Journal of Advances in Medical and Pharmaceutical Sciences
12(4): 1-7, 2017; Article no.JAMPS.32493
ISSN: 2394-1111

SCIENCEDOMAIN *international*
www.sciencedomain.org

Potential 5-Fluorouracil Encapsulated mPEG-Chitosan Nanogels for Controlling Drug Release

Dai Hai Nguyen[1,2*]

[1]Institute of Applied Materials Science, Vietnam Academy of Science and Technology, 01 TL29, District 12, Ho Chi Minh City 700000, Vietnam.
[2]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMPS/2017/32493
Editor(s):
(1) Xiao-Xin Yan, Department of Anatomy & Neurobiology, Central South University Xiangya School of Medicine (CSU-XYSM), Changsha, Hunan 410013, China.
Reviewers:
(1) Syed Umer Jan, University of Balochistan, Pakistan.
(2) S. V. Patil, Ashokrao Mane College of Pharmacy, India.
(3) Mohd Abdul Hadi, Bhaskar Pharmacy College, Jntuh University, Hyderabad, India.
(4) Janaina Aline Galvão Barros, University of São Paulo, São Paulo, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/18587

Original Research Article

Received 28th February 2017
Accepted 3rd April 2017
Published 11th April 2017

ABSTRACT

Aims: The objective of this study was to prepare and characterize poly (ethylene glycol) methyl ether (mPEG)-conjugated chitosan (CS) nanogels, mPEG-CS, at different molar ratios for 5-Fluorouracil (5-FU) delivery (5-FU-loaded mPEG-CS nanogels).
Study Design: The chemical cross-linking of those polymers were synthesized by using 4-Nitrophenyl chloroformate as coupling reagent.
Place and Duration of Study: Department of Biomaterials & Bioengineering, Institute of Applied Materials Science, Vietnam Academy of Science and Technology, between February and June 2015.
Methodology: The chemical structure of mPEG-CS was characterized by Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (^{1}H NMR).
Results: The particle sizes of 5-FU-loaded nanogels were nearly spherical in shape with diameter range of 20-50 nm, determined by transmission electron microscopy (TEM). Especially, whereas the encapsulation efficiency and loading capacity of mPEG-CS nanogels were independent of the molar ratio of mPEG, there was one factor that particularly stand out, 5-FU release behavior.

**Corresponding author: E-mail: nguyendaihai@iams.vast.vn;*

Conclusion: These results demonstrated that mPEG-CS nanogels present potential for controlled release of 5-FU working as a delivery system in cancer therapy.

Keywords: Poly (ethylene glycol) methyl ether; chitosan; 5-fluorouracil; nanogels; drug delivery system.

1. INTRODUCTION

Drug delivery system (DDS) has been an impressive subject of studying and developing as an original method that enable the release of a therapeutic substance in the body and improve its efficacy and safety for reducing side-effects in patients. The main purpose of DDS is to particularly localize and target the drug within desired therapeutic range to expected tissue and cells while maintaining the systemic level of drugs [1-4]. In order to reach those intentions, nanomaterials (NMs), an excellent candidate for desirable drug therapy, possess numerous advantages such as protecting sensitive drug molecules from reticuloendothelial system (RES) *in vivo,* increasing surface area between loaded drug and tumor tissue, improving the solubility and bioavailability of poorly soluble drugs, and possibility to tailor-making the drug release [5,6]. Self-assembled nanogels, one of the most effective NMs for ameliorating possibly drug inactivation, has the advantages of straightforward synthesis without the presence of drugs and high biocompatibility [6,7].

