Heparin - Pluronic F127 nanogel with optimal component ratio for drug delivery applications

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ABSTRACT

This study aimed to synthesize a nanogel system based on Heparin (Hep) and Pluronic F127 (F127) copolymers with optimal Hep:F127 ratio for delivering poor water soluble drug and Platinum-based drugs. Firstly, Hep-F127 conjugates were formed via disulfide bridges of cystamine molecules. The obtained Hep-F127 systems were then proved and characterized using Proton nuclear magnetic resonance (^{1}H-NMR), Dynamic Light Scattering (DLS)) methods. Cisplatin (CPT) was used as the Pt drug model, meanwhile Paclitaxel (PTX) was used as poor water-soluble drug model. The encapsulation ability for CPT and PTX was investigated and compared to choose the optimal Hep-F127 ratio. This Hep-F127 nanogel system is expected to be a good carrier for delivering drugs with different properties.

Keywords: Heparin; Nanogel; Platin drug; Pluronic F127; Poor water-soluble drug.

1. INTRODUCTION

The development of drug delivery technology has contributed to solving some main problems of chemotherapy in cancer treatment, such as side effects, low solubility, poor stability, poor distribution, and multidrug resistance [1]. Many drug delivery systems have been studied and developed, mainly nanoparticles [2]. These nanoparticles offer impressive advantages over free drugs, including encapsulation to prevent drugs exposure to off-targets and the ability to control drug release at the targeted sites. Depending on the properties of the drugs to be delivered, appropriate material components would be chosen and designed so that the obtained nanoparticles would have the highest encapsulation ability and best controlled drug release [3].

Pluronics F127, an amphiphilic tri-block copolymer have that was FDA-approved andhas been used for drug delivery applications [4]. Their molecules composed of a central hydrophobic poly(propylene oxide) (PPO) template flanked by hydrophilic poly(ethylene oxide) (PEO) chains. This structure, on the one hand makes pluronic good biocompatibility; on the other hand, it can improve the solubility of encapsulated poorly soluble drugs [5, 6]. Meanwhile, heparin, a biomolecule with masignificantrboxylic groups, has been reported to form pH-responsive complexes with platinum atoms of Platinum-based anticancer drugs [7]. These complexes make Pt-drug be released sustainably in physiological environment and rapidly in tumor microenvironment [8].

Considering the above characteristics of pluronic F127 and heparin, a drug delivery system based on these two components not only helps the system have good encapsulation for both poorly soluble drugs and Pt-based drugs, but also improve the biocompatibility and cellular uptake of the delivery system [9]. Besides, free heparin has been reported as a factor causing an increased bleeding risk. A drug delivery system based on the connection between F127 and heparin, therefore, is expected to avoid the bleeding risk caused by free heparin during circulation in the body [10].

In this study, nanogel systems were prepared from copolymers of Heparin and Pluronic F127 at different component ratios of Heprain and F127, from 1:1 to 1:20, for the delivery of poor water soluble drug and Platin drug. Of which, the Hep-F127 conjugates were formed via disulfide bridges of cystamine molecules and the obtained Hep-F127 systems were proved. The encapsulation ability for poor water soluble drug and Pt drugs was investigated and compared to choose the optimal Hep-F127 ratio. This Hep-F127 nanogel system is expected to be a promising carrier for the delivery of drugs with different properties.

2. MATERIALS AND METHODS

2.1. Materials

Heparin sodium (Hep) with MW of 12,000 - 15,000 Da, 3-amino-1-propanol (Ami), p-nitrophenyl chloroformate (NPC), 1-ethyl-3-3-dimethylaminopropyl carbodiimide (EDC), cystamine (Cys), Pluronic F127 (12400 Da) were purchased from Sigma-Aldrich. Cisplatin (CPT) and Paclitaxel (PTX) were purchased from Toronto Research Chemiscals INC. Solvents were obtained from Fisher. Deionized water (DIW) and distilled water (DW) were supplied by the Institute of Chemistry and Materials.

