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Novel amphiphilic heparin-pluronic P123 copolymers exhibiting a great potential for Cisplatin delivery

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ABSTRACT

In the study, we introduced a series of amphiphilic pluronic P123-conjugated heparin (hep-P123) copolymers which were characterized for drug delivery. In regard to the research, various grafted hep-P123 copolymers investigated its potential in the delivery of hydrophobic Cisplatin (Cis) anticancer drugs and its aquated species (CisOH) via hydrophobic interaction and complex formation, respectively. Hep-P123 was obtained via conjugation of the partially NPC-activated pluronic P123 (NPC-P123-OH) onto the aminated heparin (hep-DAB). The obtained copolymers were characterized by ^{1}H-NMR and thermal gravimetric analysis. The effect of P123 conjugation degree on size distribution of nanocarriers was evaluated by transmission electron microscopy (TEM) and dynamic light scattering (DLS). The results showed that size distribution of hep-P123 nanogels range from 62.2 ± 19.4 nm to 114.5 ± 21.7 nm by TEM and 94.4–182.4 nm by DLS. Complex formation of the CisOH was clarified by FT-IR. Loading efficiency of Cis/CisOH was evaluated by inductively coupled plasma atomic emission spectroscopy that indicated a relation between the conjugated degree of hep-P123 and amount of loaded drugs. In addition, hep-P123 exhibited a higher CisOH binding efficiency via complexation as compared to the loaded Cis by hydrophobic interaction. The CisOH and hep-P123 nanocomplex performed a high activity against NCI-H460 cancer cell growth. The obtained results offered an appropriate selection of the hep-P123 platform for dual drugs delivery of CisOH and other hydrophobic anticancer drugs which could utilize both hydrophobic interaction and complex formation.

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Introduction

Functionalization of natural polymers has recently been evolved in the biomedical field encompassing drug delivery and tissue-engineered scaffolds due to their biodegradability, biocompatibility and biological activities. Polysaccharides possess the typically chemical structure exhibiting a specific interaction with bioactive molecules that have exploited to modify with various polymers for producing several kinds of multifunctional copolymers. The grafted platforms performed a strikingly functional improvement as compared to original materials and exhibited aholistic approach for biomedical applications [1–4]. Recently, there has been some successful proof of the amphiphilic grafted platforms in drug delivery such as ThermoDox®, BIND-014, Cynviloq IG-001, Genexol-PM, etc., which have expired for extensively studying on the amphiphilic copolymers and various polysaccharides [5]. There have been other reports to confirm the effectiveness of the grafted copolymers-based drug delivery systems with dual hydrophobic and hydrophilic drugs or bioactive molecules [5–8]. The hydrophilic surface of nanocarriers could prolong the circulation time of drug delivery system along with expediting passive accumulation at tumor at via the EPR effect or directly target to the site of action resulted in enhancing drug bioavailability.

Heparin, an extract of mucosal porcine tissues, is the highest anionic-charged glycosaminoglycan among the known polysaccharides owning to dominantly possessing sulfonate and carboxylic groups. The polysaccharide possesses a broad spectrum of applications such as anticoagulation, anticancer activity, chemical affinity with various proteins (growth factors) for controlled delivery systems, etc. [9–12]. Regarding the beneficial characteristic, various heparin-based derivatives or platforms have been applied in fabrication of biomedical and artificial blood-contact devices [13–15]. In recent years, amphiphilic heparin-based copolymers have also paid much attention in drug delivery and biomedical fields due to their multifunctional performance. Sim and coworkers synthesized poly(ε-caprolactone-co-lactide)-b-poly(ethylene glycol)-b-poly(ε-caprolactone-co-lactide)-grafted heparin for controlled delivery of lysosome via ionic interactions [16]. Pluronic F127-heparin derivative effectively delivered acid fibroblast growth factor (aFCF) and protected its denature in physiological ambient [17]. An amphiphilic folate–poly(ethyleneglycol)-conjugated heparin–poly(β-benzyl-L-aspartate) copolymer was developed for targeted delivery of doxorubicin (DOX) [18]. Remarkably, the graft of thermosensitive polymer segments into heparin backbone produced platforms for the dual delivery of hydrophobic drugs and positive-charged bioactive molecules. In the copolymer solution, heparin backbone performs the electrostatic interaction with the positive-charged bioactive molecules (growth factors, genes and hydrophilic drugs) and an exclusive heat-triggered self-assembly leads to form core–shell polymeric platform which contemporaneously loaded hydrophobic drugs at the hydrophobic domain and the bioactive molecules. Choi et al. reported an amphiphilic pluronic F127-conjugated heparin copolymer as a highly efficient platform for delivering both hydrophobic indomethacin and positive-charged proteins [10]. The amphiphilic copolymer has also been well-performed in delivery of dual hydrophobic and hydrophilic drugs via hydrophobic interaction and complexation between aquated Cisplatin with hydrophilic functional groups (carboxylate and sulfate) [2, 7, 19]. In addition, our previous study indicated that a highly lipophilic pluronics-conjugated dendrimer exhibits a better efficiency in delivering hydrophobic anticancer drugs. The drug loading efficiency of the investigated pluronic increased when the copolymer owns a low hydrophilic-hydrophobic balance [20].

In the study, we introduced a series of amphiphilic pluronic P123-conjugated heparin copolymers with various grafted degrees of pluronic P123 that has not been previously reported yet. The copolymer was characterized toward delivery of the CisOH via complexation phenomenon and the Cis in hydrophobic interaction. The study also investigated cytotoxic activity against lung cancer cell growth.