In comparison with artificial polymer, chitosan (CS) obtained by deacetylation of chitin, which are suitable for the formation of nanogels due to its unlimited in biocompatibility, biodegradability, better stability, and low toxicity [8-13]. However, CS is only soluble in an aqueous acidic solution (pH< 6.5) because of a lot of amino groups on its chain, resulting in limitation of its pharmaceutical and biomedical applications [14]. As a result, a significant number of different modified CS such as *N, N, N*-trimethyl CS, *N*-acyl CS, *N*-carboxyalkyl CS, *O*-carboxyalkyl and *N*-carboxyacyl CS have been employed. However, these modifications might involve toxicity issues. To overcome these drawbacks, poly (ethylene glycol) methyl ether (mPEG), an excellent hydrophilic polymer, has been carried out to improve poor aqueous solubility of CS based on its favorable biodegradability, biocompatibility, low toxicity, low immunogenicity, and hydrophilic flexibility [15,16]. In order words, grafting mPEG onto CS chain not only optimize the biocompatibility of CS but also avoid the adsorption of protein and evade from RES [3,13,17-19]. There are several research focused on developing mPEG-CS delivery system for cancer therapy. For instance, Dong-Jun Fu and co-workers investigated the potential of mPEG grafted CS (mPEG-g-CS) to be used as nanocarriers for delivery of 5-flouroracil (5-FU). The results showed that the drug-loaded mPEG-g-CS self-assembled micelles have potential as promising nanocarriers with controlled particle size and controlled release effect for effective anti-tumor activity [15]. In addition, XiangYe Kong et al. introduced a simple new method based on free-radical polymerization initiated by potassium persulfate (KPS) to prepare the mPEG-CS diblock copolymer (mPEG-CS) as a controlled delivery system. *In vitro* cell culture assay demonstrated that mPEG-CS nanoparticles are non-toxic and cell compatible, which can be safely used as potential drug carriers for the treatment of cancer [16]. In another previous research, PEGylation of CS derivatives with PEG of different molecular weights have been also reported to improve the aqueous solubility of CS [20].

In this study, in order to examine the influence of the molar ratios of mPEG and CS on drug loading and release behavior, mPEG-CS conjugate were first prepared at different molar ratios as controlled-release systems for 5-FU, 5-FU-loaded mPEG-CS nanogels, in which CS was used as cross-linker and mPEG was designed as hydrophilic co-monomer. The obtained nanogels were then characterized by proton nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared (FTIR) and transmission electron microscopy (TEM). Moreover, the drug loading and release behavior of 5-FU-loaded mPEG-CS nanogels were also evaluated. This study is expected to create significant opportunity for drug delivery in cancer therapy.

2. MATERIALS AND METHODS

2.1 Materials

CS (Mw: 100-300 kDa), 5-FU, 4-Nitrophenyl chloroformate (PNC, Mw: 201.56 Da), Tetrahydrofuran (THF), and poly (ethylene glycol) methyl ether (mPEG, Mw: 5 kDa) were purchased from Sigma-Aldrich (St. Louis, MO,

USA). All reagents and solvents were used without further purification.

2.2 Methods

2.2.1 Synthesis of mPEG-CS

The mPEG-CS conjugate was synthesized by using PNC as intermediate under controlled conditions of temperature and vacuum environment (Scheme 1). First, 0.25 g of mPEG was melted down at 65°C under vacuum and 16 mg of PNC was later added into the mPEG solution under constant stirring for 6 h, mPEG-PNC. Next, the mixture was left to cool at 40°C, followed by addition of THF solution (5 mL) to remove an excess amount of mPEG. The obtained mPEG-PNC was added drop-wise into the CS solution (pH 5), and then the mixture was stirred for 24 h and dialyzed by dialysis membrane (MWCO 12-14 kDa, Spectrum Laboratories, Inc., USA) against deionized water (deH_2O) for 4 days at room temperature. The deH_2O was changed 5-6 times a day and the resulting solution was lyophilized to obtain mPEG-CS. The mPEG-CS conjugate was further prepared at different molar ratios (1:1, 5:1, and 10:1 of mPEG and CS) as described above, respectively.

