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Supporting Information

Mitochondrial Dysfunction Induced Apoptosis in Breast Carcinoma Cells Through pH-Dependent Intracellular Quercetin NDDS of PVPylated-TiO_2NPs

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1. Experimental section

1.1 Chemicals

Titanium (IV) isopropoxide (97% Sigma Aldrich) and sodium hydroxide (NaOH) were purchased from Merk (India), cetyl trimethyl ammonium bromide (CTAB) was from (HiMedia), PVP, Fluorescein and Quercetin (Sigma-Aldrich). The chemicals were of analytical grade and were utilized without additional purification. The glassware's were properly washed.

1.2 Preparation of Qtn-PVPylated-TiO_2NPs

To prepare spherical like TiO_2NPs, 0.2 g of CTAB was added to 100 mL of bi-distilled (BD) water, in a flat-bottomed flask. To this, 10 mL of 1% titanium (IV) isopropoxide solution was added. The whole apparatus was kept on mechanical stirrer under different stirring conditions *viz*. 500 rpm, 1000 rpm, 1500 rpm and 2000 rpm. Subsequently, the NaOH solution was added drop-wise to the cocktail. Within few minutes of agitation, the colloidal solution turned milky. The setup was retained as such for half an hour. The system was then cooled, centrifuged, dried at room temperature in a desiccator, and washed with BD water and methanol. No complex treatment (for example, high temperature and high pressure, use of an inert gas) was utilized for the synthesis. Finally, the samples of TiO_2NPs were stored in an amber color container until further use.

Qtn-loaded NPs were prepared by water/oil (W/O) method, briefly, 5 mg Qtn was dissolved in 2 mL dehydrated alcohol and ultrasonicated for 15 min. This organic phase was then slowly poured into the aqueous solution of PVP. The solution of 0.5 mL PVP in 10 mL deionized water was maintained at 35.5ºC for 2 h under stirring rate of 500 rpm. The oil in water emulsion was formed after removal of alcohol under reduced pressure. The resulting aqueous suspension containing Qtn was denoted as solution 1, which was then added to the acetic acid solution of TiO_2. By varying the ratio of solution 1 and TiO_2 solution, the Qtn-loading amount may be finely tuned. The resulting mixture was then sonicated at an energy output of 50 W for 30 min to obtain a uniform solution, denoted as solution 2, which was then added dropwise to 30 mL liquid paraffin under stirring for 12 h. The water in oil emulsion was formed without an extra emulsifier. It describes the TiO_2NPs core particles loaded with drug (Qtn) molecules on the surface of PVPylated NPs. The formation of drug Qtn-PVPylated where shown in the Fig. 1 (a).

PVP used as a stabilizer and the coating of TiO_2NPs, which protects the dissociation of from TiO_2NPs.

1.3 Characterization of Qtn-PVPylated-TiO_2NPs

The FT-IR spectrum of TiO_2NP, TiO_2NP-PVP, free Qtn and Qtn-PVPylated-TiO2NPs were recorded in KBr pellets using FTIR spectrophotometer (Jasco, Japan). The scan was performed in the range 500–4500 cm−1. XRD pattern was obtained for TiO_2NPs, and TiO_2NP-PVP by using Bruker (AXSD8) X-ray diffractometer containing Nickel-filtered Copper K_α radiation.

The synthesized Qtn-PVPylated-TiO_2NPs was sonicated for flush distribution and NPs was diluted 5 times with distilled water before being estimated. An outside structure of the Qtn-PVPylated-TiO_2NPs was tested by using TEM (JSM 6390LV, JOEL, USA). For the particles images and elemental analysis studies, the TiO_2NPs colloidal suspension was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were cast onto a sample holder; air dried and subjected to Carl Zeiss Ultra plus SEM with EDX detector and elemental mapping. The range distribution and the average size of the NPs were guessed based on DLS with the help of Sigma-Scan Pro software (SPSS Ins, Version 4.01.003). The samples were prepared by evaporating a drop of Qtn loaded TiO_2NPs nanoparticles colloidal solution on the carbon-coated copper grid. More than three micro photos of different areas were used to measure particle size distributions and >200 particles were counted and converted to percentiles to plot histogram and size distribution. The zeta potential studies were carried out to analyze the surface charge of the particles using the Zeta-sizer (Malvern) instrument. The particle size range of the nanoparticles along with its polydispersity was tried out by hiring a particle size analyzer (Malvern). Particle size was inwards based on assessing the time-dependent variation of scattering of laser light by the nanoparticles having Brownian motion.

