Gold Nanorods Protected with Thiol-end Capped Diblock Copolymer (PHEMAb-PVEAQ-SH): Synthesis and Applications in Drug Delivery

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Abstract

Theranostic nanoparticles with multifunctional ability have been emerging as a new platform for biomedical applications such as imaging, sensing and drug delivery. Despite gold nanorods (GNRs) being an excellent nanosource with multifunctional versatility, they have certain limitations in biomedical applications, which include surfactant toxicity, biological stability and controlled drug release kinetics. Hence, we fabricated thiol-end caped diblock copolymer [poly(2-hydroxyethyl methacrylate)-b-poly[(N-4-vinylbenzyl), N,N-diethylamine)]; [PHEMA-b-PVEA] *encapsulated* gold nanorods (GNRs) via RAFT polymerization techniques. pH responsive drug release ability of the synthesized biocompatible nanocomposite were also investigated .Also the success of coating was verified by fourier transform infrared (FTIR), zeta potential, transmission electron microscopy (TEM), dynamic light scattering (DLS), proton nuclear magnetic resonance (¹H NMR) spectroscopies, gel permeation chromatography (GPC) analysis and UV-Vis spectroscopy. We developed a GNRs@copolymer as narnocarier by using MTX-loading and to enhanced pharmacokinetics. The anti-cancer drug (MTX) was encapsulated into the GNRs@copolymer by the electrostatic force. The MTX-encapsulation efficiency was calculated to be 97% . Release behaviors of MTX from the nanocomposite shown that the rate of MTX release could be controlled by pH value.

Keyword: RAFT polymerization, Gold nanorods, Anti-cancer drug

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1. Introduction

Drug delivery nano-systems have become a significant tool for depreciating the capability of chemotherapeutics in late decades. Among the diverse drug delivery systems reported, nanoparticles and liposomes are prevalent and have been commercialized [1]. However, their efficiency and total number of products is still limited. Polymeric nanoprticles mediated drug delivery systems are currently applied in biomedical application [2-3]. The late developments have induced to conjugation of



This work is licensed under a <u>Creative Commons Attribution-</u> NonCommercial-NoDerivatives 4.0 International License. Polymeric micelles onto various inorganic materials such as, nanoquantum dots[4-6], nanogold [7-8] and nanomagnetic [9-11] with possible to institute a multifunctional platform such as imaging agent, theranostic agent and delivery vehicles, which could uaply be visualized by magnetic resonance imaging and optical techniques.

Among the inorganic materials described so far, a new grade of one-dimensional gold nanorods (GNRs) has attracted extensive attention in last decades for their unique potential use in electronics [12, 13], optical



[14-15] and medical properties [16-18] Applications. Therefore, the widely accepted functional versatility and biocompatibility of GNRs establish their virtual towards cellular interactions [19]. In particular, the tunable behaviors of scattering cross-sections and absorption of GNRs with different concentrated surface plasmon resonance (LSPR) fabricate GNRs an excellent candidate for solid tumor therapies such as in vivo imaging studies and photothermal ablation [20].

The longitudinal SPR of GNRs is exceedingly sensitive to the exchanges in the local environment of GNRs, making them a possible candidate for biosensors [21-23]. In particular, GNRs occupy high conjugation efficiency for various biomolecules and drugs such as antibodies [24] siRNA [25] and DNA [26] due to their higher surface energy and larger surface area. Despite GNRs being an ideal they have mainly limitations nanosource. in biomedical applications, which include toxicity of the cetyltrimethylammonium surfactant bromides (CTAB), sustainable drug releaseand and biological stability [27, 28]. Therefore, it is vital to replace the CTAB with biocompatible Polymeric nanoparticles. The most widely used methods for synthesis these type of polymers include ring-opening polymerization (ROP), [29] nitroxide-mediated polymerization (NMP), [30] atom transfer radical polymerization (ATRP), [31] and reversible addition fragmentation chain transfer (RAFT) of polymerization [32]. Among these. (RAFT) polymerization mediated by thiocarbonylthio compounds is a versatile and effective process, applicable to a broad range of vinyl monomers without need for protecting groups [33-34]. In addition, the synthesized block copolymers by using RAFT polymerization technique can be changed to thiol-end capped polymers by reduction reactions. On the other hand, the strong affinity of the thiol group toward metal nanoparticle (e.g., silver and gold) and its ability to selective and quantitate reaction with some moieties (e.g., maleimide) under physiological conditions have induced to its extensive use in

Herein, we fabricated a drug delivery nano-systems by coupling polymeric micelles, methotrexate (MTX)

numerous fields [35].