Materials and methods

Materials

Low molecular weight heparin sodium (hep, 12,000 to 15,000 daltons), 3-amino-1-propanol (Ami), silver nitrate ($AgNO_3$), 1,4-diaminobutane (DAB), *p*-nitrophenyl chloroformate (NPC), 1-ethyl-3-3-dimethylaminopropyl

carbodiimide (EDC), N-hydroxysuccinimide (NHS) were supplied by Acros Organics. Pluronic P123(5.800 MW) and Cisplatin were purchased from Sigma-Aldrich. Solvents were obtained from Scharlau's Chemicals. Cellulose dialysis membranes (MWCO 14 kDa and MWCO 3.5 kDa cutoff) were bought from Spectrum Laboratories (USA).

Synthesis of P123-conjugated heparin copolymers

Four different grafted hep-P123 copolymers were prepared with a conjugated degree of pluronic P123 via conjugation of the partially NPC-activated pluronic P123 (NPC-P123-OH) into the aminated heparin (hep-DAB) as demonstrated in Fig. 1. Synthetic processes of polymers are presented as below.

Synthesis of NPC-P123-OH

In a round flask, P123 (5.2 mmol OH) was stirred in vacuum for 30 min at 65 °C to completely remove moisture. NPC (15 mmol) was added and stirred in 6 h. After that, the mixture was stopped heating and dissolved in 20 ml of chloroform. The solution was precipitated in diethyl ether/hexane mixture (1:1 v/v). The process was repeated twice. The paste precipitant was removed solvent in a vacuum oven to obtain NPC-P123-NPC. The copolymer was characterized with ^{1}H-NMR on Bruker AC MHz spectrometer (USA).

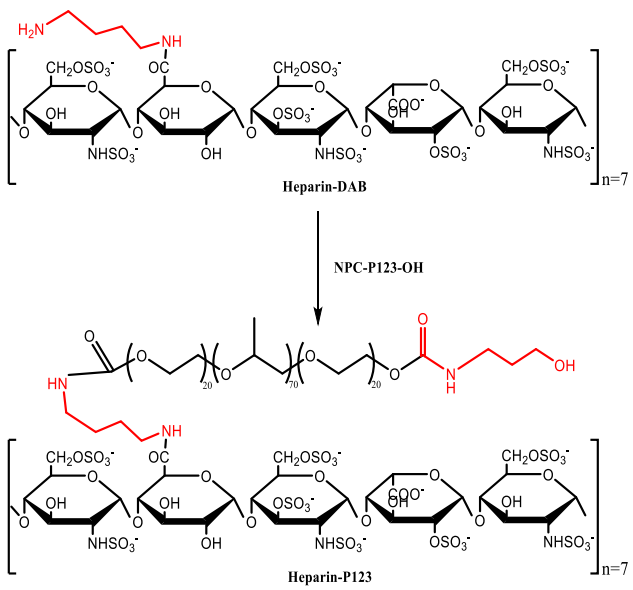

Figure 1 Preparation hep-P123 copolymers.

NPC-P123-NPC (2 mmol) was dissolved completely in chloroform (50 ml). Then, solution of Ami (1.1 mmol) in chloroform (50 ml) was added dropwise into the NPC-P123-NPC solution and stirred for 12 h at room temperature. The solution was concentrated to 20 ml and precipitated in diethyl ether/hexane mixture (1:1 v/v) for two times. The solvent was removed from the paste precipitant by a vacuum oven to obtain NPC-P123-OH for characterizing by ^{1}H-NMR on Bruker AC MHz spectrometer (USA).

Synthesis hep-DAB

The aminated heparin (hep-DAB) derivatives were prepared from heparin and DAB at different ratio of reactants (hep and DAB = 1:3; 1:7; 1:10; 1:14 mmol/mmol) using carbodiimide coupling reagent.

Synthesis of hep-P123 copolymers

In the round flasks, hep-DAB (hep and DAB = 1:3; 1:7; 1:10; 1:14 mmol/mmol) was dissolved in deionized water (DI). Then, every cold aqueous solution of different NPC-P123-OH amount was added dropwise into hep-DAB solutions. The reaction is maintained for 24 h below 20 °C. Then, the mixture was dialyzed against distilled water for 5 days using cellulose membrane (MWCO 14 kDa) and lyophilized to obtain the hep-P123 with the differently conjugated pluronic P123. For the synthetic process, grafted degree of the fed P123 was, respectively, 42.29wt/wt % (hep-P123 = 1:14), 51.33wt/wt % (hep-P123 = 1:10), 65.30 wt/wt% (hep-P123 = 1:7) and 89.68 wt/wt% (hep-P123 = 1:3). The copolymers were characterized by Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) and thermal gravimetric analysis (TGA). The TGA measurement was performed on a TGA Mettler-Toledo at a heating rate of 10 °C/min from room temperature to 800 °C.

Preparation and characterization of hep-P123 nanogels

The hep-P123 graft copolymers (20 mg) were dissolved completely in 1 ml cold DI water (around 15 °C). Then, the temperature of copolymer solutions was gradually increased to 20, 25, 30 and 35 °C to observe an optical transition from transparent

solution into the partially opaque solution that indicated a range of nanogel-formed temperature. The formation of hep-P123 nanogels was confirmed by TEM and DLS.

Preparation of hep-P123 nanogels loading Cis and its aquated form (CisOH)

In this study, we investigated Cis and CisOH loading yields of four kinds of nanogels which were prepared from four grafted hep-P123 copolymers. Amounts of fed platinum compounds were estimated regarding the remaining carboxylate and sulfate groups of hep-P123. Silver nitrate salt was utilized to produce CisOH from Cis [19, 21–24].

