

Preparation of peG-DOX nanoparticles as nano drug carrier and treatment methods to achieve the best effect

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Abstract: Among anticancer drugs, the research and development and preparation of nano drugs have always been the focus of current researchers, looking for appropriate molecular carriers that can make drugs play a better role. In this paper, PEG-DOX polymer was synthesized by using Poly(ethylene glycol) methyl ether (PEG-OH), 4-carboxybenzaldehyde, 4-(dimethyl-amino)-pyridine (DMAP), 1-(3-dimethylamino propyl)-3-ethyl carbodiimide hydrochloride (EDCI), triethylamine (TEA), hydrochloric acid, sodium sulfate, sodium bicarbonate, etc., through reasonable design and assembly, The stability, release behavior and antitumor activity of the drug were determined experimentally, to explore the best preparation scheme for the best effect of the drug.

Keywords: peG-DOX nanoparticles, Nano drugs, Anticancer drugs

1. Introduction

Cancer has always been a problem in human history. Humans started to fight against cancer since 19 century. Many people have questions about how cancer can kill you. It's something that most people worry about it at some point. Cancer is very harmful, it is started with mutation cells, these cells will continue to grow and start harming you, it is called the tumor. A tumor can appear anywhere on your body that has blood vessels. Most deathly cancers are liver cancer, lung cancer, and lymphoma. Cancer cells usually form during cell division the cell mutated. Usually, our immune system will devour the cancer cell in our body, but the cancer cell can trick the immune system to let the immune system think it is a normal cell. Because of this, the wrong mutated cell will grow bigger and bigger, and it will become a tumor. Cancer has a very high fatality rate, Scientist are trying very hard to overcome the cancer problem. It is now generally believed that there are five-way to cure cancer. Surgery, Chemotherapy, radiotherapy, immunity therapy, and targeted drugs. Surgery has a high risk, and some parts of your body can not take surgery. Chemotherapy and radiotherapy are risky also, these two therapy is killing your cancer cell and normal cell at the same time. It will cause huge harm to the patient. Immunity therapy is a great way to cure cancer, but now we have very little data and very few Clinical Trials, it is also very expensive, most people can not afford such a high price, and we are unsure if it will have side effects or not. The last is immunity therapy is the development of nanotechnology that has brought new hope for cancer diagnosis and treatment. Using nanotechnology to design functional biomaterials for targeted drug delivery can deliver drugs to the tumor site at a specific point, thus reducing the toxic and side effects of the drug on normal tissues. At the same time, magnetic materials, metal materials, quantum dots, etc. constructed by nanotechnology can significantly enhance the sensitivity and resolution of diagnosis, providing the possibility for early diagnosis and timely treatment of tumors. In recent years, due to the rapid development of nanotechnology, the nano-diagnostic and therapeutic agents used in biomedicine are constantly being updated. and therapeutic applications. However, most of the applications of nanomaterials are still in the basic research stage, especially in the process of tumor diagnosis and treatment, they are faced with problems such as biosafety, in vivo metabolism, and the stability of nanomaterial preparation, which limits the transformation to clinical medicine. . Therefore, the development of nanomaterials with excellent performance and high biosafety must be the focus and focus

of future scientific research. The nanocarrier-targeted drug delivery system, in simple terms, is to find suitable molecules to construct a nanoscale drug carrier, so that it can target and locate the action point of the drug. Targeting, the advantage of the time axis is that it can change the drug release rate, including the solubilization effect of poorly soluble drugs and the slow or controlled release effect of the drug; from the spatial axis, it can change the drug in vivo. distribution, allowing the drug to accumulate in certain target organ tissues.

2. Materials and Methods

2.1. Materials

Polyp-phenylmethyl ester (PEG-OH), 4-carboxybenzaldehyde, 4- (dimethylamino) pyridine (DMAP), 1- (3-dimethylamino propanol) -3-ethyl carbonate (EDCI), triethylamine (TEA), hydrochloric acid, sodium sulfate, sodium bicarbonate, cyclohexane, and diethyl ether were purchased from the Beijing factory. Amazon hydrochloric acid was purchased from Beijing Zhongshi Pharmaceutical Technology Development Co., LTD. All reagents were purchased by Sigma-Ardich and used unrefined.