2.2. Methods

2.2.1. Preparation of Copolymer Heparin-F127 (CHF)

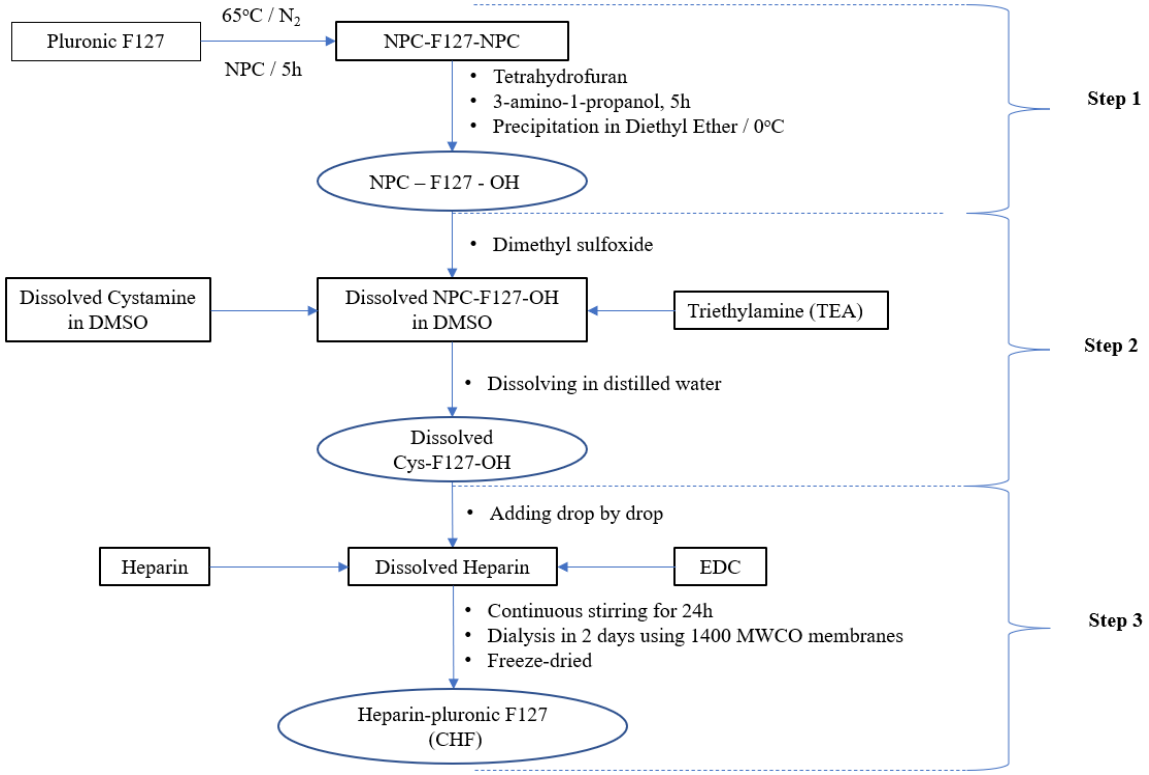

Figure 1. *Synthesis of heparin-F127 copolymer (CHF).*

Copolymer Heparin-F127 (CHF) was formed based on Hep-F127 conjugates via disulfide bridges of Cystamine molecules. The CHF synthesis was carried out through 3 steps as follows: Pluronics F127 was activated using NPC. After that, F127-Cys system was synthesized based on the urethane bonds created between NPC-F127-OH and Cystamine dihydrochloride. Finally, CHF was synthesized on the basis of amide linkage (-NHCO-) between Heparin and F127-Cys through the coupling agent EDC. Different amount of heparin with molar ratio to F127 of 1:1,

1:5, 1:10, 1:15 and 1:20 was dissolved in cold DIW in round flasks. Then, the same cold aqueous solution of F127-Cys was added dropwise into each flask. The reaction was maintained for 24 h at about 15 °C. Then, the mixture was dialyzed against DW for 5 days using cellulose membrane (MWCO 14 kDa) and lyophilized to obtain the Hep-F127 samples. The synthesis scheme of CHF system from H eparin and F127-Cys through coupling agent EDC is shown in figure 1.

2.2.2. Preparation of Nanogel Heparin-F127 (NHF)

The five CHF systems with different component ratios including 1:1, 1:5, 1:10, 1:15 and 1:20 were dissolved completely in 1 ml cold DIW (around 15 °C). Then, the temperature of the solutions was gradually increased and the optical conversion of the solution was recorded every 5 degrees. The temperature at which the solution changed from transparent to partially opaque was known as the nanogel formation temperature. NHFs were described through Zeta potential and DLS analysis.

2.2.3. Characterization

The formed conjugations of CHF systems were determined by Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR), Bruker.

The formation of nanogel was confirmed by Dynamic Light Scattering (DLS) Particle Analyzer meanwhile Zeta potential (ζ) was determined through a Helium-neon (He-Ne) laser beam with the setting detection angle of 90°, temperature of 25 °C and wavelength of 532 nm using a Zetasizer Nano SZ (SZ-100, Horiba, Kyoto, Japan).

