Synthesis and characterization of F127-Glu@ZnO nanogel material for cisplatin drug delivery application

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Received 16 Sep. 2024; Revised 10 Nov. 2024; Accepted 15 Nov. 2024; Published 6 Dec. 2024.

DOI: https://doi.org/10.54939/1859-1043.j.mst.FEE.2024.237-242

ABSTRACT

A novel F127-Glu@ZnO nanogel was synthesized for cisplatin delivery by functionalizing ZnO nanoparticles with L-glutamic acid (Glu) and grafting Pluronic onto Glu via urethane linkages. FT-IR and ^{1}H-NMR confirmed successful synthesis, while TEM characterized morphology. The nanogel exhibited pH-responsive drug release, accelerating cisplatin release in acidic environments. This suggests its potential as a smart drug delivery system for improving therapeutic efficacy and reducing side effects in cancer treatment.

Keywords: Zinc oxide nanoparticles (ZnO NPs); Glutamic acid; Cisplatin; Drug delivery system.

1. INTRODUCTION

Despite advances in cancer detection and treatment, it remains a significant health challenge. Traditional chemotherapy often employs highly cytotoxic drugs that lack selectivity, leading to severe side effects [1]. To address this, researchers are developing targeted drug delivery nanosystems [2].

Cisplatin (CPT), a widely used chemotherapeutic, exhibits high toxicity to both healthy and cancerous cells, causing drug resistance and adverse effects such as nausea, vomiting, bone marrow suppression, and organ damage [1, 3]. Innovative drug delivery strategies aim to mitigate these drawbacks [4, 5].

Zinc oxide nanoparticles (ZnO NPs) are a promising anticancer agent [6]. Their biocompatibility, antibacterial properties, and anticancer activity make them a potential platform for drug delivery [7, 8]. ZnO NPs can be used as bare nanoparticles or surface-modified with organic molecules to enhance biocompatibility [9], bioavailability, targeting [10], and controlled drug release [11].

This study developed a novel ZnO NP-based nanogel surface-modified with Glu and conjugated to Pluronic F127 via urethane linkages to enhance cellular uptake and biocompatibility. This nanogel, loaded with cisplatin (CPT), achieved a drug loading efficiency of 18.62% and encapsulation efficiency of 89.97%. The system exhibited pH-dependent drug release, with increased release in acidic environments, highlighting its potential for cancer therapy.

2. MATERIALS AND METHODS

2.1. Materials

The chemicals and materials utilized in this study include: L-glutamic acid (Glu, Bio Basic, product code: GB0221), Pluronic F127 (F127, Sigma Aldrich, BioReagent), 4-Nitrophenylchloroformate (NPC, Sigma Aldrich, for peptide synthesis), 3-Amino-1-propanol (Ami, Sigma Aldrich, for synthesis), Anhydrous ethanol (EtOH, Macklin, China), Tetrahydrofuran (THF, Fisher, analytical reagent grade), Double distilled water (DW, TDS $\leq$5).

Spherical zinc oxide nanoparticles (ZnO NPs) with a size range of 20 - 40 nm were synthesized using guava leaf extract, following a previously published procedure from our group [12].

2.2. Methods

2.2.1. Activation of terminal hydroxyl groups of pluronic F127

Pluronic F127 was activated with 4-nitrophenyl chloroformate (NPC) according to a previously reported procedure [13] with minor modifications. Briefly, F127 (1 mmol) was melted at 60 °C under nitrogen and stirred magnetically. NPC (2.5 mmol) was added, and the reaction proceeded for 6 hours. After cooling to room temperature, 10 mL of THF was added, and the mixture was stirred for an additional 4 hours. THF was then removed under reduced pressure. The resulting product (NPC-F127-NPC) was dissolved in EtOH and purified by precipitation in cold diethyl ether. Next, a solution of 1.2 mmol 3-amino-1-propanol in 5 mL THF was added dropwise to a solution of 1 mmol NPC-F127-NPC in 50 mL THF. The reaction was carried out for 24 hours at room temperature. THF was removed under reduced pressure, and the crude product was washed and precipitated in cold diethyl ether. The final product, NPC-F127-OH, was obtained after drying under vacuum at room temperature for 24 hours.

