* in distilled water was added to a round-bottom balloon, which was stirred for 20 *min* while immersed in a water bath under a nitrogen purge at a temperature of 65°*C*. A mixture of *BAC*, *BP* and *NNDEA* (26:1:55 weight ratio) was added to above solution and it was allowed to stir at this temperature under a nitrogen purge for 3.5 *h*. After turning off nitrogen flow, the mixture was allowed to polymerize for 19.5 *h*. The size distribution of the micelles was measured by dynamic light scattering (DLS) (*Malvern Instruments Ltd., Malvern, UK*).

To load *HpD* into nanomicelles, diluted solution of drug in distilled water was mixed with solution of stabilized nanomicelles and stirred gently to form a uniform solution. The resultant solution was dialyzed against water for 3 *h* (5 *kDa cut off*) to extract free drug from encapsulated drug solution. Finally, using flourimetry (*Jasco FP-6200, Tokyo, Japan*), a standard curve for encapsulated drug was ploted; to do so, excitation and emission wavelength were set at 320 *nm* and 480 *nm* respectively and by adding known amounts of drug to micellar solution, this standard curve was obtained; so it is possible to measure drug loading in nanomicelles (29). Size measurements of complex were similar to size measurements of nanomicelles. Finally, stability profile of complex was obtained for 30 *d* at 4 °*C*.

RESULTS

Figure 1 shows DLS results of nanomicelles. As it is observed, the mean diameter of nanomicelles is 14 *nm*. In this graph, vertical axis shows the intensity of scattered laser light and the horizontal axis shows size of nanomicelles and light intensity at each size is a measure of number of particles in that size. Figure 2, shows the fluorescence spectrum of *HpD*; to obtain this spectrum, flourimetric measurements were done from 300 *nm* to 600 *nm* which covers drug excitation wavelength (320 *nm*) and drug emission wavelength (480 *nm*).

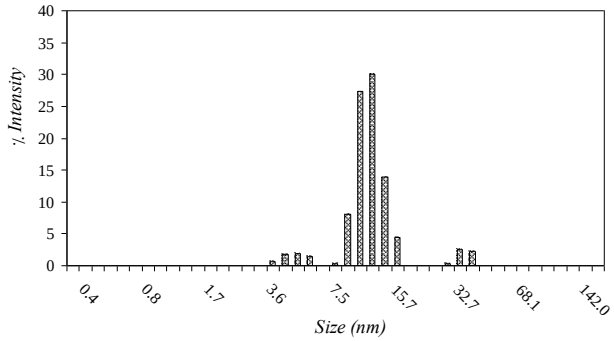

Figure 1. DLS results of micelles without drug in which mean diameter is 14 nm.

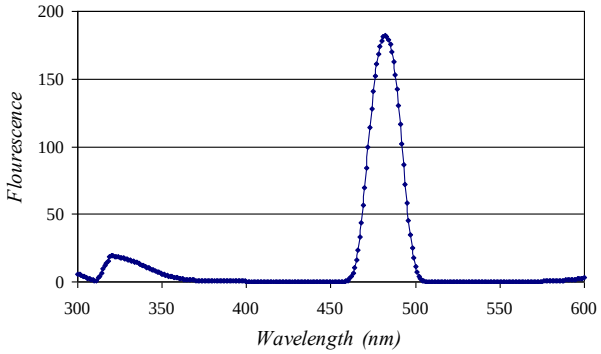

Figure 2. Fluorescent spectrum of Hematoporphyrin with its excitation and emission peaks.

According to these flourimetric characteristics of *HpD*, it is possible to draw a drug standard curve in nanomicelles as figure 3 and measure drug loading. In this graph, horizontal axis shows the amount of fluorescent emission of sample and vertical axis shows micellar drug concentration in *mg/ml* and linear regression equation of these data is presented on this graph. Using this standard curve, each time after complex synthesis, it is possible to find out drug concentration in nanomicelles from its fluorescent emission. As it is shown in this figure, maximum loading of drug in micelle is 2 *mg/ml*.