2.2.4. Study of loading capacity and loading efficiency of NHF

The drug loading capacity (DLC) and drug loading efficiency (DLE) of NHF, Paclitaxel (PTX) and Cisplatin (CPT) were used as model drugs [11, 12].

Cisplatin was dissolved in DI, named solution C. Various component ratio NHF systems were dispersed in water-cooled at 4 °C, named solution A. C was slowly dripped into A with magnetic stirring for 24 h. The mixture was then dialyzed in water for 8 h to remove the unloaded drug and lyophilized to obtain the drug-encapsulating products CPT@NHF.

Paclitaxel was dissolved in MeOH, named solution P. Various component ration NHF systems were dispersed in water-cooled at 4 °C, named solution A. P was slowly dripped into A with magnetic stirring for 24 h. The mixture was then dialyzed in water for 8 h to remove the unloaded drug and lyophilized to obtain the products PTX@NHF.

The DLC and DLE of NHF were determined by quantifying unloaded drug. CPT was determined by UV-Vis using the o-phenylenediamine (OPDA) colorimetric assay [13, 14] while PTX was determined by high-performance liquid chromatography (HPLC) [15-18]. The DLE and DLC values of CPT@NHF and PTX@NHF were calculated using the following equations:

$$\text{DLE (\%)} = \frac{m_{\text{Loaded Drug}}}{m_{\text{Initial Drug}}} \times 100 \quad (1)$$

$$\text{DLC (\%)} = \frac{m_{\text{Loaded Drug}}}{m_{\text{Materials}} + m_{\text{Loaded Drug}}} \times 100 \quad (2)$$

Where: $m_{\text{Loaded Drug}}$ is $m_{\text{Loaded PTX}}$ or $m_{\text{Loaded CPT}}$; $m_{\text{Materials}}$ is the $m_{\text{CHF raitos}}$; $m_{\text{Initial Drug}}$ is the $m_{\text{Initial PTX}}$ or $m_{\text{Initial CPT}}$

3. RESULTS AND DISCUSSION

3.1. Synthesis of CHF

The successful synthesis of the copolymer Hep-F127 of different component ratios was

proved through ^{1}H-NMR, Zeta potential and DLS experiments.

The ^{1}H-NMR spectra of the five Heparin-F127 copolymers were showed in figure 2. The characteristic resonance peaks of F127 appeared at δH = 1.25 ppm (f) and δH = 3.77 ppm (c), corresponding to the resonance of the –CH_3 proton in PPO and the OCH_2–CH_2O– proton in PEO [19]. The signals of protons –O–CH–CH_2–O– at δH = 3.27 ppm (i) and δH = 2.1 ppm (j) were characteristic signals of heparin [20, 21]. Notably, the signals of protons –CH_2–NH–(C=O)– at chemical shift δH = 3.15 ppm (a) and protons –CH_2–S– at chemical shift δH = 2.97 ppm (b) proved that heparin and F127 were successfully conjugated via disulfide bridge of Cystamine molecules [22].