and gold nanorods (GNRs) in a single biocompatible nanocomposite. Hence, gold nanorods-cored biodegradable micelles were synthesized by coating gold nanorods (GNRs) with synthesized thiol-end capped diblock copolymer [poly(2-hydroxyethyl methacrylate)-b-poly[(N-4-vinylbenzyl),N,Ndiethvlamine)]: [PHEMA-b-PVEAQ-SH]. Furthermore, the anti-cancer drug methotrexate (MTX), was attached onto the gold nanorods-cored micelles (GNRs@copolymer) by the electrostatic force and the nanocomposites formed were named MTX-loaded GNRs@copolymer The chemical structures of all samples, methotrexate loading capacity, and drug release ability of the synthesized nanocomposite were also evaluated.

2. Materials and methods

Gold (III) chloride trihydrate (HAuCl₄·3H₂O, 99%), Cetyl trimethylammonium bromide (CTAB, 99%), sodium borohydride (NaBH₄, 99%), silver nitrate 99%) and dodecanethiol (AgNO₃, (DDT). dithiothreitol (DTT), were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) Methotrexate salt (MTX) was prepared from Zahravi Pharmaceutical Company (Tabriz, Iran). 2,2'-azobisisobutyronitrile 2-Hydroxyethylmethacrylate, (AIBN), and 4chloromethyl styrene were purchased from Merck, (Darmstadt, Germany).

2.1. Synthesis of RAFT agent and N-(4-vinylbenzyl)-N,N-diethylamine (VEA) monomer

The RAFT agent, 4-cyano-4-[(phenylcarbothioyl) sulfanyl]pentanoic acid, and *N*-(4-vinylbenzyl)-*N*,*N*-diethylamine (VEA) monomer were synthesized according to the literature [36].

2.2. Synthesis of PHEMA via RAFT polymerization In an ampoule, HEMA (5 mL, 40 mmol), RAFT agent (46.0 mg, 0.16 mmol) and AIBN (1.7 mg, 0.01 mmol), 5 mL DMF were poured and degassed with several freeze–pump–thaw cycles. Then, the mixture moved in an oil bath at 70 °C for about 18 hours. Finally, the ampoule was quenched and precipitated in cold diethyl ether (50 mL). The product was dried under vacuum at room temperature (Scheme 1a).

2.3. Synthesis of PHEMA-b-PVEA diblock copolymer

In an ampoule, PHEMA (700 mg, 0.07 mmol), VEA (1 mL, 5.2 mmol) and AIBN (1.7 mg, 0.01 mmol), 5 mL DMF were poured and degassed with several freeze–pump–thaw cycles. Then, the mixture moved in an oil bath at 70 °C for about 18 hours. Finally, the ampoule was quenched and precipitated in water/methanol mixture cold (70 mL). The product was dried under vacuum at room temperature (Scheme 1b)

2.4. Synthesis of cationic quaternary ammonium (PHEMA-b-PVEAQ)

cationic quaternary ammonium (PHEMA-*b*-PVEAQ) was prepared by dissolving 0.5 g of (PHEMA-*b*-PVEAQ) in 4 mL of dry DMF under stirring, followed by dropwise addition of 5 mmol of CH₃I dissolved in 2 mL of THF. The reaction was carried out for 15 h at room temperature with magnetic stirring. The crude products were filtered, washed twice with 10 mL aliquots of cold hexane for further purification of product and dried in vacuum for 8 h to yield the (PHEMA-*b*-PVEAQ) (Scheme 1c).

2.5. Synthesis of thiol end capped PHEMA-b-PVEAQ-SH diblock copolymer

In a typical reaction, PHEMA-*b*-PVEAQ (30 mg) was dissolved in DMSO₄. Then, 4 mL of aqueous solution of NaBH₄ (1.0 M) was added. The mixture was then stirred for 4 days at 25 °C. The reaction solution was dialyzed against deionized water for 48 hours. The product was dried under vacuum at 25 °C temperature (Scheme 1c).