Preparation of CisOH

Cisplatin was dissolved in distilled water. After, $AgNO_3$ (molar ratio of Cisplatin: $AgNO_3$ = 1:2) was added. The reaction mixture was stirred in 48 h at room temperature, N_2 gas environment. The mixture was centrifuged at 3500 rpm for 10 min to remove the AgCl precipitate.

Encapsulation (complexation) of Cis (CisOH) and Hep-P123 copolymers

In a 3-neck flask, hep-P123 (500 mg) was dissolved in 50 ml cold DI water (20 °C, cover the aluminum foil to avoid light contact). Then, CisOH or Cis was added dropwise into hep-P123 solution and stirred for 24 h in nitrogen gas. The mixture was dialyzed against DI water for 20 min at 37 °C (for two times) using dialysis membrane (3500 MWCO) and then freeze-dried to obtain complex of Cis (CisOH) and hep-P123 copolymers that characterized with FT-IR, TEM and DLS. Regarding the remaining carboxylate and sulfate groups of the hep-P123, formulations of Cis (CisOH) and the grafted copolymers are shown in Table 1.

Platinum release study and cytotoxicity assay of hep-P123-CisOH complex

Platinum release study

Hep-P123-CisOH samples and control sample (CisOH) were separately dissolved in 2 ml DI water. Then, the solutions were added into every dialysis bag (MWCO 3.5 kDa) and dialyzed against 10 ml PBS buffer at pH 7.4 or 5.5, respectively. Dialyzed solutions (1 ml) were withdrawn after defined time intervals to measure platinum content by inductively coupled plasma atomic emission spectroscopy (ICP-AES, association of analytical communities) (AOAC). At the same time, 1 ml PBS buffer was supplemented into dialyzed solution for equal to the initial volume.

Cytotoxicity assay

Sulforhodamine B (SRB) colorimetric assay was used to evaluate cytotoxic behavior of the platinum samples again NCl-H460 human lung cancer cell growth. First, Cis and Cis(OH)-complexed hep-P123 nanogels were evaluated at the screening concentration and studied further on inhibitory percent of various platinum concentrations on cancer cell growth.

Results and discussion

Characterizations of the activated P123 and hep-P123 copolymers

In ^{1}H-NMR spectrum of NPC-P123-NPC, the resonance peaks appeared at δ = 7.40 ppm (c) and δ = 8.29 ppm (d) demonstrating aromatic protons of NPC as seen in Fig. 2. The typical $-CH_3$ protons of PPO and $-CH_2$ of PEO blocks appeared at δ = 1.23 ppm (a) and 3.82 ppm (b), respectively. Appearance of the peak at δ = 4.45 ppm (f) assessed the formation of NPC-CH_2 moieties in the activated

Table 1 Formulations of Cis (CisOH) and the hep-P123 copolymers

Cis (mmol)	$AgNO_3$ (mmol)	Cis (CisOH)-loaded hep-P123 formulations
0.44	0.88	hep-P123(1:3)- Cis; hep-P123(1:3)- CisOH
0.23	0.46	hep-P123(1:7)- Cis; hep-P123(1:7)- CisOH
0.16	0.32	hep-P123(1:10)- Cis; hep-P123(1:10)- CisOH
0.12	0.24	hep-P123(1:14)-Cis; hep-P123(1:14)-CisOH

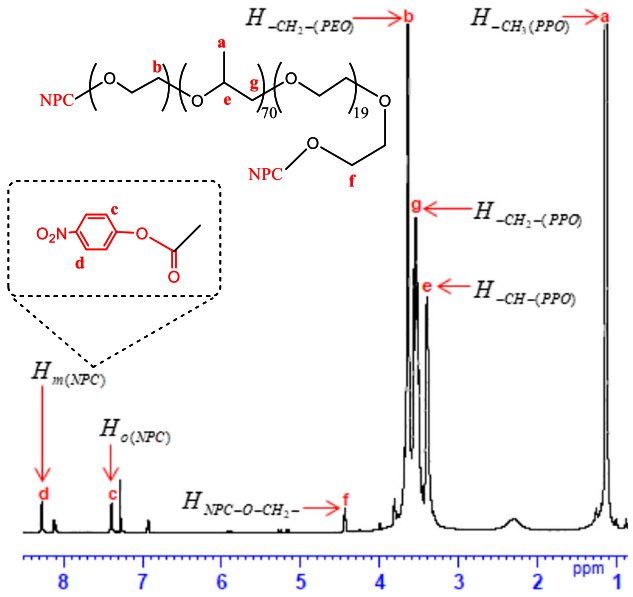

Figure 2 ^{1}H-NMR spectrum of NPC-P123-NPC.

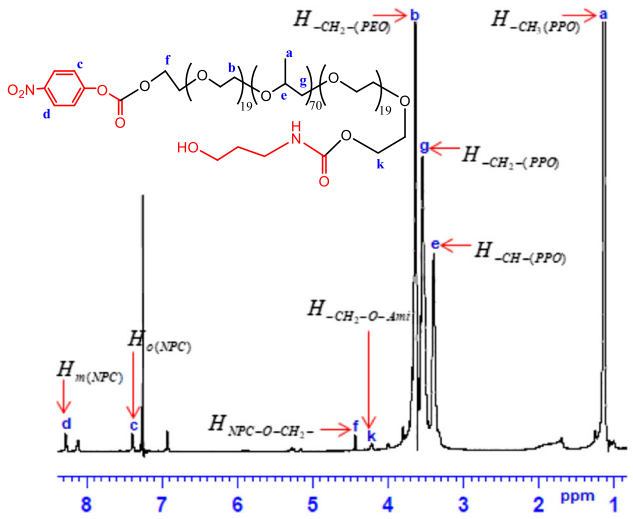

Figure 3 ^{1}H-NMR spectrum of NPC-P123-OH.