2.2. Characterizations

The magnetic resonance spectra were recorded using a brock 400MHz spectrometer. Transmission electron microscope (TEM) images were obtained from a JM-2200 FS microscope. A commercial laser spectrometer (ALV/DSS/LSS-5022F) τ digital time correlator (ALV5000) and a 22mW single-phase He-Ne laser ($\lambda_0 = 632.8\text{nm}$) were used to obtain dynamic light scattering spectra. Fluorescence was measured by xenon lamp F4600 photoluminescence spectrometer.

2.3. Synthesis of the PEG-CHO polymer

Peg-oh (0.75g, 1 easy) 4-Caboxvinzald Hyde (0.18g, 1.2 easy) EDCI (0.24g, 1.2 easy), and deep seal (25mg, 0.2 easy) were added to a 50ml circular flask. After installing the magnetic stirring rod, Add 20ml of fresh dry DCM solid, completely dissolved. After stirring at room temperature for 24h, the organic phase was collected, and cleaned with 2MHCL aqueous solution, saturated sodium carbonate solution, saturated NaCl aqueous solution, deionized water several times, and dried with anhydrous Na₂SO₄. The final product was precipitated three times in ether/cyclohexane to obtain white powder.

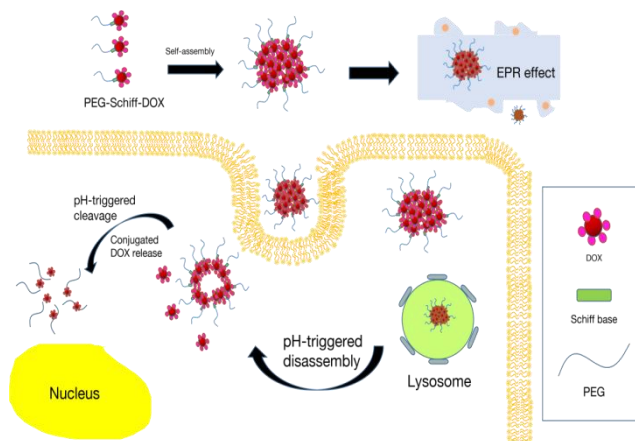


Figure1: Diagram of formation and transfer of PTX adhesive.

2.4. Synthesis of the PEG-DOX polymer

Peg-Cho (0.27g, 0.31mol), DOX · HCL (0.15g, 0.25mmol), TEA (70) μL , 0.5mmol) were dissolved in 10mL DMF solution and dissolved in nitrogen atmosphere. After the solvent was removed under vacuum, the crude product was dissolved in DCM, cleaned repeatedly with saturated NaCl solution and deionized water, and dried in anhydrous Na₂SO₄. The final product was precipitated three times in diethyl ether/cyclohexane to obtain a deep red powder.

2.5. Formation and self-assembly of the PEG-DOX nanoparticle

The PEG-DOX (5mg) was first dissolved in DMF (1mL), and then ionic water (4mL) was dropped at a rate of 0.05mL/min through an injection pump. During the self-assembly process, the 2h colloidal dispersion was further stirred at room temperature. The organic solvent was removed from the ion removal water by dialysis (MW truncation, 1kDa) for 3 days. These PEG-DOX nanoparticles were characterized by TEM and DLS.

2.6. Stability and pH-responsive degradation of the PEG-DOX nanoparticles

To determine the pH response of PEG-DOX nanoparticles, a certain amount of phosphoric acid buffered saline (PBS) with pH 5.0 and 7.4 was added to PEG-DOX nanoparticles (1mg/mL) 10mL. After gentle stirring at 37°C, the shape and size of the nanoparticles were displayed by TEM and 4hDLS.

2.7. In vitro DOX release from the PEG-DOX nanoparticles

The PH-triggered DOX emission was measured as follows. The dispersed PEG-DOX nanoparticles were placed into a dialysis membrane tube, and then in a water bath at 37°C in 30mL PBS (pH5.0 and 7.4) solution. The UV-VIS absorbance of 480nm solution was measured to determine the emission curve of DOX triggered by P. The DOX emission test was composed of formula 1 and 3 parts, and the results showed the mean standard deviation.