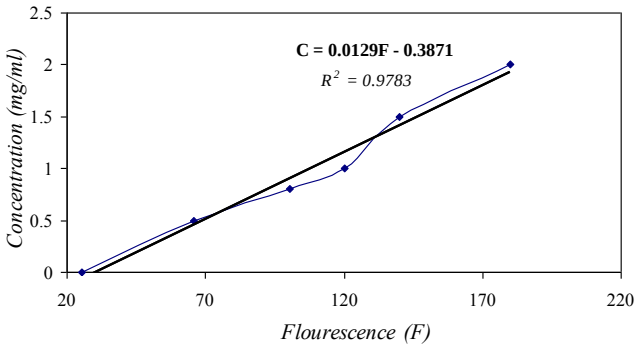

Figure 3. Standard curve for drug loading into micelles with its linear regression equation.

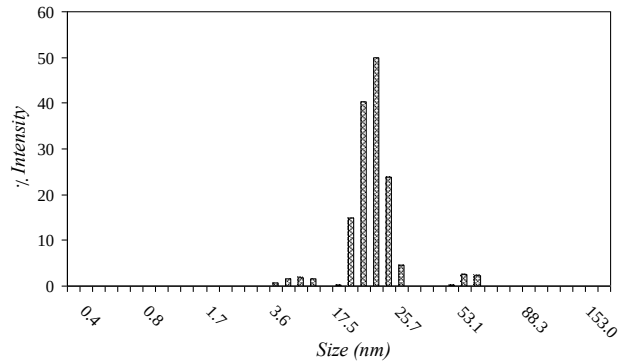

Figure 4. DLS results of complex in which mean diameter is at 23.5 nm.

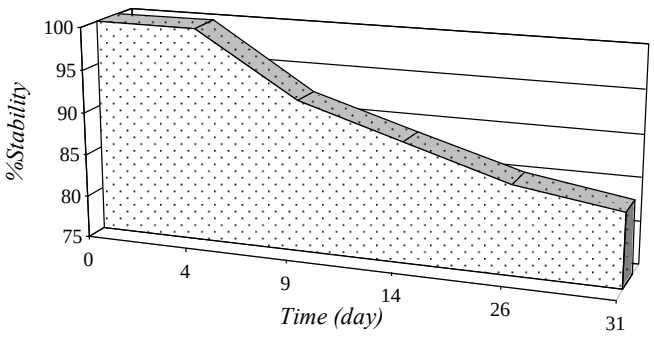

Figure 5. Stability profile of complex normalized to first day for one month.

After measurement of micellar drug loading, their size distribution was determined using DLS which results are presented in figure 4 and mean diameter of complex was obtained as 23.5 *nm*.Another important characteristic of complex is its stability versus time; to do so, fluorescent emission of drug was measured at different times from its synthesis up to one month at 4 °*C* and the amount of fluorescent emission was normalized to first day. Because drug release from complex leads to a decrease in fluorescent emission at the above mentioned emission wavelength, it is possible to use this parameter as a measure of complex stability which is shown in figure 5. In this figure, the percentage complex stability is shown on vertical axis and measurement time is presented on horizontal axis up to one month. As it is obvious from this graph, complex stability reaches a level of 80% after one month.

DISCUSSION

A polymeric drug carrier for *HpD* to be used in photodynamic therapy was presented. One of the merits of this family of polymeric drug carriers is their hydrophilic-hydrophobic structure which enables one to enhance delivery of hydrophobic drugs; besides their *PEO-PPO-PEO* structure, forms a self-assemble platform that collapses by sonication and releases their drug loading and makes them good candidates for treatment of a wide range of cancers [11-13, 30].

CONCLUSION

The presented *HpD* complex has the possibility of using in photodynamic therapy of several tumors such as GI tumors in experimental animal models. Besides, it is possible to use this complex in targeted therapy in which local sonication causes drug release in deeply sited tumors without any worry about side effects similar to ionizing radiation. Because of tumor vasculature, drug is accumulated in tumor passively and local sonication of tumor causes an enhanced drug uptake due to sonoporation.

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