P123. Activation degree of P123 was calculated up to 90% based on the formulae in previous study [25].

Figure 3 shows a new peak (*k*) at δ = 4.22 ppm corresponding to methylene group that bound to Ami moiety. The appearance accompanied by a reduction peak area of signal at δ = 4.45 ppm. The result confirmed that NPC-P123-OH synthesized successfully.

The ^{1}H-NMR spectrum of hep-P123 copolymer is shown in Fig. 4. Some typical signals of pluronic P123 appeared at δ = 1.09 ppm and δ = 3.67 ppm corresponding to -CH_3 of PPO and –CH_2 of PEO, respectively. Conjugation of hep-DAB and NPC-P123-OH

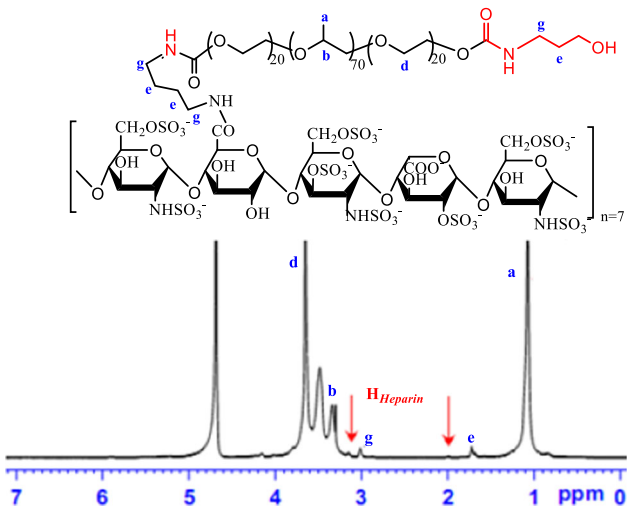

Figure 4 ^{1}H-NMR spectrum of hep-P123 copolymer.

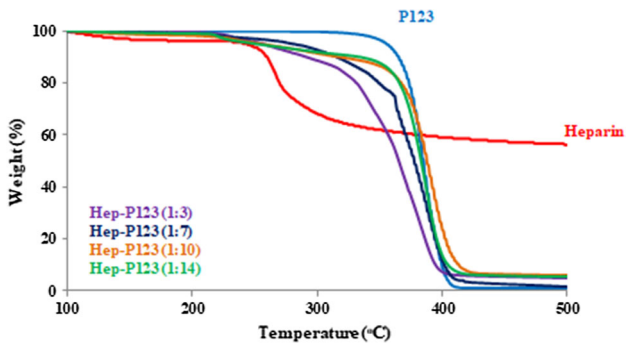

Figure 5 Thermal gravimetric analysis (TGA) of Pluronic P123, heparin and its grafted copolymers.

was determined via new signals of methylene protons (DAB) at δ = 1.74 ppm and δ = 3.03 ppm. In addition, signals of protons in heparin chain appeared at δ = 1.98 ppm and 3.22 ppm.

The thermal stability of the hep-P123 grafted copolymer together with heparin and pluronic P123 was analyzed by TGA (thermal gravimetric analysis). Figure 5 shows that the pluronic P123 stabilized up to 320 °C and began to decompose at 350 °C, followed by total mass loss above 420 °C. Heparin was stable up to 220 °C and decomposed rapidly at 260 °C, and the decomposition progresses happened slowly up to 500 °C. The hep-P123 copolymers were decomposed most of the weight from 250 to 420 °C. However, there are small amounts of decomposition at temperatures over 420 °C, whereas pluronic P123 has been completely decomposed. This demonstrated the presence of heparin in these graft copolymers.

Preparation and characterizations of the hep-P123 nanogels

In recent years, preparation of amphiphilic molecules or copolymers-based nanogels has been extensively studied for drugs delivery systems. Among the developed platforms, a popular approach is conjugation of amphiphilic copolymers onto hydrophilic polymers [26]. Pluronic F127 (a PEO-PPO-PEO triblock copolymer) also exploited to prepare several kinds of copolymer-based nanogels for drug delivery systems. Pluronic P123 exhibits a significantly higher hydrophobicity as compared to pluronic F127. The copolymer is water insoluble above 15 °C. Below the temperature, it is water soluble resulting in micelle formation. In opposite to pluronic P123, the grafted hep-P123 copolymer solutions could form stable micelles above 25 °C depending on amount of the grafted pluronic P123. Its nanogel formation made copolymer solution undergo from transference to opaque as seen in Fig. 6. Morphology and size distribution of the nanogels were observed by TEM and DLS.

The morphology of the hep-P123 nanogels observed by TEM exhibited as spherical nanoparticles ranges from 62.2 ± 19.4 nm to 114.5 ± 21.7 nm in diameter. Their size distribution ranged from 94.4 to 182.4 nm by DLS at 25 °C which depends on amount of the conjugated pluronic P123 as seen in Fig. 7 and Table 2. These evidences confirm the temperature-induced nanogel formation of the amphiphilic hep-P123 copolymers via hydrophobic interaction of pluronic P123 moieties. These results also exhibited that an increment in amount of the grafted pluronic P123 resulted in increasing a particle size of the hep-P123 nanogels. The results partially differ from some amphiphilic steroid-grafted polysaccharides in which their particle size of the nanogels slightly reduced as amount of the hydrophobic steroid-grafted moieties that was explained by the formation of dense nanoparticles increased [26, 27]. In hep-P123 copolymer structure, the hydrophilic PEO domains of the amphiphilic PEO-PPO-PEO copolymer are bulky that leads to increase a particle size of the hep-P123 nanogels as the grafted degree of the amphiphilic copolymer increased.

