

# **Near-Infrared Carbon Dots Enable Ultra-Sensitive Fluorometric Detection of Belzutifan: A Novel Approach for Real-Time Therapeutic Drug Monitoring in Cancer Treatment**

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## 2.2. Instrumentation

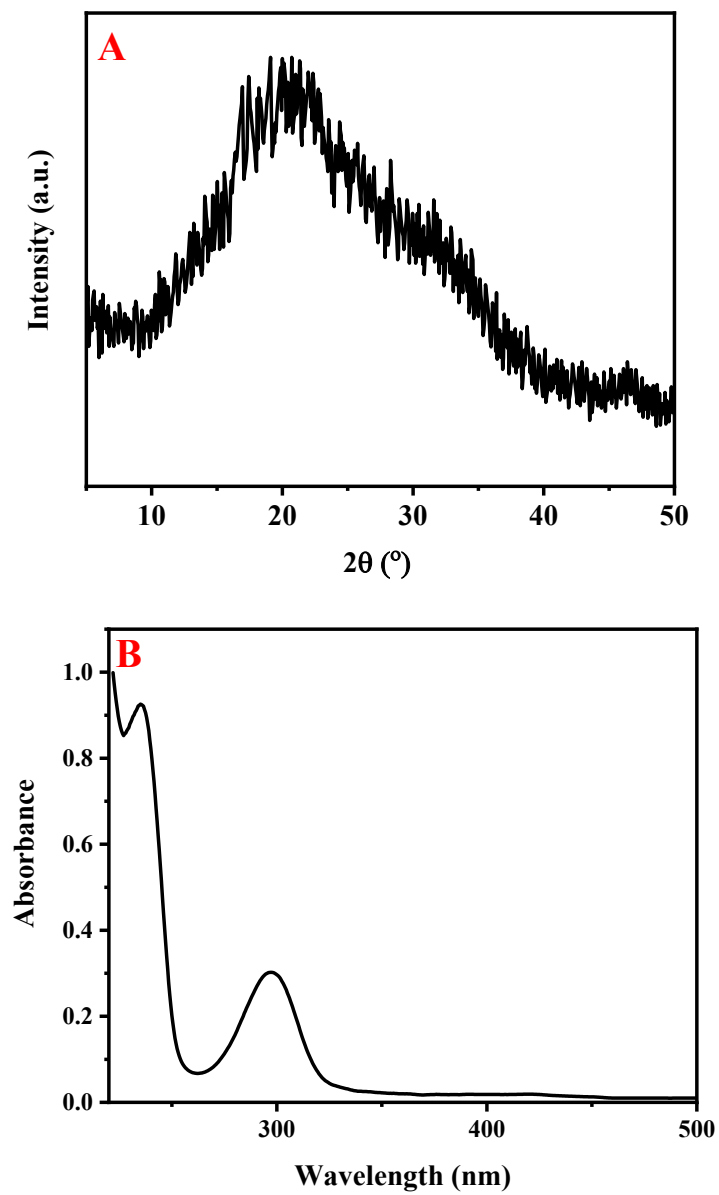
The optical characterization of the synthesized carbon dots was probed through fluorescence and UV-Vis spectroscopic methods, wherein the fluorescence emission spectra were acquired on a Shimadzu RF-5301PC spectrofluorometer by exciting the samples at 290 nm with 5 nm slit widths, while the absorption spectra were generated using a Shimadzu 1601 PC UV-Vis spectrophotometer. The structural and morphological aspects were elucidated by transmission electron microscopy using a JEOL 2100F instrument operating at 200 kV accelerating voltage to visualize the size and shape of the carbon dots, X-ray diffraction patterns recorded with a Philips PW 1700 diffractometer, and Fourier transform infrared spectroscopy performed on KBr pellets using a Nicolet 6700 spectrometer. Furthermore, the surface chemical composition and bonding states were evaluated through X-ray photoelectron spectroscopy utilizing an ESCALAB250 spectrometer from Thermo Scientific.