2.2.2. Synthesis of F127-Glu@ZnO

ZnO NPs (0.81 g) were dispersed in 200 mL each of EtOH and distilled water by sonication. Glu (0.74 g) was added and stirred vigorously for 30 minutes until dissolved. After further sonication for 60 minutes, the product was collected by centrifugation (10,000 rpm, 5 minutes) and washed with distilled water to remove unreacted Glu. The Glu-functionalized ZnO was redispersed in 200 mL of distilled water, then 2 g of NPC-F127-OH was added. The mixture was stirred at room temperature for 48 hours. The final product, F127-Glu@ZnO, was obtained after centrifugation, washing, and drying under vacuum at 40 °C for 24 hours.

2.2.3. Characterization of materials

The successful synthesis of F127-Glu@ZnO was confirmed by spectroscopic techniques. FT-IR spectra (Tensor II, Brucker) were recorded using the KBr pellet technique in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. ^{1}H-NMR spectra (AvanceNEO, Bruker, 600 MHz) were acquired in D_2O. The morphology of the material was observed using transmission electron microscopy (TEM, JEM 2100). The concentration of CPT in solution was determined by the standard curve method using UV-Vis spectroscopy at a wavelength of 705 nm.

2.2.4. Drug loading experiments

F127-Glu@ZnO (250 mg) was dispersed in 250 mL of distilled water (DW) by sonication and cooled to 4 °C. CPT solutions (100 mL) at various concentrations were added dropwise under continuous stirring for 24 hours. After water removal by vacuum evaporation, the solid was redispersed in 100 mL DW and dialyzed against DW (3500 Da membrane) for 8 hours, with dialysate changes every 4 hours. Free CPT in the dialysate was quantified by UV-Vis spectroscopy (705 nm) after complexation with o-phenylenediamine (OPDA). Drug loading efficiency was evaluated by drug loading entrapment (DLE) and drug loading capacity (DLC).

$$DLE\ (\%) = \frac{m_{0(CPT)} - m_{1(CPT)}}{m_{0(CPT)}} \times 100\% \quad (1)$$

$$DLC\ (\%) = \frac{m_{0(CPT)} - m_{1(CPT)}}{m_{0(CPT)} + m_C - m_{1(CPT)}} \times 100\% \quad (2)$$

where: DLE - Entrapment efficiency of CPT (%); DLC - Drug loading of CPT (%); $m_{0(CPT)}$ - Initial mass of CPT (mg); $m_{1(CPT)}$ - Mass of free CPT (mg); m_C - Mass of F127-GA@ZnO carrier (mg).

2.2.5. Drug release test

In a 50 mL Erlenmeyer flask, 20 mg of F127-Glu@ZnO/CPT was dispersed in 20 mL of PBS buffer. This suspension was then subjected to dialysis using a membrane with a molecular weight cut-off of 3500 Da against 100 mL of PBS buffer at the same pH. At predetermined time intervals,

3 mL aliquots of the dialysate were withdrawn for CPT release quantification and immediately replaced with an equal volume of fresh PBS buffer. The cumulative drug release was calculated using the following formula:

$$Q = C_n V_s + V_t \sum_{i=1}^{n-1} C_{n-1} \quad (3)$$

where: Q is the amount of CPT released from the nanocarrier; C_n is the concentration at time t; V_s is the volume of PBS; V_t is the volume of the sample; $\sum_{i=1}^{n-1} C_{n-1}$ is the concentration of CPT released over time.

3. RESULTS AND DISCUSSION

3.1. Results of pluronic F127 activation

FT-IR spectra of F127 and NPC-F127-OH (figure 1a) show characteristic peaks for -CH_2 and -CH_3 stretching vibrations (2972 cm^{-1} and 2878 cm^{-1}), bending vibrations (1340-1470 cm^{-1}), and C-O and C-O-C stretching vibrations (1000-1150 cm^{-1} and 840-960 cm^{-1}). NPC-F127-OH also exhibits peaks for C=O stretching in the chloroformate group (1770 cm^{-1}), NO_2 group (1590 cm^{-1}), and a pronounced hydroxyl peak (3500 cm^{-1}). The emergence of a peak at 1645 cm^{-1} (-NHCOO-) confirms partial substitution with 3-amino-1-propanol, indicating successful synthesis of HO-F127-NPC.