2.8. CCK-8 assay

MCF-7 cells were used to study the cytotoxicity of nanoparticles by CCK-8 assay. The cells were seeded onto 96 well plates with a density $\times 10^4$ cells /200 μ L of DMEM containing 10% of the side and back and incubated for 24 hours. Media replacement 90 μ After fresh DMEM medium containing 10% side slurry in L, suspensions of various binder concentrations were added to PBS (PH7.4) solution, culture was removed from the cell culture plate after 24 h incubation and immediately placed in fresh culture 100. μ L and cck-8 component solution 10 μ L, stirring evenly, heating in CO2 medium for 4h. Finally, pour L of solution into the 96 well plates. An enzyme scale was used to read the optical density at 450nm per well.

3. Results and Discussion

3.1. Analysis of synthetic results

The synthesis roadmap is shown in Figure 1 and the ¹H NMR spectrum of PEG-CHO is shown in Figure 2. Analysis shows that the ratio of the hydrogen of the benzene ring in the synthesized PEG-CHO corresponds to the methyl peak at the end of PEG, which can be inferred from the successful synthesis of PEG-CHO. ¹H NMR spectrum of PEG-DOX shows that the peak shape of the DOX drug molecule is more disordered and short, using the methyl on the end of PEG As a starting point for the analysis, the methyl peak corresponded essentially to the area of the main chain peak, while the peaks in the low-field region were thought to originate from the benzene ring and the Schiff base bond with a total of eight H. The areas corresponded essentially after integration, and new peaks attributed to the Schiff base bond were observed at $\delta = 8.1 \sim 8.5$ ppm. This indicates the successful preparation of PEG-DOX.[1]