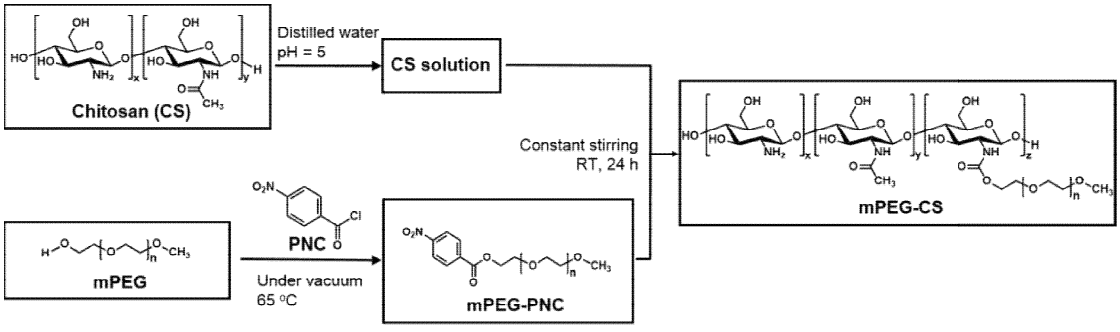
2.2.2 Characterization

For the purpose of investigating the presence of mPEG on CS, FTIR analysis (Nicolet Nexus 5700 FTIR, Thermo Electron Corporation, Waltham, MA, USA) of CS, mPEG, and mPEG-CS was carried out with KBr pellets in 500-4000 cm^{-1} range. ^{1}HNMR spectrum of mPEG-CS was obtained on a Bruker Avance 500 (Bruker Co., USA). The size and morphology of 5-FU-loaded mPEG-CS nanogels were imaged by TEM (JEM–1400 TEM; JEOL, Tokyo, Japan) at an accelerating voltage of 300 kV. The sample was prepared by placing a drop of solution in deH_2O (1 mg/mL) onto a carbon-copper grid (300-mesh, Ted Pella, Inc., USA) and air-dried for 10 min.

2.2.3 Drug loading content (DLC) and drug loading efficiency (DLE)

5-FU have been loaded into the mPEG-CS nanogels by equilibrium swelling technique. Initially, 10 mg of 5-FU and 100 mg of mPEG-CS conjugate were dissolved independently in deH_2O and then mixed together. The mixture was sonicated for 60 min and magnetic stirred for 24 h. The solution was later dialyzed by dialysis membrane (MWCO 3.5 kDa, Spectrum Laboratories, Inc., USA) against deH_2O to remove unloaded 5-FU. The 5-FU-loaded mPEG-CS nanogels was obtained under solid phase by lyophilization.

Three different 5-FU-loaded mPEG-CS samples were dissolved independently with a solution of 1 mL of CH_3COOH (0.25 M) and 1 mL of acetonitrile, followed by sonication for 30 min and filtration. These experiments were taken in triplicate for high performance liquid chromatography (HPLC) analysis. The total 5-FU contents in mPEG-CS were measured using the Shimadzu Prominence LC-20A series HPLC system (Shimadzu, Kyoto, Japan). The injected volume was 20 µL and the mobile phase (acetonitrile: water = 97: 3) was delivered at 1.00 mL/min. A reversed-phase column (Fortis Amino, 150×4.6 mm, 5 µm pore size, Fortis Technologies Ltd., Cheshire, UK) was used and column effluent was monitored with a UV detector at 260 nm. The calibration curve for quantification of 5-FU in mPEG-CS was found to be linear over the standard 5-FU concentration range of 0-100,000 ng/mL with a high correlation



Scheme 1. Synthetic route for mPEG-CS

coefficient of R^2= 0.999. The %DLC and %DLE were calculated using following equations. The %DLC and %DLE were calculated using following equations:

DLE (%) = (weight of drug in particles/ weight of drug feed initially) × 100.

