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Copper-gold nanoparticles: Fabrication, characteristic and application as drug carriers

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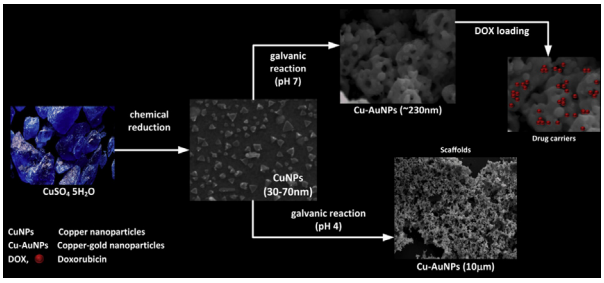
HIGHLIGHTS

- Porous copper-gold nanostructure as a cytostatic drug carrier was prepared.
- Kinetics and thermodynamics of drug adsorption were studied.
- DOX-loaded copper-gold nanoparticles showed a pH-controlled release rate.
- DOX-loaded copper-gold NPs caused inhibition cell proliferation of cancer cells.
- The Cu-Au NPs could serve as a theranostic platform for biomedical applications.

GRAPHICAL ABSTRACT



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ABSTRACT

In this investigation, the fabrication of porous core/shell nanostructures consisting of copper (core) and copper-gold nanoalloy (shell) for medical applications is presented. As a core triangular-shaped copper nanoparticles were used. The porous bimetallic nanoshell was prepared via galvanic reaction in the presence of oil-in water emulsion. It was proved that porous nanoalloy layer can be prepared at pH 7 and in the presence 0.1% and 0.5% oil-in water emulsion. The porous structure fabrication was mainly determined by volume fraction of hexadecane to acetone in the oil-in water emulsion and Zeta-potential of emulsion droplets (pH of emulsion). The influence of emulsion droplets size before galvanic reaction on porous structure preparation was negligible. It was found that doxorubicin could be easily introduced and released from porous core/shell nanostructures, due to spontaneous adsorption on the copper-gold nanoporous surface. The *in vitro* test showed that cytotoxic effect was more prominent once the doxorubicin was adsorbed on the porous copper-gold nanocarriers. It was demonstrated, that doxorubicin-loaded copper-gold nanostructures caused inhibition cell proliferation and viability of cancer cells, in a concentration-dependent manner. The results indicates that presented coper-gold nanocarrier have potential to be used in targeted cancer therapy, due to its porous structure and cytotoxic effect in cancer cells.

1. Introduction

Nowadays, metal nanoparticles (MNPs) have been investigated as a possible drug carriers for cancer therapeutics, radiotracers as

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well as contrast reagents [1]. The last decade has exposed various copper-based nanocrystals like copper sulfide or copper selenide, which create a new class of photothermal agent for brain imaging by using positron emission tomography (PET) or integrating PET with photothermal therapy (PTT) [2]. The studies reported by Zhao et al. have demonstrated the potential of copper-64-alloyed gold nanoparticles as a platform for oncological PET imaging [3]. The authors have proven that the direct incorporation of ^{64}Cu into the lattices of gold nanoparticles afforded stable radiolabeling and precise control of the specific activity of copper-64-alloyed gold nanoparticles. The radiolabeled ^{64}Cu-doped PdCu@Au core/shell tripods for use in PET have been the subject of Pang et al. research [4]. These core-shell tripods conjugated with $_{D}$-Ala$_1$-peptide T-amide (DAPTA) were capable of specific targeting of the C-C chemokine receptor 5 (CCR5) to the tumor cells in mouse model. Radiotracers are able to image a metabolism, proliferation, perfusion and drug/receptor interactions. The use of nanoparticles as alternative radiotracers will allow to obtain additional information about tumor cell metabolism [5]. The biomechanism of metal/metal oxide nanoparticles interaction with cells has been investigated by Teske and Detweiler [6]. It has been showed that properties of metal oxide nanoparticles can be modified by attachment of various ligands, such as carboxylic acids, phosphonates or silanes. For example, phosphonate-metal oxide nanoparticles have longer half-lives in organisms in compare to single metal oxide nanoparticles. However, nanoparticles modified by these organic ligands are able to produce toxic metabolites which can harm tissues and/or destroy nanoparticles [7].

Metallic nanoparticles have attracted tremendous attention for their biomedical applications such as medical diagnostics, drug delivery and self-therapeutics. For example, selenium nanoparticles (SeNPs) receive more attention as doxorubicin (DOX) nanocarriers due to their biocompatibility, low-toxicity and also antioxidant activity [8]. The results obtained by Lu et al. indicated that nanomaterials consisting of selenium nanoparticles conjugated with folate (FA) are able to overcome multidrug resistance cancer cells [8]. The interesting application of nanoparticles was demonstrated by Zhang et al. [9]. They have prepared porous composite consisting of mesoporous carbon and copper oxide nanoparticles for the purification of hemoglobin from human blood. The selective adsorption of a hemoglobin was achieved by adjusting the acidity of the mixture consisting of copper-oxide-carbon composite and protein solution at pH 7.

