pH-Sensitive Polymeric Nanoparticles for Effective Delivery of Doxorubicin

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Abstract. pH-responsive micelles beam nanoparticles were created using the self-assembly of PEG-Shiff-DOX medication. Under normal circumstances, these nanoparticles have great storage stability for more than a week, however they will degrade fast in a weak acidic environment. We saw a process-dependent drug release behaviour. This could lead to higher intracellular drug concentrations and a more sustained effect. The anti-tumor efficacy of nanoparticles on HeLa cells is superior to that of free DOX, according to the CCK-8 analysis. The potential for creating conversion DOX formulations for the treatment of cancer with these pre-drug-based nano-drugs is enormous.

Keywords: pH-responsive; Prodrug; Polymeric nanoparticles; drug delivery; Schiff base.

1. Introduction

Because of their potential to enhance drug solubility, improve therapeutic efficacy and reduce side effects, polymer-based nanodrugs have been extensively studied in cancer therapy over the past few decades [1-4]. They have several advantages, including prolonged cycle time by evading glomerular filtration, improved pharmacokinetic properties, and enhanced tumour accumulation through enhanced penetration and retention (EPR) effects [5-6], compared with small-molecule anticancer drugs. Among the different types of nanoparticulate, prodrug, micellar, vesicular, nanogel and liposomal nanoparticulate [7], prodrug nanoparticulate have attracted more attention due to their clear and simple structure and great potential in clinical translation.

The reported PEG-DOX prodrug has a number of inherent defects, including a relatively low drug loading capacity (DLC) and an incomplete release of the drug. In order to ensure the successful release of the drug into the cells, it is important that the cross-link between the drug and the polymer must be lystocellular in the environment of the tumour cells. Redox-sensitive disulphide and acid-unstable acetal linkages have been used to prepare stimulating polymeric DOX prodrugs due to the abnormal conditions in tumour cells, such as high glucose sulphide concentration and low pH [18-22]. Furthermore, for the drug release system, the DLC and the drug release kinetics are of great importance. Increasing the DLC not only reduces costs but also decreases the need for excipients, reducing the risk of toxic side effects. So much energy has been put into finding new DLC-rich carriers. The concentration of the drug in the cells and the time of action of the drug are related to the kinetics of drug release.

The reported PEG-DOX prodrug has a few flaws that are inherent, such a poor drug carrying capacity (DLC) and insufficient drug release. The connective body between medications and macromolecules must be lystocellular in the environment of tumour cells to enable effective intracellular drug release. Redox-sensitive disulfide and acid-unstable acetal linkages have been used to produce stimulating polymeric DOX prodrugs [18-22]. These prodrugs take advantage of the abnormal conditions in cancer cells, such as high glucose sulfide concentration and low pH. DLC and the kinetics of drug release are also very important to the drug release mechanism. Higher DLC results in cheaper costs as well as a reduction in the amount of carrier materials needed, which lowers the danger of hazardous side effects. People have spent money because of this. The pH-triggered acetal Schiff base breaks in the acidic environment of the lysosome region of the cell, leading to the breakdown of nanoparticles. Subsequently, the encapsulated DOX is quickly released,The hidden Schiff base is then exposed to the acidic environment and finally the fully released conjugated DOX is available for tumour therapy.