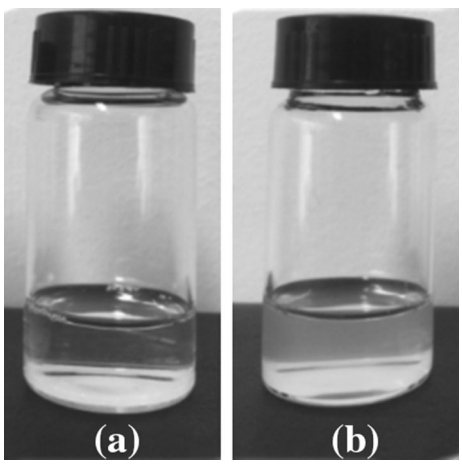

Figure 6 Photograph images of hep-P123 (1:3) copolymer at 20 °C (**a**) and 25 °C (**b**).

Characterizations of the platinum hep-P123 nanogels

The Cis and CisOH-loaded hep-P123 nanogels were characterized by FT-IR and ICP-AES. Figure 8 shows FT-IR spectra of hep-P123-CisOH and Cis-loaded hep-P123 nanoparticles in which a broad peak at 3295.73 cm^{-1} (A) was stretched of N–H bond corresponding to IR absorption frequencies of amine groups in Cis [28]. For hep-P123-CisOH, it is very interesting that new absorption band appeared at 1711.79 cm^{-1} (B) indicated complexation of CisOH and carboxylate groups of heparin leading to a shift the infrared absorption of C=O in carboxylate group from 1632.05 cm^{-1} up to 1711.79 cm^{-1} as seen in Fig. 8. In addition, two new infrared absorption bands of hep-P123-CisOH spectra (at 1297.89 cm^{-1} (C) and 846,03 cm^{-1} (D)) differed from two assigned absorption bands to axial deformation of S=O bonds of hep-P123 spectra that a complexation of sulfate and sulfonate moieties (on heparin) with CisOH resulted in a change in the IR absorption frequencies of the groups. The phenomenon could be observed on FT-IR spectra of the complexed heparin [19, 29]. The obtained results significantly confirmed a complex formation of anionic groups on heparin with aquated species of Cis.

Platinum loading efficiency was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). In the study, a priority of various hep-P123-based nanogels complex or load CisOH and Cis were investigated, respectively. A greater amount of the grafted pluronic P123 increased hydrophobicity of heparin-P123 resulting in an increment in the Cis loading efficiency and a