2.6. Synthesis of GNRs using seedless growth method

HAuCl₄ (5.0 mL; 1.0 mM), 5.0 mL cetyltrimethylammonium bromide (CTAB; 0.2 M) and AgNO₃ (250 L;4.0mM) was added to flask and pH of this solution was regulated about 1-1.15. Then, 70 L ascorbic acid (78.8 mM) was added until the solution was liberate. Without delay NaBH₄(15 L; 0.01 M) was discharged to the solution and stirred for 6 hours.

diblock 2.7. Synthesis of GNRs-DDT using phase transfer ligand exchange

CTAB as a cationic surfactant is highly toxic and it is essential to remove of GNRs for many biological applications. Briefly, 1 mL GNRs solution was place into contact with 2 mL DDT. After 40 s sonication, 4 mL acetone were added and organic layers was seprated with added 2 mL toluene. The solution was centrifugated for 15 min and dried in vacuum at room temperature (Figure 1)



Figure 1. Image of Phase transfer procedure with gold nanorods and dodecanethiol as organic ligand

2.8. Preparation of MTX-loaded GNRs@copolymer

MTX (10 mg) and GNRs@copolymer (100 mg) was dissolved in 10 mL deionized water .The content of the ampole was sonicated and stirred for about 48 hours. Finally, the MTX- loaded GNRs@copolymer were collected by centrifugation at 8000 rpm for about 15 minutes. The encapsulation efficiency of the prepared GNRs@copolymer was calculated by using UV-vis spectroscopy at 290 nm.

2.9. In vitro release study

In the *in vitro* drug release experiment, MTX-loaded GNRs@copolymer was placed in PBS (0.01 molL⁻¹, 100 mL) at pHs (7.4, 5.4 and 4). The release solution was stirred at 300 rpm individually at 37 °C, and then 2 mL of buffer solution was collected in different times to evaluate characterization with UV-Vis spectrophotometer at 290 nm.

The percentage of the cumulative content of released MTX was determined from the standard calibration curve. The obtained absorbance of MTX was commuted to their concentration according to the



Figure 2. The FT-IR spectra of the PHEMA (a), and *PHEMA*-*b-PVEA* (*b*)

calibration curve of MTX in the same buffer percent of drug released from nanocomposite was calculated by Equation (1)

Cumulative released drug (%)

$$= \frac{\text{amount of drug in released}}{\text{amount drug loaded in GNRs nanocomposite}}$$
$$\times 100 \quad (1)$$

2.10. Characterization

Fourier transform infrared (FTIR) spectra of the samples were taken on a shimadzu 8101 M FTIR (Shimadzu, Kyoto, Japan) between the wavenumbers of 400 and 4000 cm⁻¹. The samples were prepared by grinding the dry powders with potassium bromide (KBr), and compressing the mixture into disks. The spectra were recorded at room temperature. Proton nuclear magnetic resonance (1H-NMR) spectra were recorded at 25°C using a FT-NMR (400 MHz) Bruker



Figure 3. The 1H NMR *spectra* of the PHEMA (A), PHEMA-b-PVEA (B) and PHEMA-b-PVEAQ-SH (C).

spectrometer (Bruker, Ettlingen, Germany). The samples for 1HNMR spectroscopy was prepared by dissolving about 10 mg of sample in 1 mL of deuterated dimethyl sulfoxide (DMSO-d6) and chemical shifts were reported in ppm units with tetramethylsilane (TMS) as an internal standard. Size exclusion analyses were carried out using a Waters 1515 (USA) gel permeation chromatography (GPC) instrument equipped with Breeze 1515 isocratic pump and 7725 manual injector. Size exclusion analyses were carried out using a Waters 1515 (USA) gel permeation chromatography instrument equipped with Breeze 1515 isocratic pump and 7725 manual



Figure 4. GPC chromatogram of poly(HEMA) and poly(HEMAb-VEA) samples.

injector. Dimethylformamide (DMF) was applied as eluent at a flow rate of 1 mL min⁻¹ and column temperature of 25 °C. Ultraviolet-visible (UV-Vis) spectroscopy was planned by a Shimadzu 1650 PC UV-Vis spectrophotometer (Shimadzu, Kyoto, Dynamic (DLS) Japan). light scattering measurements were performed by Nanotrac WaveTM (Microtrac, San Diego, CA, USA) at room temperature. Samples were prepared as 0.5% (w/v). Transmission electron microscopy (TEM) images of the GNRs was taken on a Philips CM10-TH microscope (Phillips, Eindhoven, The Netherlands) with a 100 kV accelerating voltage.

3. Result

In the past decade, AuNPs have been attracted intense research over because of their singular properties and many applications in various areas including optics, biomedical materials, and electronics. The surface of GNRs has widely been modified with synthetic polymers by thiol chemistry. In this context, thiol-end caped a diblock copolymers are one of the advances and innovations in the concepts of drug delivery system, in part due to their remarkable and smart physicochemical characteristics [37].