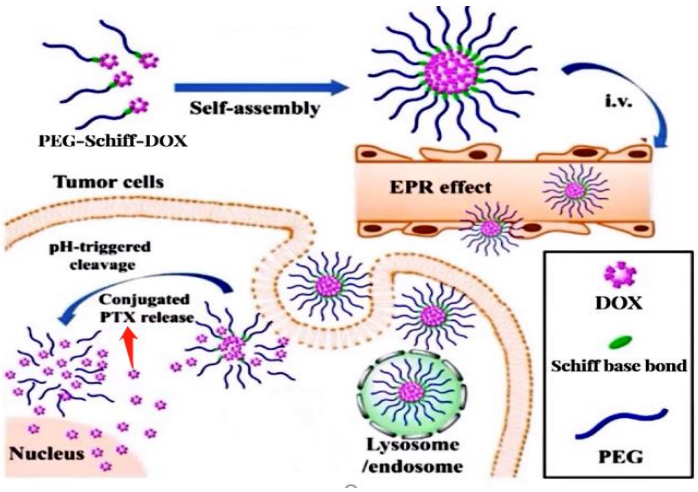

Figure 1. Schematic showing how PTX-loaded micelles form and release

2. Materials and methods

2.1. Reagents and raw materials

You may buy doxorubicin hydrochloride, carboxybenzaldehyde, 4-dimethylaminopyridine, Anhydrous N, N-dimethylformamide (DMF) and anhydrous dimethylsulfoxide (DMSO) are commercially available. The water utilized for the experiment is ultra-pure, and all other chemical reagents were directly acquired from Heilongjiang Chemical Factory.

2.2. Advanced Hockey 400 (400 MHz) spectrometer for 1H NMR.

The deuterated reagents are deuterated acetone-D6, dimethyl sulphoxide (DMSO-D6), deuterated chloroform (CDCl3), and TMS is the internal standard, measured at 25 oC. Transmission electron microscopy (TEM) was performed using a JEM-2200FS (JEOL, Japan) electron microscope at an accelerating voltage of 100 kV. Pour three litres of the sample onto a copper mesh (300 mesh) wrapped in carbon paper. Use filter paper to remove excess liquid. A Gatan multi-scan CCD was used to capture the images and Digital Micrograph was used to process the images taken with an electron microscope.

Malvern Zetasizer Nano ZS dynamic light scattering particle size analyser. Dynamic Light Scattering (DLS).The sample cell for measuring particle size is a quartz cuvette that is fitted with a 633 nm He Ne laser with a 173°detection angle.

Shimadzu TU1901 UV Vis Spectrophotometer for UV Vis.

2.3. Synthesis of pH responsive prodrugs

The two processes for the production of pH-responsive prodrugs are shown in Figure 2.

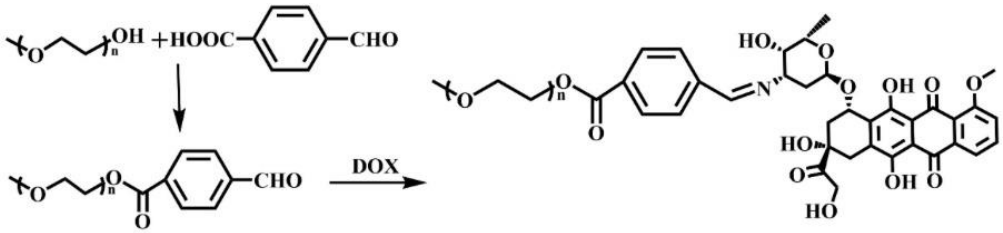

Figure 2. Synthesis Scheme of PEG-Schiff DOX

2.3.1 Synthesis of PEG-CHO

Add PEG-OH (375 mg, 0.5 mmol) while nitrogen is being protected after dissolving para carboxybenzaldehyde (150 mg, 1 mmol), EDCI (191.7 mg, 1 mmol), and DMAP (61 mg, 0.5 mmol) in ultradry DCM. A total of three washes with 1 M HCl, saturated NaHCO3, and saturated salt water

were performed after the system was agitated at 37? for 24 hours. Gather organic materials, spin-dry it, filter it, then dry it with anhydrous magnesium sulfate. 86% was the final output.

2.3.2 Synthesis of PEG-Schiff DOX

Doxorubicin (50 mg, 90 M), TEA (70 L), and PEG-CHO (100 mg, 110 M) were dissolved with the reaction over night in 3 mL of anhydrous DMF. It was dissolved in a significant volume of DCM, extracted three times with saturated salt water, and precipitated in cold ether after the reaction solvent was removed through rotary evaporation. 78% was the final output.