## 2.4. Quantum yield determination of NIR-CDS

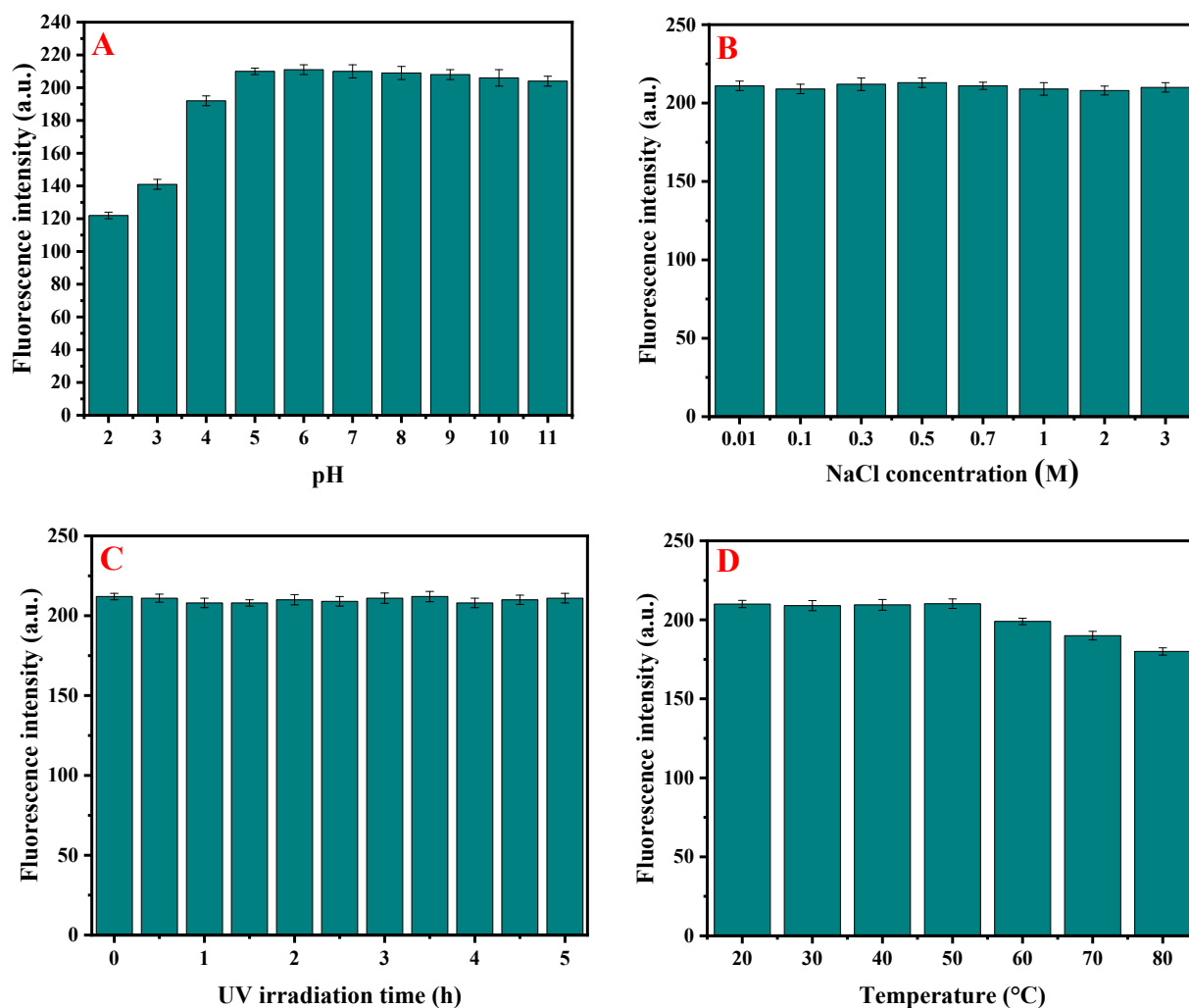
The quantum yield (QY) of NIR-CDS was calculated according to the following equation using quinine sulfate (QS) as a reference in 0.1 M H<sub>2</sub>SO<sub>4</sub> (QY = 54 %). By measuring the absorbance (less than 0.05) and emission spectra of a certain concentration of NIR-CDS and quinine sulfate at the excitation wavelength at 360 nm, the absorbance and fluorescence integral area were substituted into the following formula:

$$\phi_{NIR-CDS} = \phi_{QS} \times \frac{F_{NIR-CDS}}{F_{QS}} \times \frac{A_{QS}}{A_{NIR-CDS}} \times \frac{\eta_{NIR-CDS}}{\eta_{QS}}$$