3.1. Characterization of diblock copolymer and GNRs@copolymer

The FTIR spectra of the PHEMA and P(HEMA-*b*-VEA) are shown in Figure 2. As seen, the FTIR spectrum of PHEMA can be listed as vibration of carbonyl group at 1724 cm^{-1} , aliphatic C–H stretching vibrations at 2869 and 2937 cm⁻¹, C–O–C stretching vibration at 1253 cm⁻¹ and 3471 cm⁻¹ is related to the hydroxyl group of the PHEMA (Fig.1a). The FTIR spectrum of the PHEMA-*b*-PVEA diblock coplolymer can be listed as: aliphatic and aromatic C–H stretching vibrations at 3100-2800 cm⁻¹ region, aromatic C=C stretching vibration at 1454 cm⁻¹, and hydroxyl stretching vibration as a broad strong band centered at 3445 cm⁻¹ (Fig. 2b)

The successful preparation of the PHEMA homopolymer is confirmed by ¹HNMR in Fig. 2A. The chemical shifts of O<u>C</u>H₂-CH₂-OH, OCH₂-<u>C</u>H₂-OH, OCH₂-CH₂-O<u>H</u> protons and RAFT agent backbone are observed at 3.56, 4.02, 4.83 and 0.85-2.10 ppm, respectively.

The successful preparation of the PHEMA-*b*-PVEA diblock copolymer is confirmed by ¹HNMR in Fig. 2B. The chemical shifts of CH₃, -CH₂ protons and RAFT agent backbone are observed at 0.85-2.10 ppm, respectively. The protons of the N-CH₂, -CH₂OH, and -OCH₂ groups are appeared at 2.37, 3.50 and 3.55 ppm, respectively. The aromatic proton is shown at 6.70-7.30 ppm.

The synthesis of the PHEMA-*b*-PVEAQ-SH diblock copolymer is confirmed by the unappearance of chemical shifts at 7.90 ppm, which is related to the aromatic protons of RAFT agent, and the chemical shifts at 3.8 is related to the N⁺–CH₃ protons of the PVEAQ block. All other chemical shifts are labeled on ¹HNMR spectrum of the PHEMA-*b*-PVEAQ-SH diblock copolymer (Fig. 3C).

The GPC chromatograms of PHEMA and PHEMA*b*-PVEA are demonstrated in Fig. 4. The polydispersity index (PDI) values of PHEMA and PHEMA-*b*-PVEA were obtained 1.14 and 1.19, respectively. The copolemr synthesized via RAFT technique was relatively low, suggesting a good control (Table 1).



Figure 5. UV/Vis spectrophotometer spectra of GNRs-CTAB and GNR@polymer (a), TEM image of GNRs (b) GNRs@copolymer (c), DLS diagrams of GNRs@copolymer and Zeta potentials of as-prepared GNRs-CTAB, GNRs@copolymer .

3.2. Preparation and characterization of GNRs@copolymer

GNRs@copolymer was readily obtained by reacting GNRs-DDT with thiol end capped PHEMA-*b*-PVEA through formation of the Au-S bond. Here, we first applied dodecanethiol to elimination CTAB bilayers, mixing the GNRs-CTAB with dodecanethiol resulted in a dislocation of bound CTAB on the nanorod surface. Addition of acetone actuated an aqueousorganic phase separation where GNRs were elicited into dodecanethiol (top black portion), leaving CTAB in the bottom aqueous solution. The aqueous phase turned clear, indicating that phase transfer ligand exchange performed successfully. The optical properties were investigated by UV-vis spectroscopy. As is well known, GNRs have two separate localized surface plasmon resonance (LSPR) bands at 515 nm and a strong longitudinal plasmon band around 782 nm due to the oscillation and resonance of surface plasmon along the short and long axes of the GNRs. After coating the surface with diblock copolymer, the wavelength slightly red shifted to 802 nm owing to the change in the local refractive index arising from the polymer shell. The absorption intensity increased in the visible region (515 nm) due to light scattering of polymeric shell [38]. The plasmon band remained narrow and well defined during the coating process, which suggested



Figure 6. In vitro release profiles of MTX-loaded GNRs@copolymer at various pHs (37 °C).

that the GNRs@copolymer preserved well the optical properties of the encapsulated GNRs and ruled out any possible aggregation (Fig. 5a)

The TEM image (Fig. 5b) shows, the obtained GNRs had a uniform and well-defined structure with an average length of 30 ± 5 nm. After coating, gold nanorods with thiol-functionalized diblock copolymer (PHEMA-b-PVEAQ -SH), can be seen pale shadow around GNRs (Fig. 5 c), which further confirm the self-assembly behavior of the synthesized diblock copolymer around GNRs.

we were evaluated the size of synthesized GNRs@ polymer by DLS measurements. The size of this GNRs@ polymer has been to 270 nm. Zeta potential is usually applied to verify the surface charge and stability of nanoparticles in solution (Fig. 5d). The sequential absorption of oppositely charged molecules onto gold nanorods' surfaces inverted the charge of gold nanorods after each coating step. As shown in Fig. 5e, the zeta potential of GNRs-CTAB is 20 mV because of the presence of a bilayer of CTAB on the gold nanorod surface. After coating with diblock copolymer PHEMA-b-PVEAQ-SH, surface charges of GNRs@copolymer were exhibited to become 13 mV. It can be clearly discrened that GNRs were individually and homogeneously coated by diblock copolymer shell and there was no sign of aggregation.