2.3.3 Stability and pH Responsiveness of Nanodrugs

Test the particle size distribution of the nanodrug by dynamic light scattering after dissolving it in a PBS buffer solution with a pH of 7.4 (0.5 mg/mL), and then test again after two weeks to compare the changes.

The nano medicine should be dissolved in a PBS buffer solution with a pH of 5.0 (0.5 mg/mL), then placed in a water bath at 37˚C for two hours to determine its particle size distribution using dynamic light scattering.

2.4. Nanoparticle stability and pH-dependent degradation

The storage stability of the nanoparticle suspensions were assessed using differential scanning calorimetry (DSC), comparing the particle size distributions before and after 6 months of storage at room temperature. In order to study the pH-dependent degradation behaviour of the nanoparticles, the lyophilized powder of the nanoparticles was redispersed in phosphate buffer at pH = 5.0 at a concentration of 1 mg/ml.

3. Results and discussion

3.1. Analysis of chemical structures

Figure 3 displays the PEG-CHO 1H NMR spectrum. The proportion of hydrogen in the benzene ring of the synthesised PEG-CHO, according to analysis, matches to the methylpeak at the end of the PEG, demonstrating the success of the PEG-CHO synthesis.

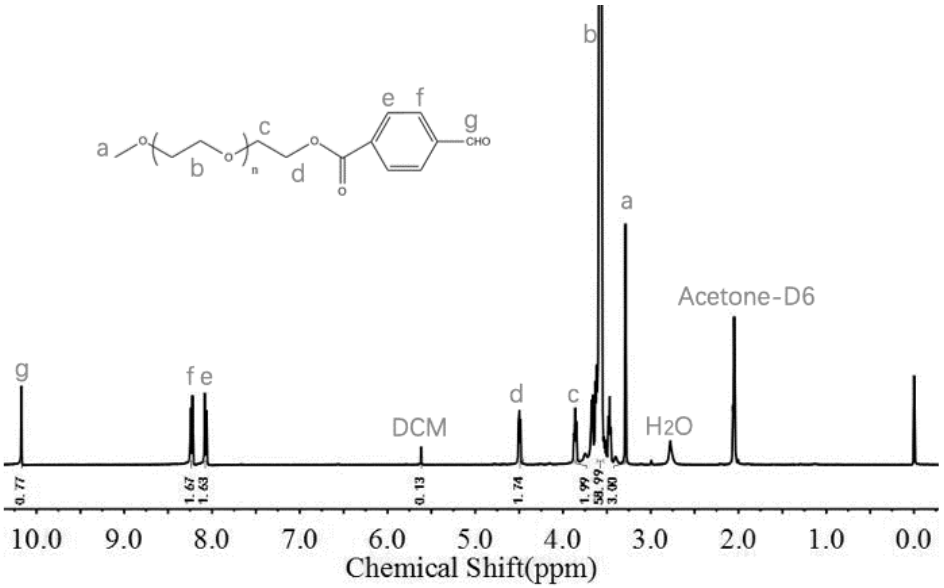

Figure 3. ^{1}H NMR Spectrum of PEG-CHO

The PEG-Schiff-DOX 1H NMR spectrum (Figure 4) reveals that the drug molecule's peak shape is comparatively disordered. The methyl peak corresponds to the region of the main chain peak when the methyl group at the end of the PEG is used as the analysis's starting point. The area after integration supposedly corresponds to the area = 8 and the peaks in the low field zone are said to

result from an overall 8H on the benzene ring and Schiff base bond.At 8.1 ppm, a single new peak ascribed to Schiff base bonding groups was discovered, demonstrating the effective manufacture of PEG-Schiff DOX polymer and verifying the development of Schiff base connections.