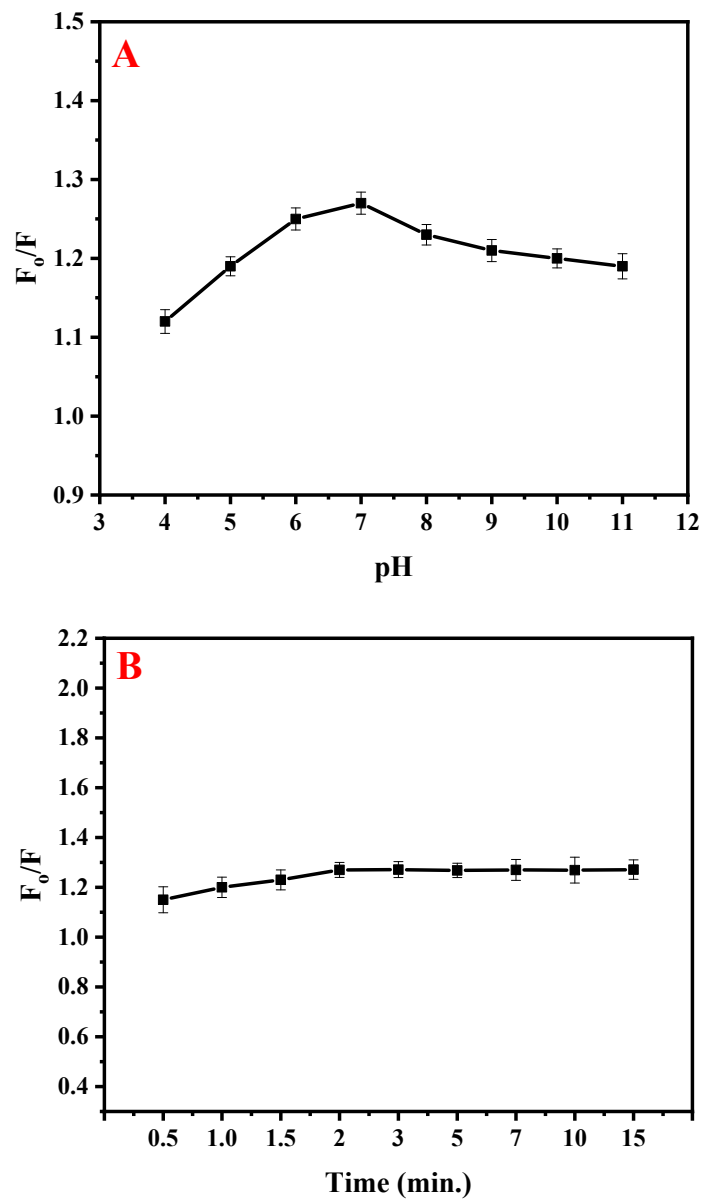
$\Phi_{\text{NIR-CDS}}$  represents the quantum yield of NIR-CDS,  $\phi_{\text{QS}}$  represents the quantum yield of QS,  $F_{\text{NIR-CDS}}$  is the fluorescence intensity of NIR-CDS,  $F_{\text{QS}}$  is the fluorescence intensity of quinine sulphate,  $A$  refers to the absorbance value and  $\eta$  refers to the refractive index of the solvent (double distilled water). The synthesized NIR-CDS were dissolved in distilled water ( $\eta = 1.33$ ) and quinine sulfate was dissolved in 0.1 M  $\text{H}_2\text{SO}_4$  ( $\eta = 1.33$ ).