1.4 Drug loading and encapsulation efficiency of Qtn-PVPylated-TiO_2NPs

Studies on the determination of drug loading and encapsulation efficiency of nanocombinations were performed as reported elsewhere.[1] The drug loading studies, a stock solution of Qtn (free base) was prepared in methanol. The drug was internally loaded into the nanocombinations by adding a predetermined volume of Qtn stock solution to the PVPylated TiO_2NPs solution followed by mixing to ensure uniform distribution. The amount of drug packed per milligram of TiO_2 was determined by redissolving a known amount of the nanocombinations in methanol and then analyzing the drug content by high-performance liquid chromatography

(HPLC) assay.[2] Finally, the Qtn loaded nanocombinations were lyophilized and stored in a freezer to prevent the unexpected leaking of Qnt. The reverse-phase consisted of 1.0% (v/v) triethylamine in deionized distilled water added to methanol in the ratio of 11.89 (v/v). The injection volume was 20 µL and the flow rate of the mobile phase was 1.2 mL/min. Qtn was detected at 352 nm using a UV detector. The amount of Qtn loading (%) in nanocombinations was determined from a calibration curve of the drug in acetone. The encapsulation efficiency of the drug was calculated as the mass ratio of the amount of the drug entrapped in nanocombinations to that used for nano preparation. To quantify the amount of encapsulated Qtn from nanoformulation, 2 mg of NPs was dissolved completely in 1 mL of 0.5 N NaOH solutions. The drug loading and encapsulation efficiency were determined by the following equation:

$$\text{DLC (\%)} = \frac{\text{The weight of the drug in NPs}}{\text{Weight of the NPs}} \times 100$$

$$\text{E.E (\%)} = \frac{\text{Weight of the drug in NPs}}{\text{Weight of the feeding drug}} \times 100$$

Where W_0 is the weight of drug enveloped in the TiO_2NPs, W is the weight of TiO_2NPs, and W_1 is the amount of drug added in the system.

1.5 *In vitro* drug release kinetics

The drug release profiles of Qtn from Qtn loaded nanocombinations was investigated at pH 7.4 (pH of physiological blood), 6.0 (pH of the environment around the tumor), and 4.5 (approximate pH in endosomes or lysosomes), were studied using a dialysis tube. Qtn-loaded TiO_2 nanoparticles were dispersed in PBS (pH 7.4, 5 mL) and transferred into a dialysis bag (Spectra/Por®; Spectrum Laboratories, Inc., Rancho Dominguez, CA). The dialysis bag was then immersed in 95 mL of PBS at pH 4.5, 6.0, or 7.4. The dispersion in tubes was then put in an orbital shaker shaking at 120 rpm in a water bath at 37 and 42 °C. At designated time intervals, the suspension was centrifuged at 15000 rpm for 20 min. The pellet was drained and resuspended in fresh medium to continue the drug release process. The supernatant was filtered through a 0.22 µm membrane filter to ensure that the filtrate was free of nanocombinations and the concentration of the drug was determined by HPLC as described above. The amount of released Qtn in the medium was then determined at 352 nm.