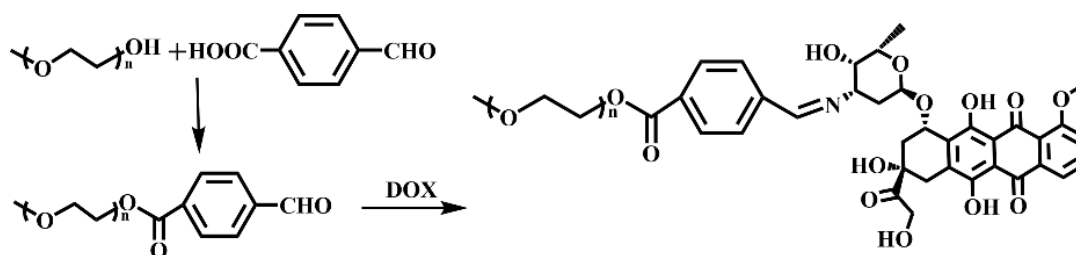


Figure 2: Synthesis protocol for PEG-DOX

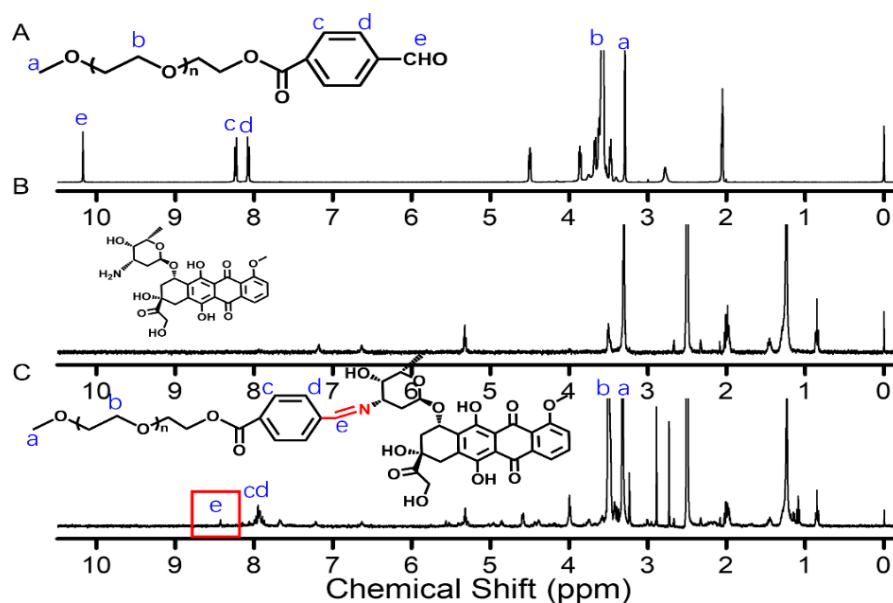


Figure 3: ^1H NMR spectra of PEG-CHO, DOX and PEG-DOX

3.2. Assembly morphology and degradation behaviour of PEG-DOX nanoparticles

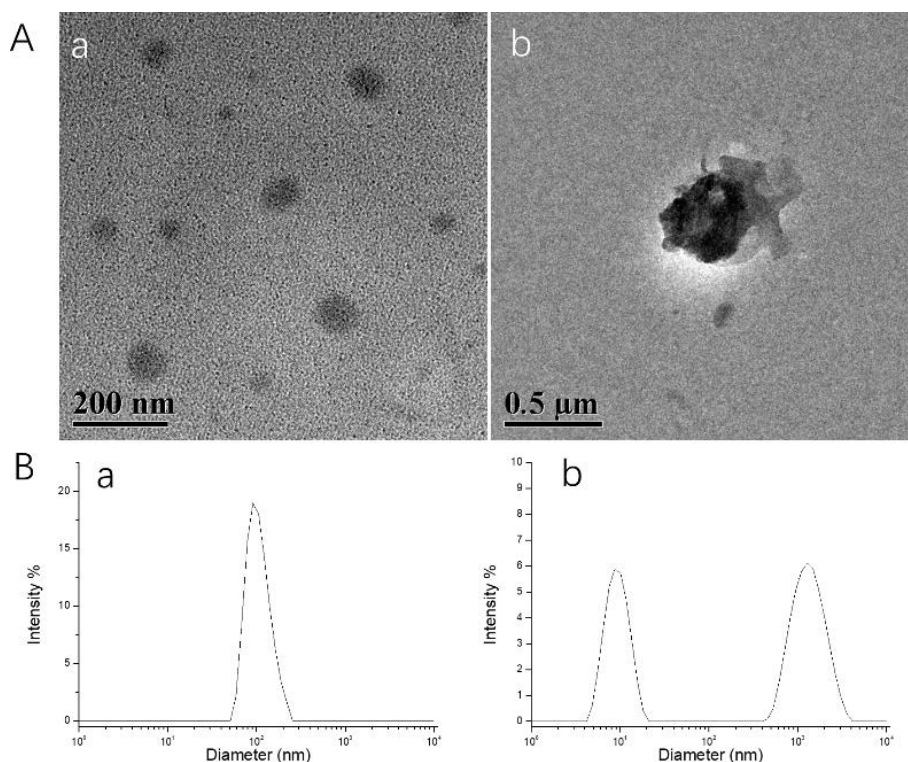


Figure 4: Morphology and particle size of PEG-DOX nanoparticles at (A) $\text{pH}=7.4$ and (B) $\text{pH}=5.0$

PEG-DOX was able to assemble spherical micelles below 200 nm in size in an aqueous solution at $\text{pH}=7.4$ (Figure 3A) and when the nanoparticles were placed in an aqueous solution at $\text{pH} 5.0$, the morphological structure of the nanoparticles was significantly disrupted with an amorphous structure. The pH responsiveness of the nano-drug is determined by the change in particle size after two hours of shaking at 37 degrees Celsius in a buffer with a pH of 5.0. Figure 3B shows that there was a significant change in the particle size of the PEG-DOX nanoparticles before and after the acid treatment, with some of the nanoparticles having completely disintegrated. The narrowing of the particle size distribution indicates that the inhomogeneous nanoparticles disintegrate in the presence of acid and release the drug.[2][3]