It should be pointed out that the unique properties of metal nanoparticles are related to their small size and shape (such as rods, plates, cubes, wires etc.). The dimensions of NPs (nanoparticles) prepared are associated with method of their synthesis [10]. Various methods are known to metal NPs production, i.e. chemical [11] and hydrothermal reduction [12], radiation method [13], vacuum vapour deposition [14] or/and laser ablation [15]. Usman et al. have reported synthesis of spherical shaped copper NPs in the presence of chitosan as a stabilising agent due to its chelate metals ability [16]. The proposed chemical reduction method allowed to obtain copper NPs with average mean sizes below 75 nm and an fcc crystal structure, however this reaction followed at high temperature (120–190 °C) [16]. Preparation of stable copper nanorods has been the subject of Panigrahi and co-workers studies [17]. It was demonstrated that copper NPs can be synthesise in simple reaction of copper sulphate with glucose in alkaline conditions, without any additional stabiliser or/and capping agent. Although, in order to prevent copper oxidation, reaction was carried out under nitrogen atmosphere. Josi et al. have reported the synthesis of pure and stable copper nanoparticles by using gamma radiation from ^{60}Co source in the presence of nonionic surfactants such as BRIJ 97 (cetyltrimethylammonium bromide) [12]. It has played dual role: first as a reductant and second as a stabilisers due to the oxyethylene group of BRIJ 97. The one-step method for copper nanorods and nanowires preparation under vacuum conditions has been described by Liu and Bando [13]. It was proved that NPs of copper prepared through this method were free of defects. Moreover the authors confirmed that this simple procedure can be also employed to produce other one-dimensional structure of various metals.

Based on recent reports it should be pointed that chemical reduction is the most preferred due to simple procedure and low cost production [18]. The selection of reducing agent is crucial from environmental point of view. Copper salts can be reduced in aqueous solution by using hydrazine, sodium borohydride or polyol and in non-aqueous media and under an inert atmosphere [19]. However, these reductors are costly, toxic and harmful to the environment and human health [20,21].

In this study, we have developed a new green method for the preparation of triangular-shaped copper nanoparticles in air conditions. The copper NPs were synthesised by chemical reduction using non-toxic and environmental friendly reducing agent, i.e. ascorbic acid. Moreover, in order to prevent aggregations of nanoparticles, polyvinylpyrrolidone has been applied as a capping agent. To the best of our knowledge, the use of ascorbic acid for green synthesis of triangular-shaped copper nanoparticles has not been reported.

The main goal of this report was designing a porous core/shell nanostructure with a core of copper nanoparticles and a porous shell of copper-gold nanoalloys. The bimetallic shell was obtained via galvanic reaction carried out in oil-in-water emulsion (Pickering emulsion). Nowadays there are only a few application of copper nanoparticles in medicine. Most studies suggest that copper nanomaterials should soon find many applications in various fields of medicine [22]. In this report, it was demonstrated, that core/shell nanostructure could be easily loaded by cytostatic and applied as drug delivery nanocarriers. Thus, several experiments focused on introduction and release of doxorubicin were performed, including kinetic and thermodynamic studies of drug adsorption. In order to assess the cytotoxicity of copper-gold and copper-gold-drug nanostructure the *in vitro* tests in human fibroblasts and HeLa cells were carried out.

2. Experimental part

2.1. Materials

Copper (II) sulphate pentahydrate (purity 99.995%), polyvinylpyrrolidone (PVP, purity 98–99%), L - ascorbic acid (purity >99.0%), chloride hydrate (purity 99.999%), hexadecane (purity 99%) and doxorubicin hydrochloride (DOX, purity 99%) were purchased from Sigma-Aldrich. Sodium hydroxide (purity 99.8%), gold(III) and acetone (purity 99.5%) were purchased from Avantor. All reagents were used without further purification. All the reactions were performed by using Milli Q water.

2.2. Synthesis of copper nanoparticles

Copper nanoparticles (Cu NPs) were prepared by two-step chemical reduction of copper sulphate using ascorbic acid. In typical procedure, 10 mL of aqueous solution of copper (II) sulphate pentahydrate (2%) was added to a 10 mL of 1% solution of polyvinylpyrrolidone (PVP). The solution of sodium hydroxide was added dropwise to adjust the pH to 7–8. The mixture was stirred for 1 h at room temperature in the presence of air. After stirring, the mixture was kept for 10 min at 65 °C and then the ascorbic acid solution (6%, 10 mL) was added gradually to the reaction mixture. The colour of the solution changed from yellow to dark orange.