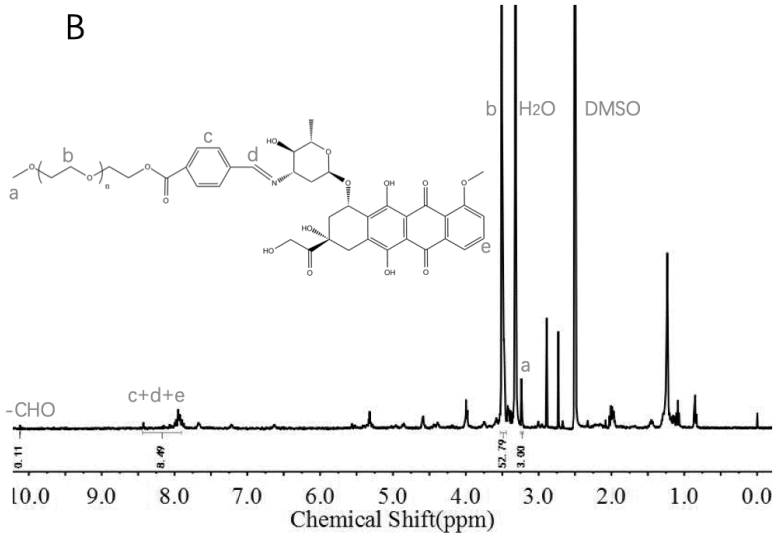

Figure 4. [1]H NMR Spectrum of PEG-Schiff-DOX

3.2. Self-assembly and degradation behavior of PEG-Schiff DOX nanoparticles

In an aqueous solution with a pH of 7.4, PEG-Schiff DOX may build spherical micelles smaller than 200 nm (Figure 5A). The shape and structure of the nanoparticles are drastically altered when they are dissolved in an aqueous solution with a pH of 5.0, resulting in an amorphous form. When nanomedicine is shaken at 37°C for two hours in a buffer solution with a pH of 5.0,variations in particle size indicate how sensitive the nanomedicine is to pH.

Figure 5B demonstrates the PEG Schiff DOX nanoparticles' particle size altered dramatically before and after being exposed to acid, and how some of the particles entirely dissolved. Narrow particle size distribution suggests that heterogeneous nanoparticles break down and release medicines when acid is applied.

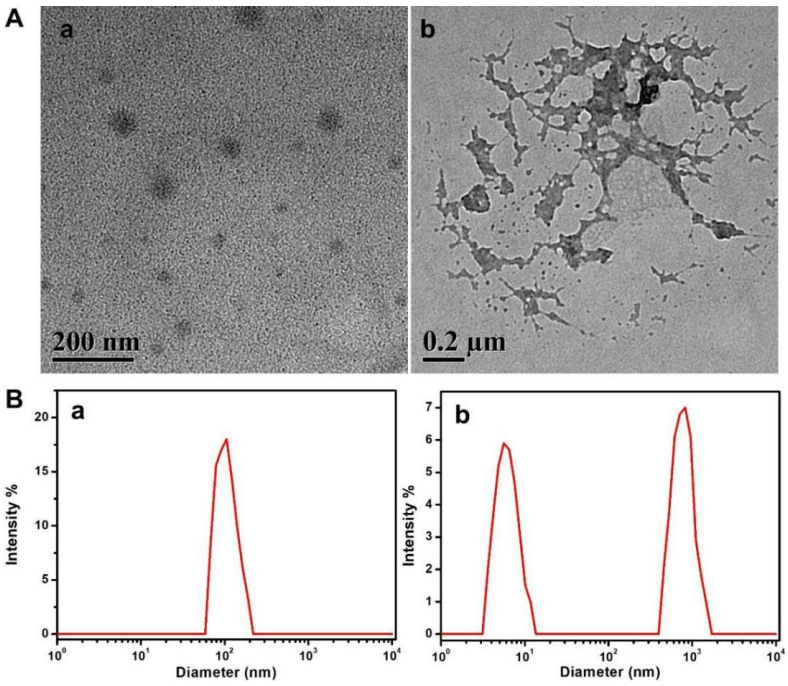

Figure 5. Morphology and size of PEG-Schiff-DOX nanoparticles at (A) pH=7.4 and (B) pH=5.0

The UV detection results show that the outcomes of drug release behavior are more logical. According to Figure 6, When pH was 5.0, PEG-Schiff DOX nanoparticles produced a substantial amount more medication than when pH was 7.4.