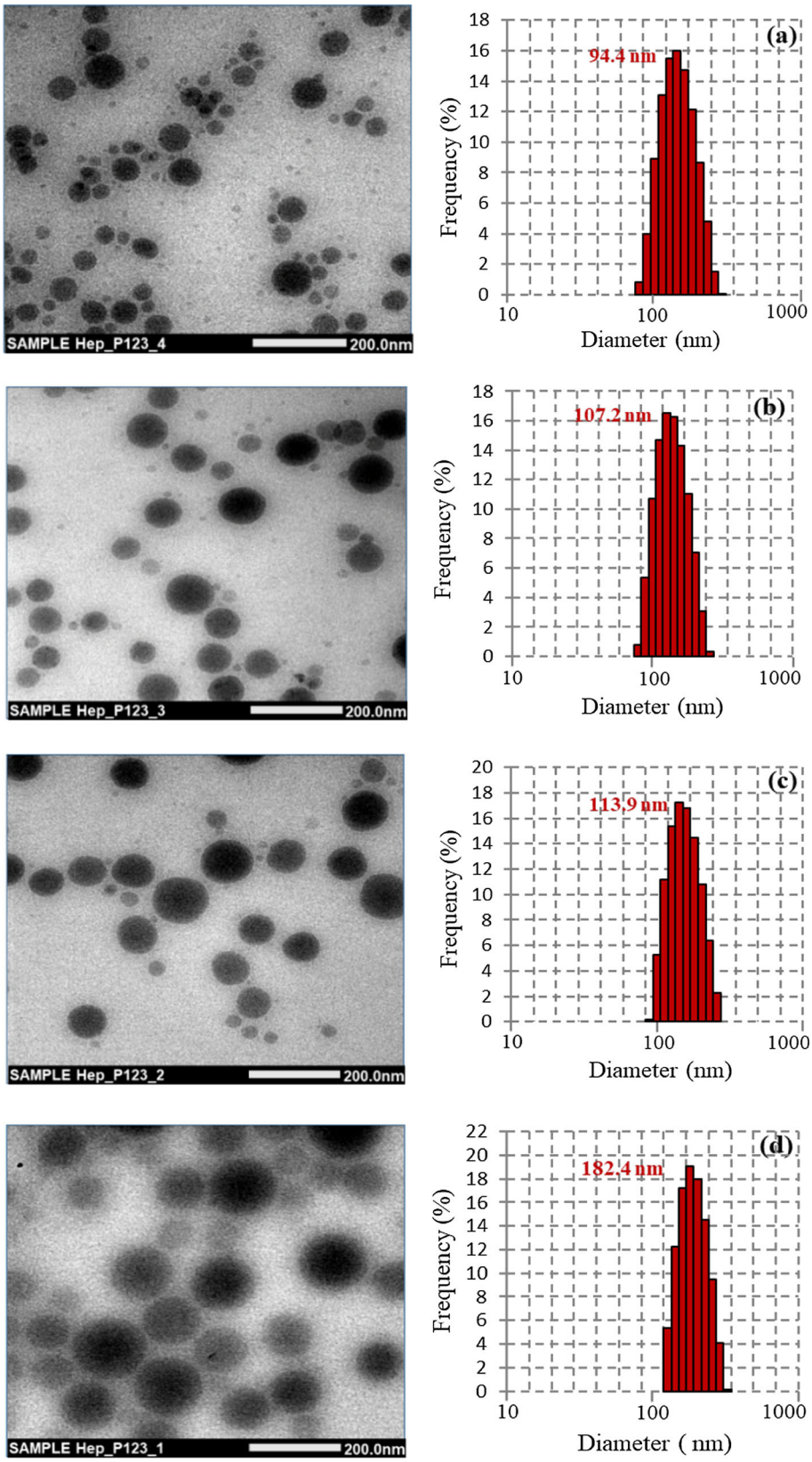

Figure 7 Morphology and size distribution of hep-P123 nanogels by TEM (right images) and DLS (left images): **a** hep-P123 (1:3); **b** hep-P123 (1:7); **c** hep-P123 (1:10); **d** hep-P123 (1:14).

Table 2 Size distribution (DLS) of the hep-P123 nanogels

Grafted copolymers	DLS (nm)	TEM (nm)
hep-P123 (1:3)	94.4	62.2 ± 19.4
hep-P123 (1:7)	107.2	87.3 ± 15.1
hep-P123 (1:10)	113.9	104.0 ± 15.5
hep-P123 (1:14)	182.4	114.5 ± 21.7

decrement in complexation with CisOH. Results shown in Table 3 clarified relation of every hep-P123 copolymer with platinum loading efficiency. In general, percent of Cis loaded into hep-P123 nanogels via hydrophobic interaction increased in the higher hydrophobic heparin-P123. The result agreed with our previous study in which a highly lipophilic pluronics-conjugated dendrimer exhibits a better loading efficiency of the hydrophobic anticancer drug. However, there is slightly difference in Cis loading efficiency of hep-P123 (1:3) and hep-P123 (1:7) nanogels. This could be explained by a portion of Cis hydrolyzed to form CisOH in the Cis loading process [30]. The aquated species complexed with abundancy of the anionic groups in the hep-P123 resulted in a slightly higher Cis loading efficiency as compared to hep-P123 (1:7) nanogels. Opposite to the above results, CisOH loading efficiency of the hep-P123 nanogels decreased as the grafted degree of pluronic P123 increased. The result was agreed with some previous studies that a higher amount of anionic groups could induce an increment of platinum loading content in nanocarriers [19, 21, 22]. This is easily seen in a demonstrated structure of hep-P123 containing abundancy of anionic groups (Fig. 9).

Regarding to the evaluation, it could be concluded that use of CisOH was a higher loading efficiency in the hep-P123 copolymers than that of Cis and the highest value was seen at hep-P123 (1:3)-based nanogel. The CisOH-complexed hep-P123 nanogels did not show a significant change in particle size as shown in Fig. 10. The size distribution of four CisOH-

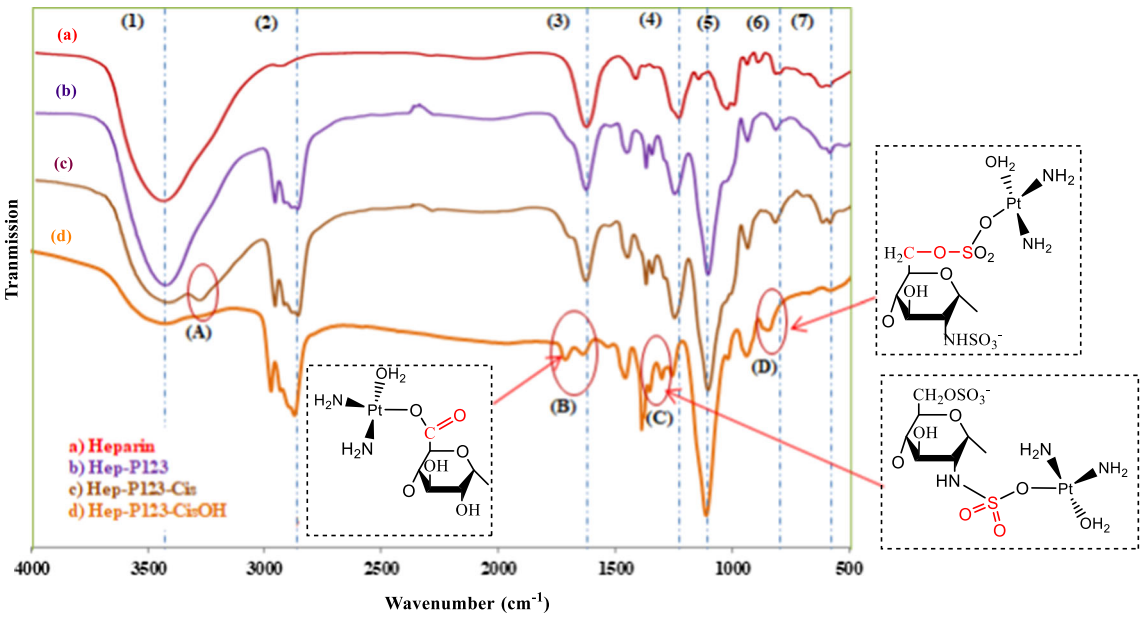

Figure 8 The FT-IR spectrum of hep-P123-Cis and hep-P123-CisOH.