DLC (%) = (weight of drug in particles/ weight of particles and drug) × 100.

2.2.4 *In vitro* release

The release of 5-FU-loaded mPEG-CS nanogels was dialyzed (MWCO 3.5 kDa) into a vial containing phosphate buffer saline (PBS) and 2 % (v/v) Tween 80 (12.5 mL, pH 7.4). The release medium (0.5 mL) was withdrawn at predetermined time intervals (2, 4, 6, 8, 10, and 12 h), filtered (pore size = 0.20 µm) and replaced with an equivalent volume of fresh medium. These experiments were repeated three times for the HPLC analysis.

3. RESULTS AND DISCUSSION

3.1 Characterization of mPEG-CS Conjugate and 5-FU-loaded mPEG-CS Nanogels

The mPEG-CS was characterized by ^{1}H NMR analysis (Fig. 1). The solvent peak of D_2O was found in 4.74 ppm. Typical peak at 3.4-3.94 ppm (H-b, H-c, H-d, H-e, H-2, and H-3) were assigned to methylene protons of CS saccharide units and repeat units in mPEG. Peaks at 2.95 ppm (H-c) and 3.37 ppm (H-1) were attributed to –CH-NH- from CS and $–OCH_3$ from mPEG, respectively. The peaks at 1.95-1.98 ppm (H-f) appeared, which were assigned to $–NH-CO-CH_3$. The presence of all these resonance signals imply that mPEG-CS conjugate was successfully prepared.

The FTIR spectra of CS (i), mPEG (ii), and mPEG-CS are presented in Fig. 2. The spectrum of CS reveals distinctive absorption bands at 1656 cm^{-1} (amide I), 1594 cm^{-1} ($-NH_2$ bending), and 1400 cm^{-1} (amide III) (Fig. 2i). The absorption bands at 1150 cm^{-1} (asymmetric stretching of the COOOC bridge), 1092 cm^{-1}, and 1042 cm^{-1} (skeletal vibration involving the COO stretching) were assigned to its saccharine structure. In addition, the increased intensity of the peaks at around 2910 cm^{-1} and 2830 cm^{-1}, and 1094 cm^{-1} show the CH_2 groups, and C-O-C stretch of mPEG, respectively (Fig. 2ii). As shown in Fig. 2iii, the CS amide peaks slightly shifted to 1631 cm^{-1} and 1529 cm^{-1}, respectively. The shifts were possibly due to hydrogen bonding between amide carbonyl with mPEG hydroxyl. Besides, the increased intensity of the peaks at around 2924 cm^{-1} and 1100 cm^{-1} indicated the CH_2 groups and C-O-C stretch of mPEG. These results also indicate that the amino groups of CS were substituted by mPEG groups.

As shown in Fig. 3, the 5-FU-loaded mPEG-CS nanogels were nearly spherical in shape and their particle size was in the range of 20-50 nm. According to previous reports, nanoparticles are ranging from 10-200 nm in size that have enormous potential advantages, including higher drug efficiency, enhanced therapeutic efficacy and cytotoxicity as well as facilitating penetration of drugs through various biological barriers such as the mucosal membrane and tumor vasculature. Furthermore, the use of drug-encapsulated nanoparticles has been shown to overcome drug-resistant cancer cells by facilitating entry into cells through surface binding and endosomal uptake routes, as opposed to the normal route of administration of the drug alone [21]. Therefore, the obtained nanogels can serve as potential drug nanocarriers to deliver 5-FU.

3.2 Drug Loading and Release Behavior

The drug loading efficiencies of 5-FU-loaded mPEG-CS were 11.5% at mPEG-CS (1:1), 10.0% at mPEG-CS (5:1), and 10.39% at mPEG-CS (10:1). There were no significant differences in 5-FU loading among the three ratio groups. These results show that the 5-FU loading was independent of different molar ratios of mPEG-CS.