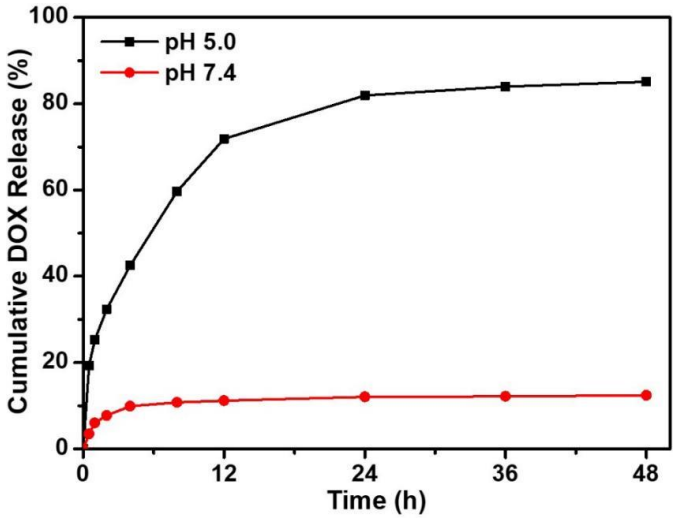

Figure 6. In vitro release curve of gel containing PEG Schiff-DOX nanoparticles

Figure 7 illustrates the outstanding anti-tumor effects of PEG-Schiff DOX nanoparticles, particularly when the concentration of loaded DOX approaches 100 M. With a cell survival rate of less than 8%, PEG Schiff-DOX had a significantly inhibiting impact on tumor development, indicating that the produced PEG-Schiff-DOX is a viable targeted formulation for therapeutic use.

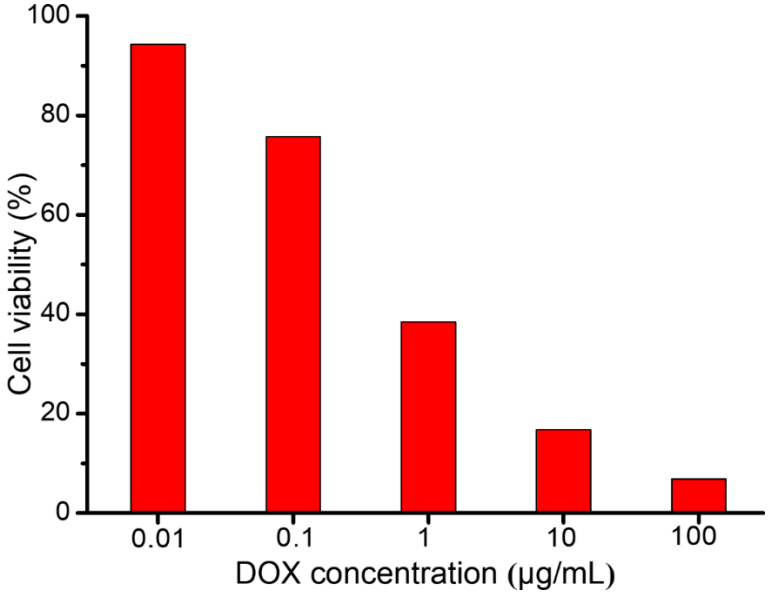

Figure 7. Evaluation of the anti-tumor effect of PEG-Schiff-DOX nanoparticle

4. Conclusion

In conclusion, we created an amphiphilic PEG-Shiff-DOX prodrug and synthesized it. We next used the prodrug and free DOX to self-assemble unique pH-responsive micellar nanoparticles. These prodrug-based nanoparticles are notable for having the following benefits: (1) distinct and well-defined structures; (2) high drug concentration; (3) excellent loading capacity for active ingredients; (4) Good shelf life (can be stored at room temperature for more than 1 week); (5) superior tumor inhibition compared to free DOX; 6) negligible or low drug leaks in neutral circulation, (7) endosomal pH-triggered release of pure DOX. These nanoparticles based on prodrug offer a viable alternative for creating DOX formulations that are translated.

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