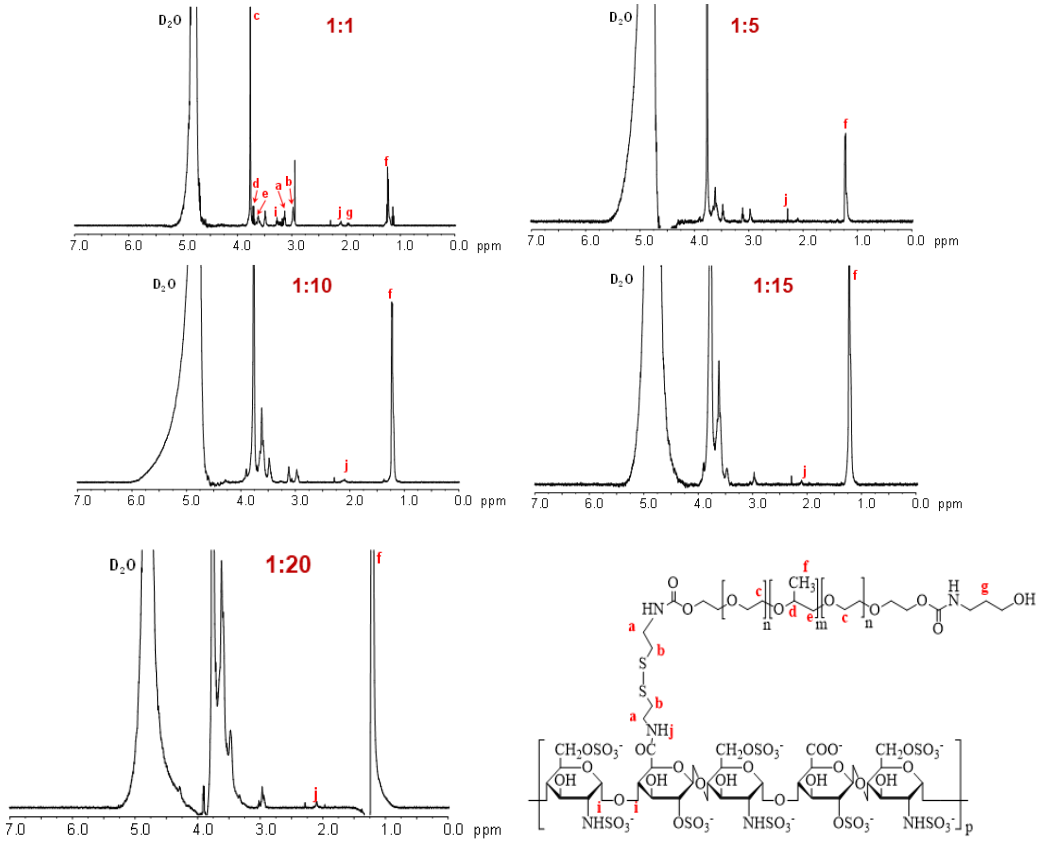

Figure 2. *^{1}H-NMR spectra of CHF Hep-F127 at five ratios of Hep:F127.*

To confirm the successful synthesis of CHF Hep-F127, zeta potential and DLS size of the nanogel systems formed from CHF Hep-F127 (NHF) at different component ratios were determined and compared to those of F127.

It could be seen from figure 3 that zeta potential of F127 micelles approximated zero meanwhile those of NHF systems were negative. The zeta potential of NHF (1:1) was the most negative with the value of -16.26 mV. When the F127 proportion in the CHF formula increased, the zeta potential of corresponding NHF increased and that of NHF (1:20) reached the maximum value at -9.66 mV. This proved that the presence of F127 in the CHF copolymer system reduces the negative zeta potential of heparin. In addition, it was possible to predict that the composition of F127 in CHFs increased gradually with the F127 ratio in the synthesized formula.

The DLS size of F127 micelles and NHF nanogel at different Hep:F127 ratios were determined and presented in figure 4. It could be seen that the size of the NHF nanogel increased with the increase of the F127 proportion. As the F127 proportion increased, the amount of F127

in the CHF copolymer increased, leading to an increase in the size of NHF nanogel. This result was completely consistent with the zeta potential results.