The *in vitro* release behavior of 5-FU from self-assembled nanogels was carried out in PBS (pH 7.4) at 37°C (Fig. 4). The release of 5-FU from mPEG-CS (1:1) was 23% at 2 h, and then increased to 39% at 4 h. The release behavior of 5-FU could be explained by the release of a hydrophilic drug loosely bound on the surface of mPEG-CS nanogels. Thereafter, the drug was a relatively slow release up to 12 h of 41%, only 2% 5-FU was released during that period of time. The percentages of 5-FU released from mPEG-CS (5:1) were 17% at 2 h and 33% at 4 h. After 12 h, 37% 5-FU was slowly delivered. Compared with mPEG-CS (10:1), the drug was rapidly freed up 94% at 12 h. In aqueous medium, the pores

at the surface of the mPEG-CS nanogels are diffused through by the water uptake; accordingly, loosely bound drug would be delivered. Based on these results, mPEG-CS (1:1) and mPEG-CS (5:1) might be suitable for sustained and controlled DDS, rather than mPEG-CS (10:1), the highest performance mPEG.

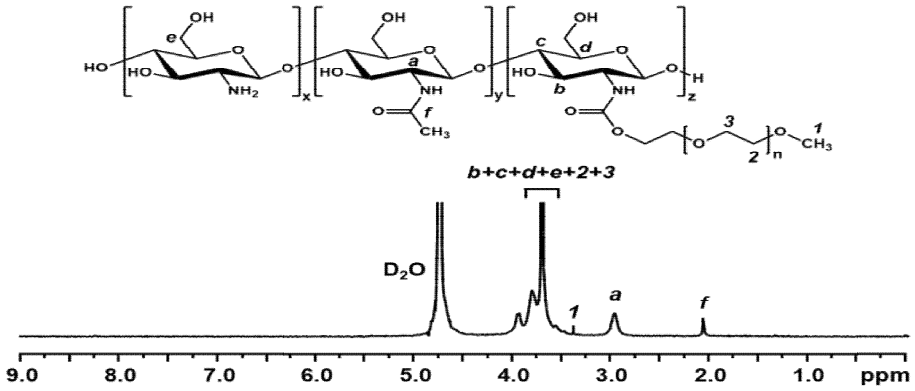


Fig. 1. ^{1}H NMR spectrum of mPEG-CS

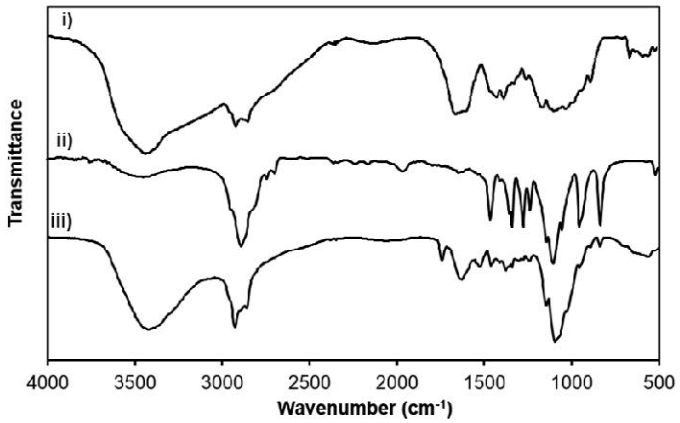


Fig. 2. FTIR spectra of (i) CS, (ii) mPEG and (iii) mPEG-CS

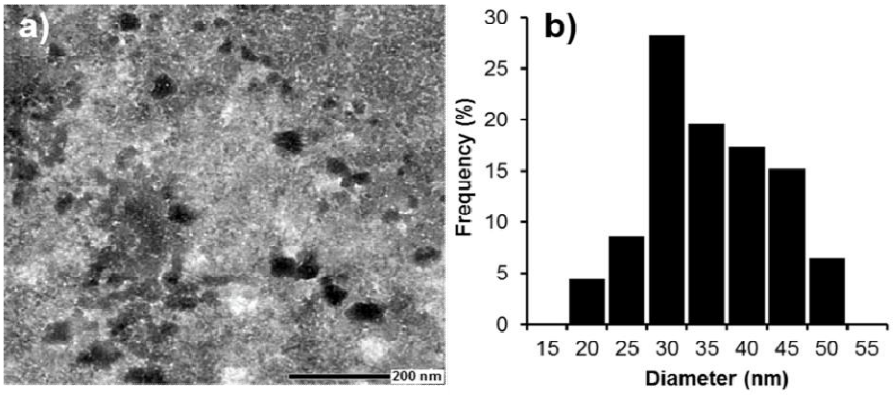


Fig. 3. (a) TEM image and (b) particle size distribution of 5-FU-loaded mPEG-CS nanogels

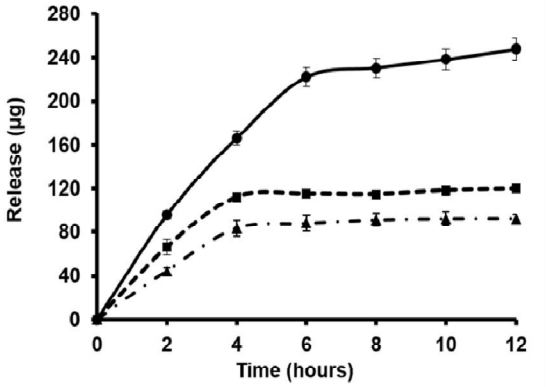


Fig. 4. Drug release of mPEG-CS at molar ratio of 1:1 (dashed line); 5:1 (dash-dot line); 10:1 (solid line), respectively

4. CONCLUSION

We successfully developed mPEG-CS conjugate and 5-FU-loaded mPEG-CS nanogels as controlled delivery systems. The obtained nanogels were spherical in shape with diameter range of 20-50 nm, demonstrating promising applications for targeted cancer therapy. Although the geometrical characterization of the nanogels show promise for drug loading, the encapsulation efficiency of 5-FU was relatively low. Interestingly, the drug release profile of 5-FU showed that the highest percentage of mPEG over the modified CS nanogels is, the fastest is the drug release behavior. Consequently, mPEG-CS nanogels present a potential for developing suitable nanomedicine carriers in cancer treatment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