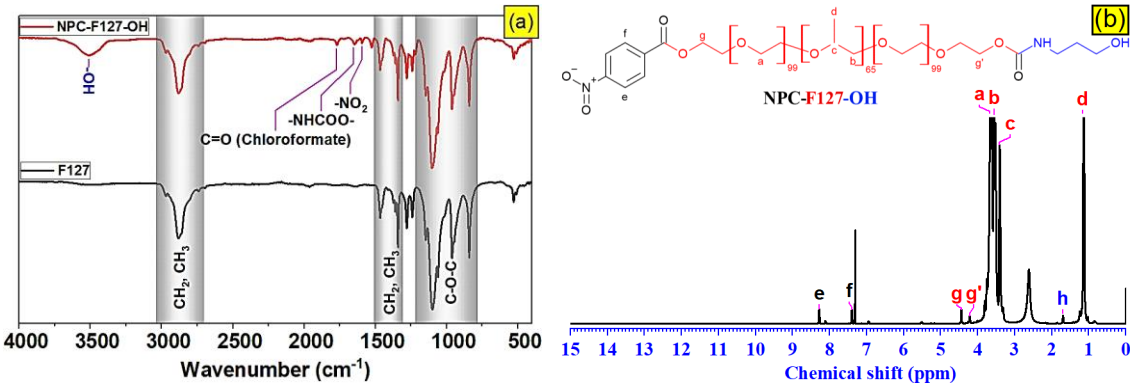

Figure 1. *FT-IR (a) and ^{1}H-NMR spectra of NPC-F127-OH.*

The ^{1}H-NMR spectrum (600 Hz, D2O) of the modified F127 product (figure 1b) shows characteristic signals for PEO protons (a) at δ = 3.55-3.64 ppm, PPO protons (b, c, and d) at δ = 3.52-3.54 ppm, 3.4 ppm, and 1.1 ppm, respectively, and NPC aromatic protons (e and f) at δ = 8.2 ppm and 7.3-7.4 ppm. Replacement of NPC by 3-amino-1-propanol (Ami) in NPC-F127-OH is evidenced by the signal at δ = 1.68-1.70 ppm (h). Attachment of Ami and NPC to F127 is confirmed by signals at δ = 4.43-4.45 ppm (g, -CH_2-O-NPC) and δ = 4.216 ppm (g', -CH_2-O-CO-Ami) [14].

3.2. Synthesis and Characterization of F127-Glu@ZnO nanogel

FT-IR and ^{1}H-NMR spectroscopy confirmed the successful synthesis of F127-Glu@ZnO (figure 2a). The FT-IR spectrum exhibits characteristic peaks for ZnO NPs (420 cm^{-1}), Glu (1240 cm^{-1}, 1278 cm^{-1}, 1406 cm^{-1}, 3015-3290 cm^{-1}, 3330 cm^{-1}), and Pluronic (842 cm^{-1}, 1102 cm^{-1}, 1342 cm^{-1}, 1061 cm^{-1}, 3252 cm^{-1}, 1359 cm^{-1}, 1466 cm^{-1}, 2971 cm^{-1}, 2880 cm^{-1}). The absence of peaks at 1770 cm^{-1} and 1590 cm^{-1} indicates successful conjugation of NPC with Glu on the ZnO nanoparticles.

The ^{1}H-NMR spectrum (600 MHz, D2O, figure 2b) shows characteristic F127 signals at δ(a) = 3.67 ppm, δ(b) = 3.50 ppm, δ(c) = 3.40 ppm, and δ(d) = 1.13 ppm. Signals at δ(n) = 7.22 ppm (-

NH), δ(i) = 4.48 ppm (-CH- adjacent to -NH), δ(j) = 2.08 ppm and δ(k) = 2.44 ppm (-CH_2- on Glu side chain), δ(m) = 6.92 ppm (-NH on Ami), and δ(h) = 1.72 ppm (-CH_2- on Ami backbone) confirm the presence of Glu and Ami. The presence of signal (n) and downfield shifts of signals (i), (j), and (k) indicates successful conjugation of NPC-F127-OH to Glu on the ZnO nanoparticles via a urethane linkage.