Table 3 Cis and CisOH loading efficiencies on Hep-P123 nanocarriers

Grafted copolymers	Cis		CisOH	
	% Pt (w/w)	% Cis (w/w)	% Pt (w/w)	% Cis (w/w)
hep-P123 (1:3)	5.80	8.92	19.70	30.3
hep-P123 (1:7)	3.99	6.14	13.50	20.76
hep-P123 (1:10)	7.64	11.75	10.20	15.69
hep-P123 (1:14)	8.64	13.29	5.49	8.44

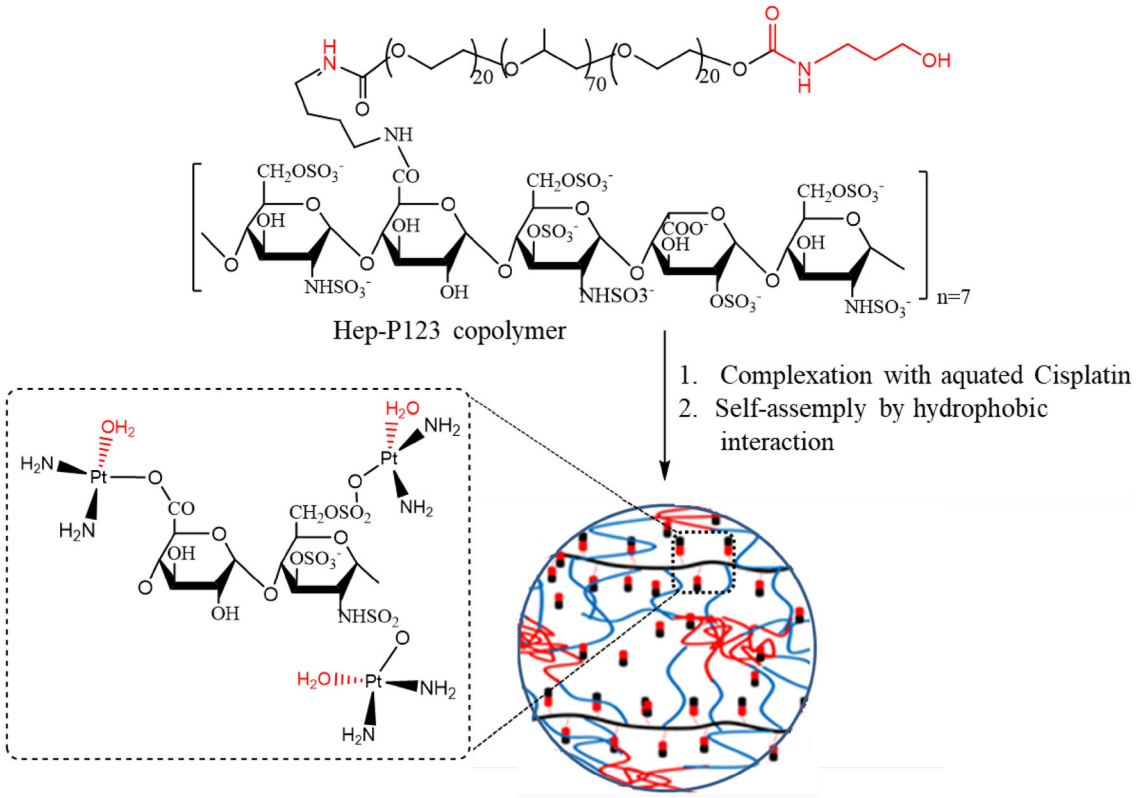

Figure 9 Demonstration of formations of hep-P123-CisOH complex and its nanogel.

complexed nanogels produced ranges from 90 nm to 150 nm. With a highest platinum loading hep-P123 (1:3) copolymer was utilized for further studies.

Platinum release profile and cytotoxicity of the hep-P123-CisOH (1:3) nanogels

Up to know, metabolism of Cisplatin have been well-understanding. In physiological condition, the drug is gradually hydrolyzed into monoaqua–diaqua-Cisplatin which binds to guanine and adenine in DNA resulting in interfering their replication. The hydrolyzation of Cisplatin or platinum complexes and their pharmacodynamics were also reported [21, 22, 30, 31]. In the study, platinum release profile and its cytotoxicity were evaluated to clarify potential of hep-P123-CisOH nanogels in drug delivery systems. Figure 11 shows that more than 60% (wt/wt) of CisOH was gradually released after 24 h in both different pH conditions, while CisOH released out of dialysis bag over 90% after 3 h. This is probably a rapid hydrolyze rate of the anionic groups and CisOH complexes as well as a loose nanogel network due to a low amount of the conjugated pluronic P123. The profile shows that released CisOH at pH = 5.5 (approximately 75%) was higher than that of pH = 7.4 (65%) after 24 h. The CisOH released behavior could indicate that the hep-P123-CisOH complex was quickly hydrolyzed in acidic condition. It was reported that some platinum complexes are less stable in this condition [32, 33]. The behavior could be useful in the drug delivery system because pH at some tumor sites ranges from 6.0 to 7.0 which is lower than that of normal tissue [34]. The platinum particles showed a plateau of the drug release at 60–70% that was explained by some non-reversible bindings of amine/amide (in hep-P123 structure) and aquated Cisplatin [21].