2. Results

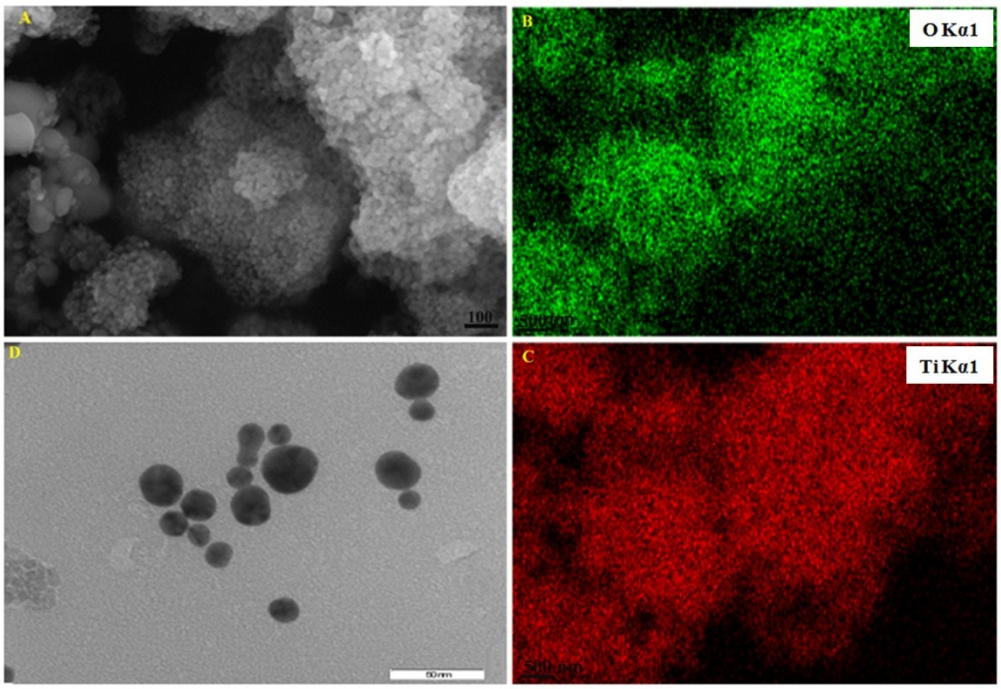

Fig. S1: Summary of SEM-EDS analysis results of TiO_2NPs: (A) an SEM image showing the distribution of (B) O and (C) Ti in a mass of TiO_2NPs. (D) TEM image of unmodified TiO_2NPs.