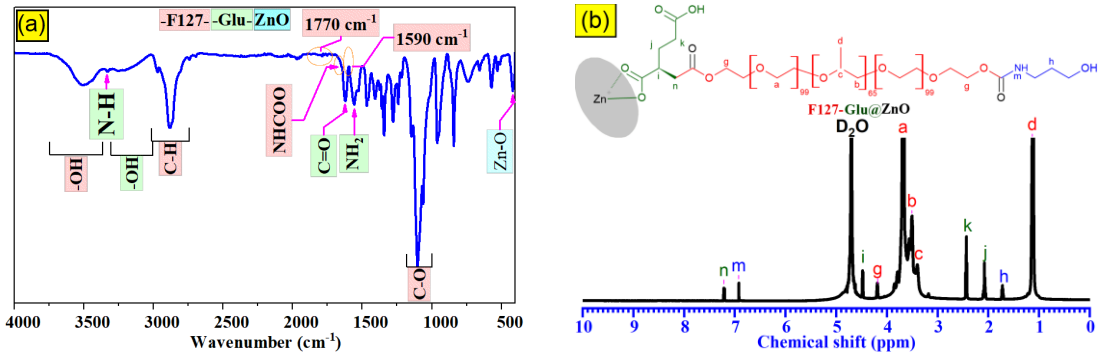

Figure 2. *The FT-IR spectrum of F127-GA@ZnO.*

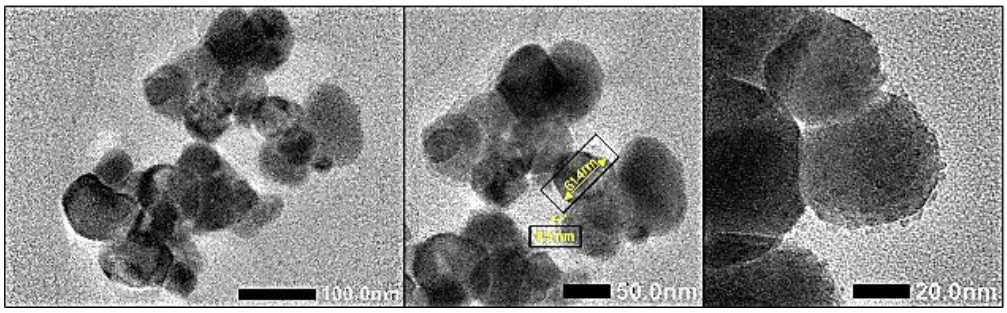

Figure 3. *TEM image of F127-Glu@ZnO.*

TEM images at different magnifications (figure 3) reveal that the F127-Glu@ZnO material exhibits a spherical nanoparticle morphology with an average size of approximately 61.4 nm. A low-contrast shell surrounding the nanoparticles, likely attributable to the F127-Glu organic layer, is observed with an estimated thickness of about 8.9 nm. At higher magnification, small spherical dots are visible, which may be due to the self-assembly of F127 molecules into micelles on the surface of the ZnO nanoparticles.

3.3. CPT Drug Loading of F127-Glu@ZnO

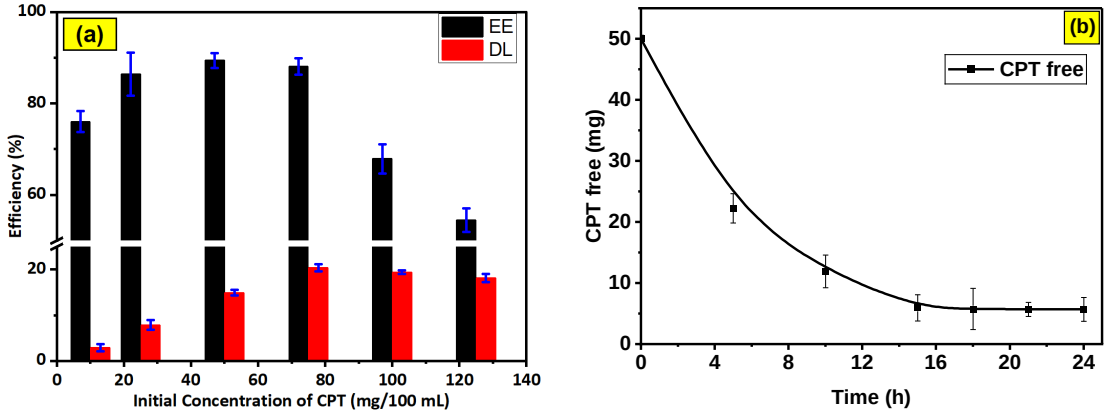

Figure 4. *Effect of CPT initial concentration on drug loading efficiency (DLE) and drug loading capacity (DLC) (a) and investigation of CPT drug loading time (b).*

Increasing the initial CPT concentration from 10 mg/100 mL to 50 mg/100 mL enhanced both the drug loading efficiency (DLE) and drug loading content (DLC) of the carrier (figure 4a). This is attributed to increased CPT diffusion onto the carrier surface at higher concentrations, reducing free CPT in solution and maximizing DLE at 89.79%. However, at 50 mg/100 mL CPT, the carrier reached its loading capacity, causing DLE to decline with further CPT increases due to excess free CPT. Similarly, DLC increased with initial CPT concentration but plateaued at ~ 15.22% at 75 mg/100 mL, indicating a finite loading capacity. Drug loading kinetics (figure 4b) show rapid loading in the first 5 hours, reaching equilibrium after 15 hours, which is determined as the effective loading time.