3.3. In vitro drug release study

All the GNRs were individually and homogeneously coated by the GNRs@copolymer shell and there was no sign of aggregation. The MTX-encapsulation efficiency was obtained to be 97.2% in the case of this study.

The zeta potential of GNR@copolymer was changed to -11.0 mV, which further confirmed the successful encapsulation of gold nanorods by the triblock copolymer shell. Also the surface charge of GNR@copolymer is negative, which benefits the adsorbtion of cationic molecules. When MTX was loaded on GNRs@copolymer , the zeta potentials were more positive at about 1.45. This result is because of the positive charge of MTX amino groups (at pH below their pKa= 8.3), increased the zeta potentials to much positive values. All of these results corroborated that MTX had been successfully introduced to the nanocomposite.

Gold Nanocomposites have been most applied as drug delivery for the ability to target a specific site, physical parameters, size of the carrier particles and physiological parameters [48]. MTX was applied as an anticancer drug to test the loading and controlled release behaviours of MTX and the capability for drug adsorption by GNRs@copolymer as a nanocarrier. MTX may be loaded into nanocarriers by an ionic interaction between the carboxylate anion of MTX and protonated amino groups of the nanocarrier at pH 7.4 [39-41].

The release behaviors of the MTX-loaded GNRs@copolymer were investigated at 37 °C. The release rate in a buffer solution at pH 7.4 (T=37° C) was quite low and about 29.4 wt% was released after 150 hours. At the pHs of 4 and 5.4 at the same condition, due to the partly release rate of MTX was accelerated and reached 42.8 and 34.4 wt%, respectively (Fig). When the pH value was reduced to the pKa value of MTX about 4-5, the carboxylate anion of the MTX was protonated. The uncompleted release of MTX might be related to the second pKa of MTX (4.8) which caused some of MTX to be deprotonated and, remain interacted with the nanocarrier.



Scheme 1. synthesis route of PHEMA, PHEMA-b-PVEA and thiol end capped PHEMA-b-PVEAQ-SH.



Scheme 2. The photograph of the MTX- loaded GNRs@copolymer.

Table 1. Characteristics of poly(HEMA) and poly(HEMA-b-VEA) samples			
Sample	Mna (GPC)	Mwa	PDIa
Poly(HEMA)	10400	11701	1.14
Poly(HEMA-b-VEA)	24000	30800	1.19

M_n; number average molecular weight, M_w; weight average molecular weight, PDI; polydispersity index determine from ^a GPC.

4. Conclusion

In this study, a novel drug delivery system, gold biodegradable nanorods-cored micelles were prepared by coating gold nanorods (GNRs) via synthesized thiol-end capped diblock copolymer [poly (2-hydroxyethyl methacrylate)-b-poly[(N-4vinylbenzyl),N,N-diethylamine quaternary)]; [PHEMA-b-PVEAQ-SH]. The molecular weights of PHEMA and [PHEMA-b-PVEA segments were obtained to be 10400 and 24000 gmol-1, respectively from GPC. The TEM images for the nanomicelle, nanorods and GNRs@copolymer demonstrated spherical, rod and pale shadow around GNRs shapes, respectively, and average diameter of 100 and 30 ± 10 nm. Methotrexate (MTX), as model anticancer drugs, was loaded effectively to GNRs@copolymer through electrostatic interactions with high loading efficiency (97.2%). The release rate in a buffer solution at pH 7.4, 5.4 and 4 (T=37° C) was released 29.4%, 34.4 wt% and 42.8 wt%, 5 10 15 20 25 30 35 40 45 0 50 100 150 Released drug (%) Time (h) pH=4 pH=5.4 pH=7.4 respectively after 150 hrs. Drug release studies corroborated that GNRs@copolymer-MTX is an excellent candidate for cancer therapy in result a low MTX release in the simulated blood stream at body conditions (pH 7.4). Also it having a high drug release at acidic conditions (pH 5.4 and 4.0). It may be prognoses that GNRs@copolymer -MTX could be utilited as a superlative candidate for drug delivery.

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Conflict of interest

The authors declare that they have no conflict of interests.

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