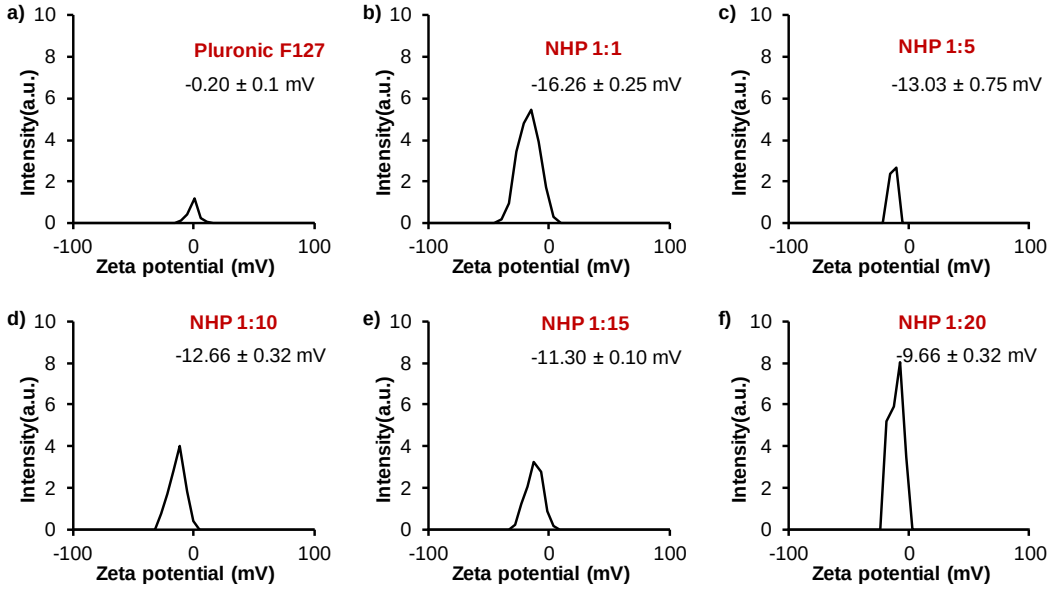

Figure 3. *Zeta potential of NHF Hep-F127 at different component ratios.*

In terms of the size of a nanomaterial in drug delivery application, NHF (1:1) and NHF (1:5) nanogels can be considered as potential carriers, as their size are close to the optimal value of 100 nm. This optimal size was reported to be suitable for carrying a sufficient amount of drug, high absorption capacity, as well as avoiding clearance during administration [23-24]. In terms of the ability to encapsulate drugs, the ratio of the two components Heparin and F127 in the NHF nanogel play important roles. Therefore, NHF nanogels with different component ratios need to be evaluated for drug loading capacity and drug loading efficiency in order to select appropriate Hep:F127 ratios.

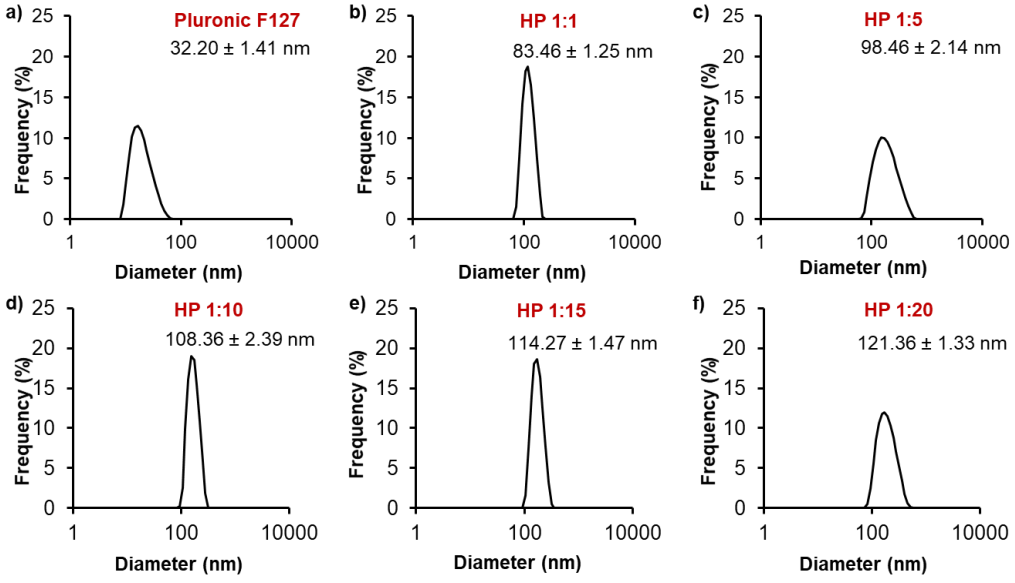

Figure 4. *DLS diameter of CHF Hep-F127 at different component ratios.*

3.2. Drug loading capacity and loading efficiency of NHF

The DLC and DLE of NHF systems for CPT and PTX are presented in figure 6. The results showed that when the Hep:F127 ratios increased from 1:1 to 1:20, the encapsulation ability of NHF gradually decreased for CPT and slightly increased for PTX. In detail, DLC and DLE for CPT reached the highest values at the Hep:F127 ratio of 1:1 (16.2% and 85.2%, respectively), meanwhile those for PTX increased when the Hep:F127 ratios changed from 1:1 to 1:5 and remained stable for the other Hep:F127 ratios (about 18% and 92%, respectively). This was due to the encapsulation of CPT through the complexation with heparin molecules in NHF system. Therefore, the higher the proportion of heparin in NHF system, the better the CPT encapsulation ability of the NHF system. Furthermore, PTX is a poorly soluble drug that can be well encapsulated in the PPO blocks of the F127 molecules. Consequently, the higher the proportion of F127 in the NHF system, the better its ability to encapsulate PTX. However, there was no significant difference among the NHF samples from 1:5 to 1:20. This might be due to the stereospecific interference effect which lead PTX being mainly encapsulated into the PPO units of the outer F127 molecules of the NHF systems.