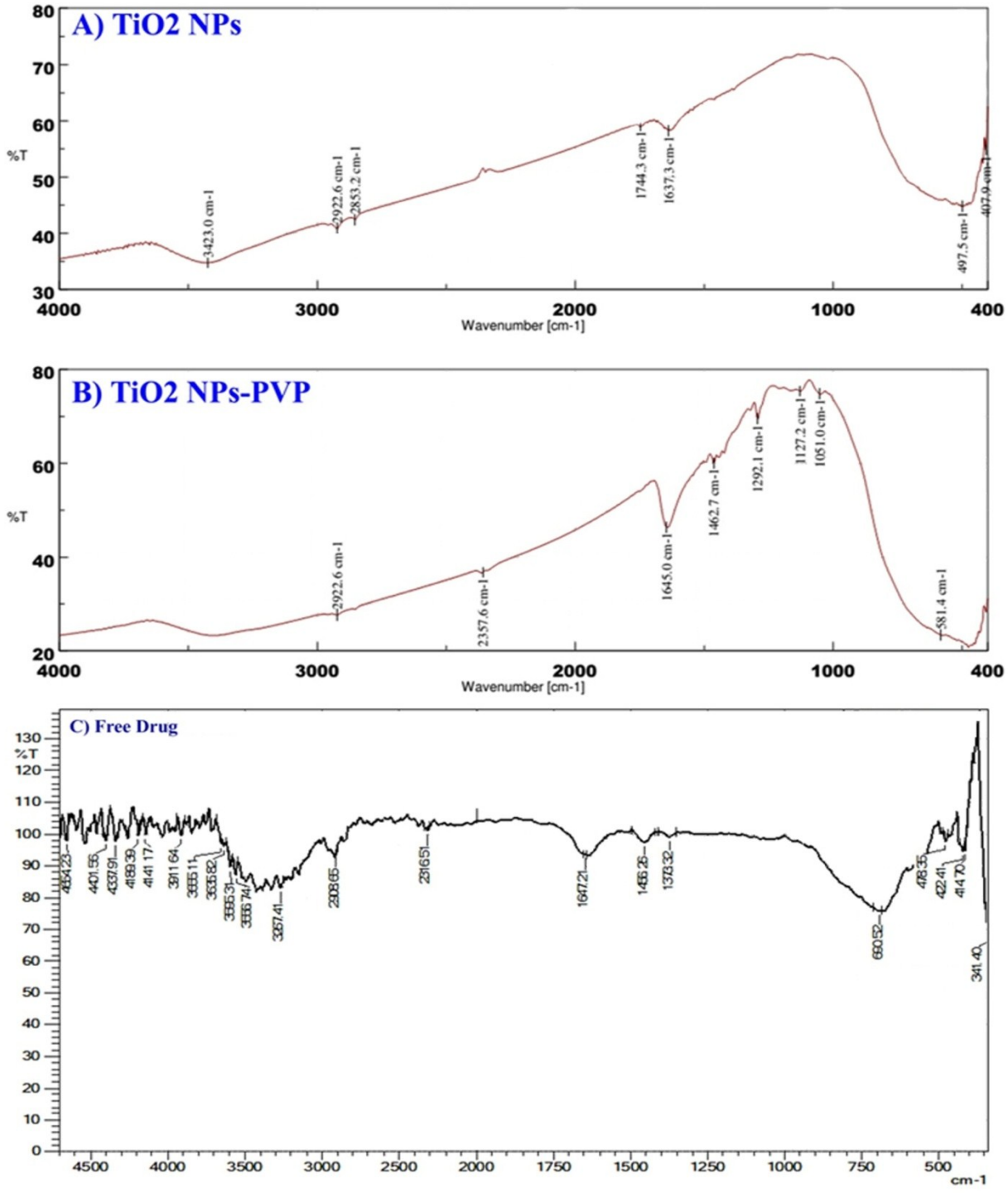

Fig. S2 (**A**)**.** Fourier transform infrared spectroscopy (FTIR) of characteristic peaks of TiO_2NPs (**B**)**.** FTIR spectrum of characteristic spectrum of TiO_2NPs-PVP. (**C**)**.** Fourier transform infrared spectroscopy (FTIR) of characteristic peaks of free drug Qtn.

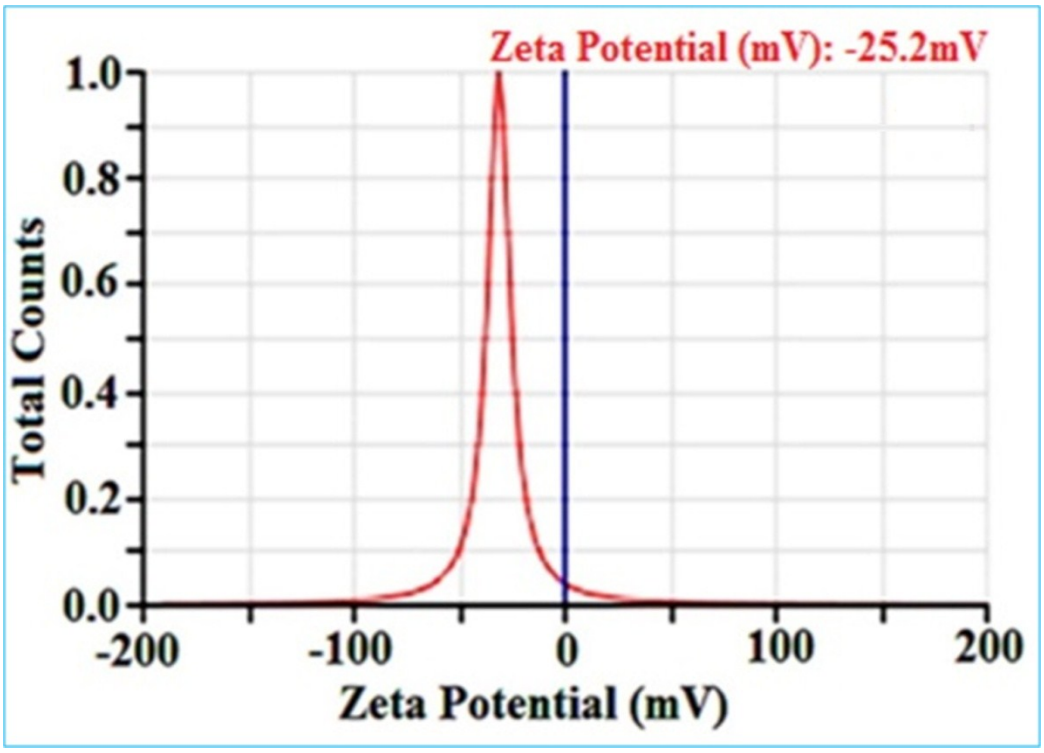

Fig. S3: Surface zeta potential graph showing negative zeta potential value for Qtn-PVPylated-TiO_2NPs.

References

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