This research was supported by Institute of Applied Materials Science, Vietnam Academy of Science and Technology.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Goldberg M, Langer R, Jia X. Nanostructured materials for applications in drug delivery and tissue engineering. Journal of Biomaterials Science, Polymer Edition. 2007;18(3):241-68.
2. Nguyen DH, Choi JH, Joung YK, Park KD. Disulfide-crosslinked heparin-pluronic nanogels as a redox-sensitive nanocarrier for intracellular protein delivery. Journal of Bioactive and Compatible Polymers. 2011; 26(3):287-300.
3. Nguyen DH, Lee JS, Choi JH, Lee Y, Son JY, Bae JW, et al. Heparin nanogel-containing liposomes for intracellular RNase delivery. Macromolecular Research. 2015;23(8):765-69.
4. Nguyen TTC, Nguyen CK, Nguyen TH, Tran NQ. Highly lipophilic pluronics-conjugated polyamidoamine dendrimer nanocarriers as potential delivery system for hydrophobic drugs. Materials Science and Engineering: C; 2016.
5. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. ACS Nano. 2009;3(1):16-20.
6. Nguyen DH, Lee JS, Choi JH, Park KM, Lee Y, Park KD. Hierarchical self-assembly of magnetic nanoclusters for theranostics: Tunable size, enhanced magnetic resonance imagability, and controlled and targeted drug delivery. Acta Biomaterialia. 2016;35:109-17.
7. Batrakova EV, Kabanov AV. Pluronic block copolymers: Evolution of drug delivery concept from inert nanocarriers to biological response modifiers. Journal of Controlled Release. 2008;130(2):98-106.
8. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro-and nanoparticles in drug delivery. Journal of Controlled Release. 2004; 100(1):5-28.
9. Hoang DQ, Tran TV, Tran NQ, Nguyen CK, Nguyen TH, Truong MD, et al. Functionalization of Fe3O4 nanoparticles with biodegradable chitosan-grafted-mPEG for paclitaxel delivery. Green Processing and Synthesis. 2016;5(5):459-66.
10. Hoang Phuc D, Thi Hiep N, Ngoc Phuc Chau D, Thi Thu Hoai N, Chan Khon H, Van Toi V, et al. Fabrication of hyaluronan-poly (vinylphosphonic acid)-chitosan hydrogel for wound healing application. International Journal of Polymer Science. 2016;2016.