3.4. Drug release study

Drug release from F127-Glu@ZnO/CPT (figure 5) was pH-dependent. In pH 7.4 PBS, free CPT showed rapid release (51.84 ± 6.21)% within 1 hour and (94.69 ± 2.47)% after 6 hours, reaching (96.45 ± 0.37)% after 24 hours. In contrast, F127-Glu@ZnO/CPT exhibited slower release at pH 7.4: (23.56 ± 0.61)% after 12 hours, (38.70 ± 0.85)% after 48 hours, and (51.31 ± 1.14)% after 96 hours.

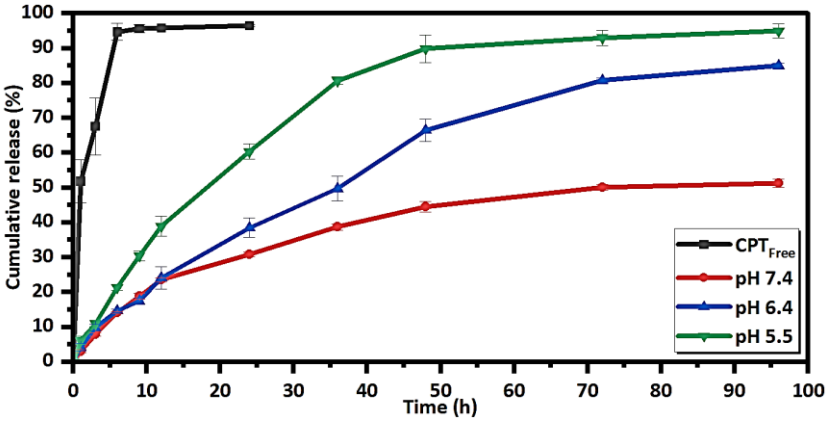

Figure 5. *Drug release profiles of F127-Glu@ZnO/CPT.*

At pH 6.4, CPT release was sustained for 72 hours, reaching (80.87 ± 0.71)% and (85.00 ± 0.74)% after 72 and 96 hours, respectively. At pH 5.5, the release was significantly faster, reaching (80.71 ± 1.16)% after 48 hours and (94.97 ± 2.09)% after 96 hours. This result demonstrates the pH-responsive nature of F127-Glu@ZnO/CPT, with acidic environments enhancing both the rate and efficiency of drug release.

4. CONCLUSIONS

The successful synthesis of the F127-Glu@ZnO nanogel material was confirmed through FT-IR and ^{1}H-NMR spectroscopic analyses. TEM images revealed a nanoparticle morphology with an average size of approximately 61.4 nm, encapsulated by an organic shell with an estimated thickness of 8.9 nm. Investigations into the CPT drug loading capacity of this material demonstrated an optimal drug entrapment efficiency (DLE) of 89.79%, while the drug loading capacity (DLC) could reach up to 18.62%. Drug release experiments clearly demonstrated a pH-dependent release profile, with both the rate and efficiency of drug release increasing in more acidic environments (lower pH). These results highlight the significant potential of the F127-Glu@ZnO material for CPT drug delivery in cancer therapy applications.

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TÓM TẮT

Tổng hợp và đặc trưng của vật liệu nanogel F127-Glu@ZnO cho ứng dụng phân phối thuốc cisplatin

Một loại nanogel F127-Glu@ZnO mới đã được tổng hợp để vận chuyển cisplatin bằng cách chức hóa các hạt nano ZnO với L-glutamic acid (Glu) và ghép Pluronic lên Glu thông qua liên kết urethane. Phổ hồng ngoại biến đổi Fourier (FT-IR) và phổ cộng hưởng từ hạt nhân proton (^{1}H-NMR) đã xác nhận sự tổng hợp thành công, trong khi kính hiển vi điện tử truyền qua (TEM) được sử dụng để khảo sát hình thái của vật liệu. Nanogel thể hiện khả năng giải phóng thuốc đáp ứng với pH, tăng tốc độ giải phóng cisplatin trong môi trường axit. Điều này cho thấy tiềm năng của nó như một hệ thống vận chuyển thuốc thông minh để cải thiện hiệu quả điều trị và giảm tác dụng phụ trong điều trị ung thư.

Từ khoá: Hạt nano kẽm oxit; Axit glutamic; Cisplatin; Chất mang thuốc.