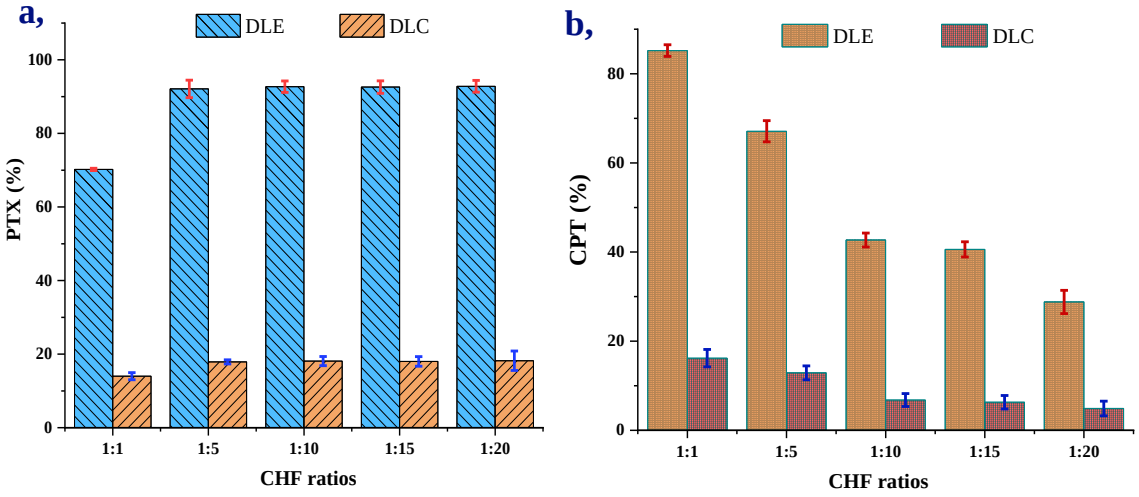

Figure 5. *Loading capacity and loading efficiency for a) PTX@NHF, b) CPT@NHF.*

From the experimental results to determine the drug encapsulation ability, together with the DLS size results, it could be seen that NHF (1:1) and NHF (1:5) were the two best drug-carrying systems for CPT and PTX, respectively. Depending on the properties of the desired encapsulated drug, the ratio 1:1 or 1:5 could be selected. Moreover, these results make a very important meaning for studies that develop a dual drug encapsulation system consisting of one poorly soluble drug and one Pt drug.

4. CONCLUSIONS

Nanogel Hep-F127 system with five component ratios was investigated to define the optimal formula to deliver drugs with different properties. The five NHF systems, which were proved through ^{1}H-NMR, Zeta potential, DLS, have monodisperse spherical shapes with DLS diameters range from 83 to 121 nm. The proportion of F127 in the obtained NHF systems increased when the F127 ratio in the Hep-F127 synthesis formula increased. The Hep:F127 optimal ratio was defined as 1:1 for CPT delivery with DLE and DLC were 16.2% and 85.2%, respectively. Meanwhile, that was defined as 1:5 for PTX delivery with DLE and DLC were 92% and 18%, respectively. These findings suggested that NHF (1:1) and (1:5) would be potential nanocarriers for effective delivery of poor water-soluble drug and Pt drug in cancer treatment. Moreover, dual drug encapsulation and drug release profile of these optimal NHF should be further investigated.

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TÓM TẮT

Heparin - Pluronic F127 Nanogel với tỷ lệ thành phần tối ưu cho các ứng dụng mang thuốc

Nghiên cứu tổng hợp copolyme trên cơ sở Heparin (Hep) và Pluronic F127 (F127) với tỷ lệ Hep:F127 tối ưu để nang hoá hai hệ thuốc Paclitaxel và cisplatin. Sự tạo thành copolyme của liên hợp Hep-F127 được hình thành thông qua các cầu nối disulfide của các phân tử cystamine. Các hệ Hep-F127 thu được đã được nghiên cứu đặc trưng cấu trúc bằng phương pháp cộng hưởng từ hạt nhân Proton (1H-NMR), thế Zeta và tán xạ ánh sáng động (DLS). Dược chất Cisplatin (CPT) được sử dụng cho mô hình nang hoá dược chất tan trong nước, trong khi Paclitaxel (PTX) được sử dụng cho mô hình nang hoá thuốc kém tan trong nước. Khả năng nang hoá CPT và PTX của hệ nanogel đã được khảo sát để lựa chọn tỷ lệ Hep-F127 tối ưu trong từng mô hình.

Từ khóa: Nanogel; Cisplatin; Pluronic F127; Paclitaxel.