11. Phuong NT, Ho VA, Nguyen DH, Khoa NC, Quyen TN, Lee Y, et al. Enzyme-mediated fabrication of an oxidized chitosan hydrogel as a tissue sealant. Journal of Bioactive and Compatible Polymers: Biomedical Applications. 2015;30(4):1-12.
12. Khon C, Van Toi V, Dai Hai N, Bao BC. Fabrication of hyalunonan-poly (Vinylphosphonic acid)-chitosan hydrogel for wound healing application. International Journal of Polymer Science. 2016;2016:9.
13. Nguyen DH, Tran NQ, Nguyen CK. Tetronic-grafted chitosan hydrogel as an injectable and biocompatible scaffold for biomedical applications. Journal of Biomaterials Science, Polymer Edition. 2013;24(14):1636-48.
14. Chan P, Kurisawa M, Chung JE, Yang YY. Synthesis and characterization of chitosan-g-poly (ethylene glycol)-folate as a non-viral carrier for tumor-targeted gene delivery. Biomaterials. 2007;28(3): 540-49.
15. Fu DJ, Jin Y, Xie MQ, Ye YJ, Qin DD, Lou KY, et al. Preparation and characterization of mPEG grafted chitosan micelles as 5-fluorouracil carriers for effective anti-tumor activity. Chinese Chemical Letters. 2014; 25(11):1435-40.
16. Kong X, Li X, Wang X, Liu T, Gu Y, Guo G, et al. Synthesis and characterization of a novel MPEG–chitosan diblock copolymer and self-assembly of nanoparticles. Carbohydrate Polymers. 2010;79(1): 170-75.
17. Zhu S, Qian F, Zhang Y, Tang C, Yin C. Synthesis and characterization of PEG modified N-trimethylaminoethylmethacrylate chitosan nanoparticles. European polymer journal. 2007;43(6):2244-53.
18. Kulkarni AR, Lin YH, Liang HF, Chang WC, Hsiao WWW, Sung HW. A novel method for the preparation of nanoaggregates of methoxy polyethyleneglycol linked chitosan. Journal of Nanoscience and Nanotechnology. 2006;6(9-1):2867-73.
19. Nguyen DH, Lee JS, Bae JW, Choi JH, Lee Y, Son JY, et al. Targeted doxorubicin nanotherapy strongly suppressing growth of multidrug resistant tumor in mice. International Journal of Pharmaceutics. 2015;495(1):329-35.
20. Dong AJ, Feng MH, Qi HY, Li SS, Deng LD. Synthesis and properties of O-carboxymethyl chitosan/methoxy poly(ethylene glycol) graft copolymers. J Mater Sci Mater Med. 2008;19(2):869-76.
21. Masarudin MJ, Cutts SM, Evison BJ, Phillips DR, Pigram PJ. Factors determining the stability, size distribution, and cellular accumulation of small, monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to the passive encapsulation of [14C]-doxorubicin. Nanotechnology, science and applications. 2015;8:67-80.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18587