Figure 12 shows the platinum nanogel exhibiting a high proliferative activity against NCI-H460 human lung cancer, although its activity was significantly lower than that of Cis. The half maximal inhibitory concentration (IC50) of Cis performed at 0.54 ± 0.02 μg/ml, while the value of platinum nanogel was calculated at 0.98 ± 0.02 μg/ml. It is meaning that the cytotoxicity of CisOH significantly reduced by its complexation and hep-P123 as well as its slow release behavior from the nanogel. These

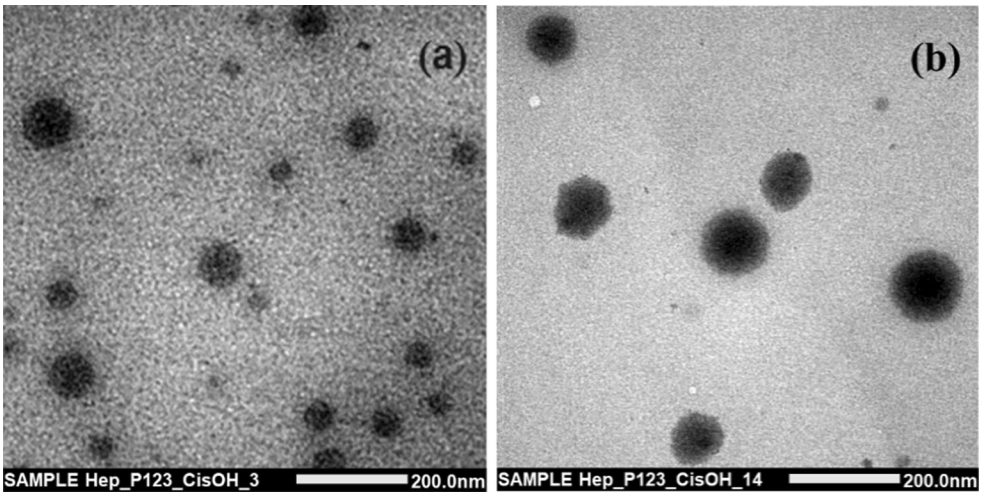

Figure 10 TEM images of **a** hep-P123-CisOH (1:3) and **b** hep-P123-CisOH (1:14) nanogels.

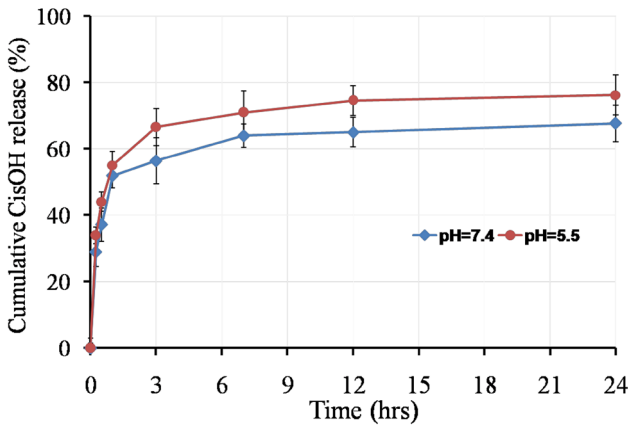

Figure 11 The release profiles of CisOH from the platinum nanogels at pH = 5.5 and 7.4.

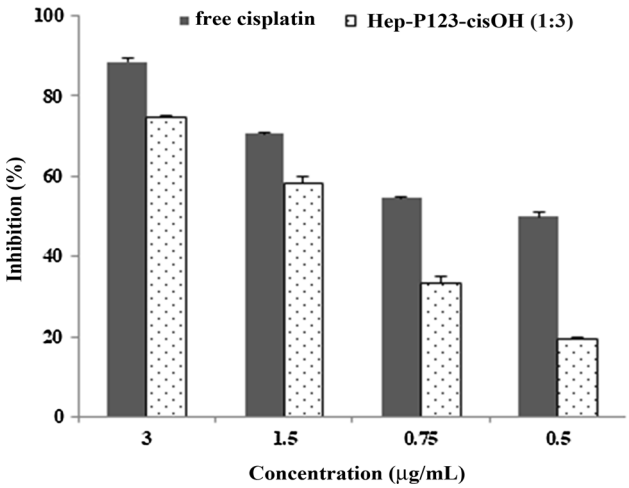

Figure 12 In vitro cytotoxicity study of the hep-P123-CisOH (1:3) nanogel and free cisplatin.

obtained results exhibit potential of the hep-P123-based nanogels for delivering Cis under its aquated species. In addition, the results from cytotoxicity assay of the hep-P123 (1:3) nanoparticles showed that the platform did not perform inhibitory effect ($-2.51 \pm 2.44\%$) at screening concentration 100 ppm.

Conclusion

Four of differently grafted hep-P123 copolymers were successfully prepared and characterized for delivering hydrophobic cisplatin anticancer drug and its aquated species via hydrophobic interaction and complex formation, respectively. Size distribution of the nanogels ranges from 62.2 to 182.4 nm by TEM and DLS which was depended on the grafted degree of the amphiphilic copolymer. The study indicated that the hep-P123 nanogels had a higher loading efficiency of CisOH via complexation in comparison with Cis loading by hydrophobic interaction. The hep-P123 (1:3) owns abundancy of the anionic groups gave a highest complexing efficiency with CisOH. Furthermore, those nanocarriers reduced cytotoxicity of Cis against human lung cancer cell growth. Our additionally preliminary experiment on delivery of dual bioactive compounds (curcumin-a hydrophobic model and aquated cisplatin) also indicated that the hep-P123 nanogels performed high loading efficiency of these molecules (data not shown here). The study could pave a way for an appropriate selection of the amphiphilic hep-P123 platforms for dual drugs delivery consisting of CisOH and other hydrophobic

anticancer drug which could utilize both hydrophobic interaction and complex formation.

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