

**Preparation of Zwitterionic Polypeptides and Biomedical Application**

Huayu Tian, Xiuwen Guan, Yanhui Li, Zixue Jiao, Xuesi Chen

Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
thy@ciac.jl.cn

**ABSTRACT SUMMARY**

A novel drug delivery system was developed by electrostatic binding of pH-sensitive charge conversional PEI-poly (L-lysine)-poly (glutamic acid) (PELG), polyethylenimine (PEI) and pH-sensitive modified doxorubicin (DOX). DOX was confirmed to be efficiently delivered into cancer cells, and the PELG/PEI/CAD system showed higher drug release and endocytosis in lower pH (tumor tissues), which would lead to higher killing effect on cancer cells.

**EXPERIMENTAL METHODS**

The PELG was synthesized by ring opening polymerization. Firstly, the N-carboxyanhydride of ε-benzoxycarbonyl-L-lysine (Lys (Z)-NCA) and N-carboxyanhydride of γ-benzyl-L-glutamate (BLG-NCA) were synthesized. Secondly, PEI, Lys (Z)-NCA and BLG-NCA were dissolved in dried chloroform and stirred for 72 h at 30 °C. After reaction, the mixture was deposited with excess diethyl ether. After filtration, PEI-poly (benzoxycarbonyl-L-lysine)-poly (benzyl-L-glutamate) (PEI-PLys (Z)-PBLG) was dried. The benzoxycarbonyl and benzyl of protected PEI-PLys (Z)-PBLG were removed to obtain PELG.

The synthesis of CAD was conducted by dissolving certain amount of DOX and cis-aconitic anhydride (CA) in DMF, stirred for 24 h at 25 °C in dark. After reaction, the product was extracted and obtained by lyophilization.

The PELG/PEI/CAD complex solution was prepared by mixing PEI and CAD aqueous solution together and vortex. After incubation, PELG was added to the previous solution to obtain PELG/PEI/CAD ternary complex.

The zeta potentials of the complexes were measured by zeta potential/BI-90Plus particle size analyzer. In vitro drug release behavior was also studied. The cytotoxicities of complexes were assessed with methyl thiazolyl tetrazolium (MTT) assay. Cell uptake studies were carried out by flow cytometry (FCM) and confocal laser scanning microscopy (CLSM).

**RESULTS AND DISCUSSION**

The \(^1\)H NMR and GPC results demonstrated that the PELG was successfully synthesized. CAD was characterized by \(^1\)H NMR, FT-IR spectra and mass spectrometry (MS).

The zeta potential study showed the PELG/PEI/CAD complexes were negatively
charged in pH 7.4, while in acidic pH (pH 6.8, 6.4 and 5.8), they were positively charged (+22.1~+25.7 mV), which would help to interact with negatively charged cell membranes, leading to higher cell uptake efficiency.

The cumulative drug release of PELG/PEI/CAD complexes increased with the pH decreasing (Figure 1). In pH 7.4, about 49.2% drug released in 24 h, and the cumulative drug release reached to 69.9% in pH 6.4. In acidic environment the pH-sensitive cis-aconityl linkage of CAD could be cleaved and DOX would be released.

![Figure 1](image1.png)

**Figure 1.** Drug release of PELG/PEI/CAD in different pH values.

The PELG/PEI/CAD showed significant differences in cytotoxicity between different pH values (Figure 2). In acidic tumor area, the complexes showed higher killing effect on cancer cells. In pH 7.4, the complexes had relatively lower toxicity which could reduce the side effects on normal tissues. No significant changes were observed in the negatively charged PGA/PEI/CAD system.

FCM and CLSM results showed PELG/PEI/CAD had higher cell uptake in pH 6.8 than 7.4. A lot of DOX was observed in cells, indicating the pH-sensitive charge conversional system had excellent ability to deliver DOX into cells. It was worthy to note that more DOX was found in nuclei in pH 6.8. This benefited from the combined effect of pH-sensitive charge conversional shielding PELG and pH-sensitive cleavable modified DOX.

![Figure 2](image2.png)

**Figure 2.** Cytotoxicities of PELG/PEI/CAD and PGA/PEI/CAD in different pH values.

**CONCLUSION**

A pH-sensitive charge reversal shielding system was designed to deliver anti-tumor drug doxorubicin. Both PELG and CAD have pH-responsive characteristic, and DOX can be effectively delivered into the cancer cells to inhibit their growth. According to this study, we can believe that the system is a promising delivery system in biomedical application.

**REFERENCES**


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