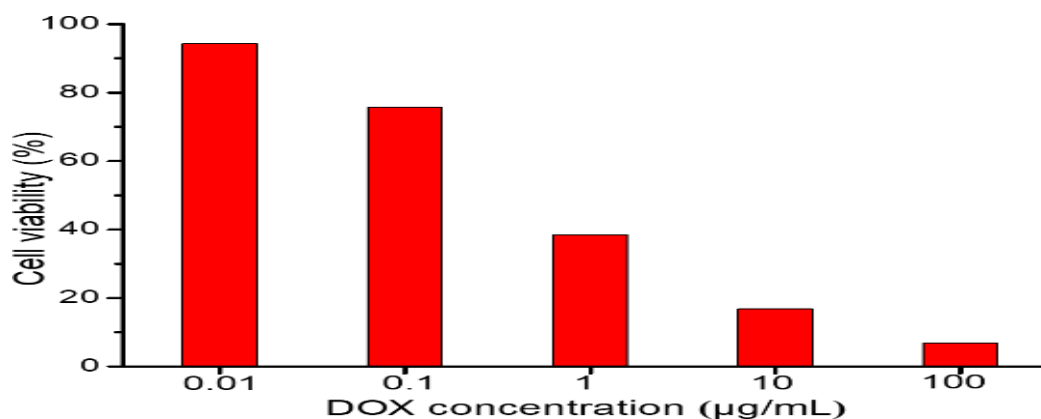


Figure 5: Anti-cancer cellular activity of PEG-DOX nanoparticles

3.3. Drug release behavior

DOX is bound to PEG via an acid-unstable junction, and DOX is released by cleavage in the micro-acidic microenvironment of tumor tissue. To confirm that bound DOX can be released in situ, we used UV to detect the degradation fraction of PEG-DOX. The results of the drug release behavior were more intuitive.

We performed in vitro drug release experiments in phosphate buffer at pH 7.4 and acetate buffer at pH 5.0, respectively, and the drug release profiles are summarised in Figure 4. As seen from the UV assay results. As shown in Figure 4, there was almost no drug release from PEG-DOX nanoparticles loaded at pH = 7.4, while the release behavior was significantly enhanced at pH = 5.0.

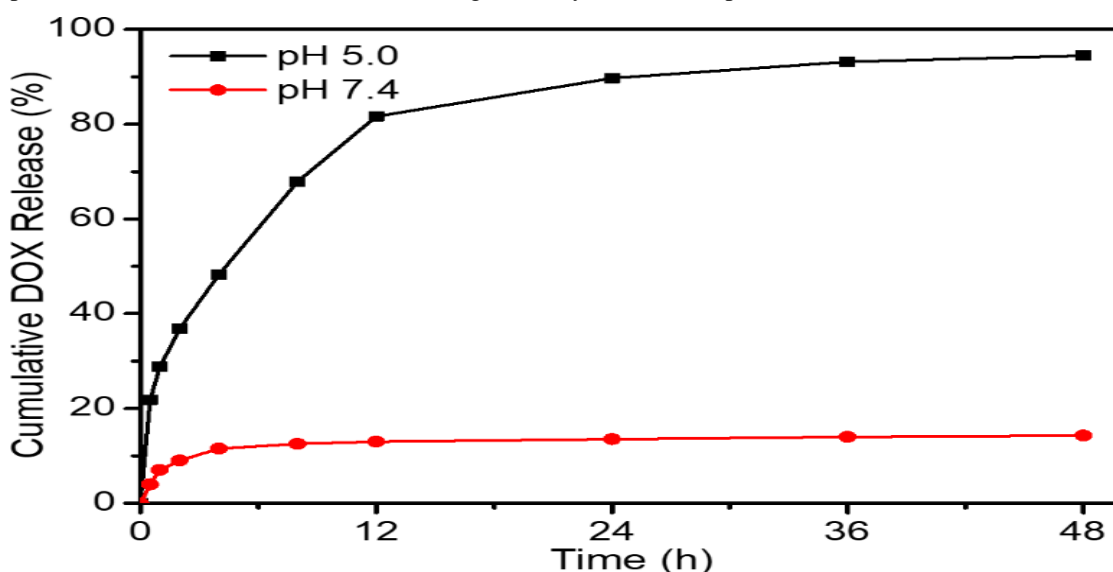


Figure 6: In vitro release profile of PEG-DOX-loaded nanodrugs

3.4. Antitumour activity

In vitro cytotoxicity of nanoparticles against MCF-7 cells was assayed by the CCK-8 assay. Figure 6 shows the efficient antitumor activity against MCF-7 cells after 24 h incubation. Viability of MCF-7 cells was correlated with DOX concentration. At low concentrations, PEG-DOX nanoparticles had similar toxicity to low concentrations of free DOX. At higher concentrations, DOX molecules readily enter the cell and cell membrane by passive diffusion, whereas PEG-DOX nanoparticles are localized by intracellular action and then escape within the lysosome, followed by drug distribution in the cytoplasm and nucleus. The passive diffusion of free DOX molecules in this situation is substantially faster than the internalization process of the nanopolymers discussed above, indicating a relatively stronger anti-tumor capacity.

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