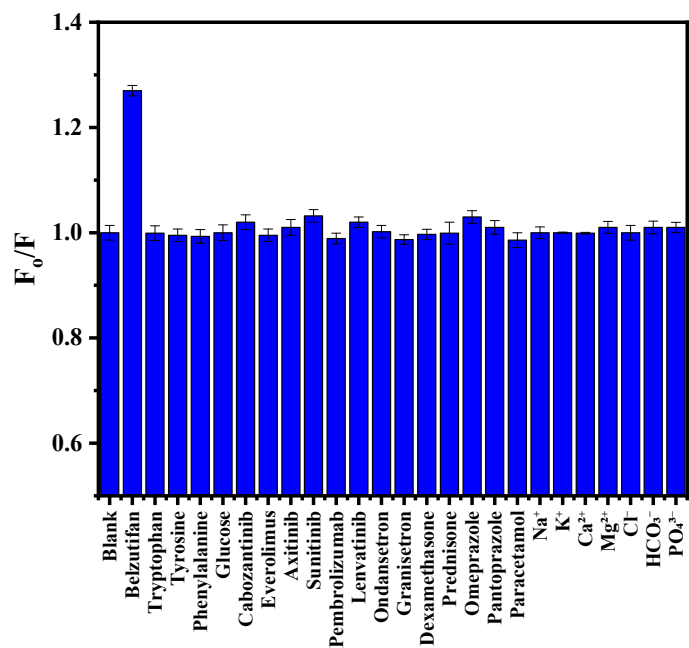
**Fig. S1.** (A) XRD pattern and (B) UV visible spectrum of NIR-CDs.



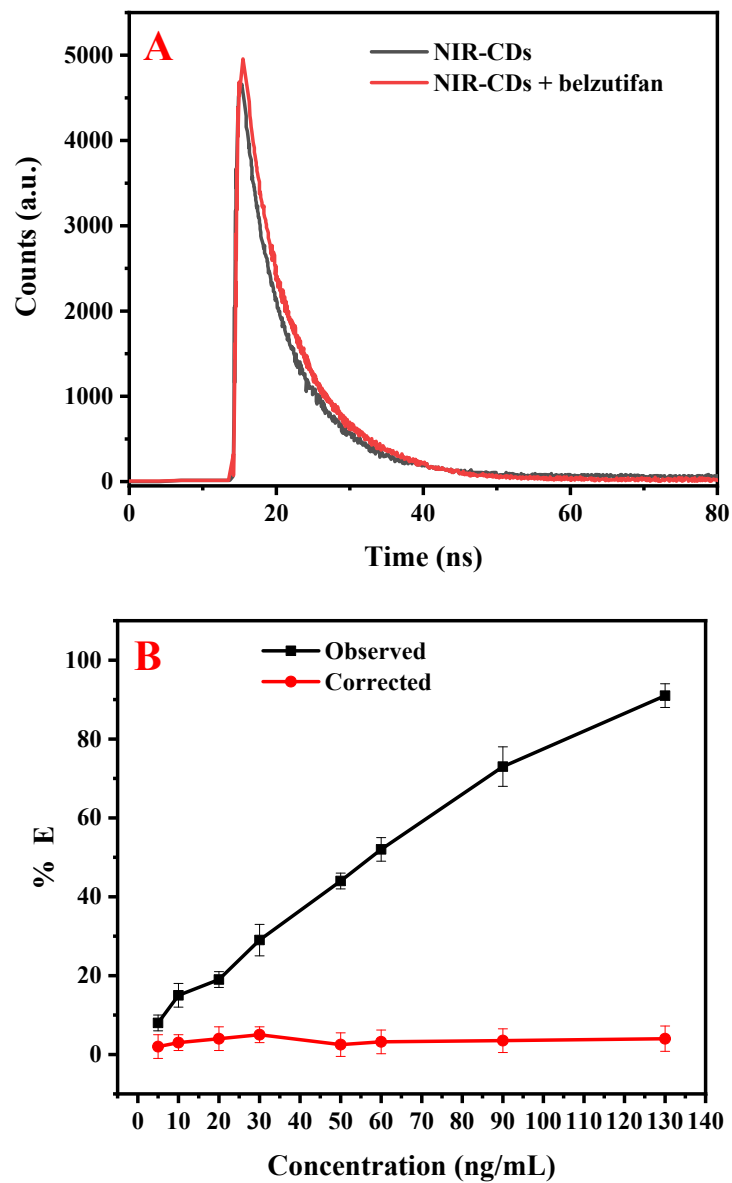
**Fig. S2.** Stability of the fluorescence response of the prepared NIR-CDs under various conditions: (A) effect of pH (2–11), (B) effect of NaCl concentration (0.1–3.0 M), (C) effect of UV irradiation time (0–5 h) and (D) effect of different temperatures (20 – 80 °C).



**Fig. S3.** (A) The effect of pH (4.0 to 10.0), (B) different reaction time (0.5 to 15.0 min.) on  $F_0/F$  of NIR-CDs after addition of belzutifan.

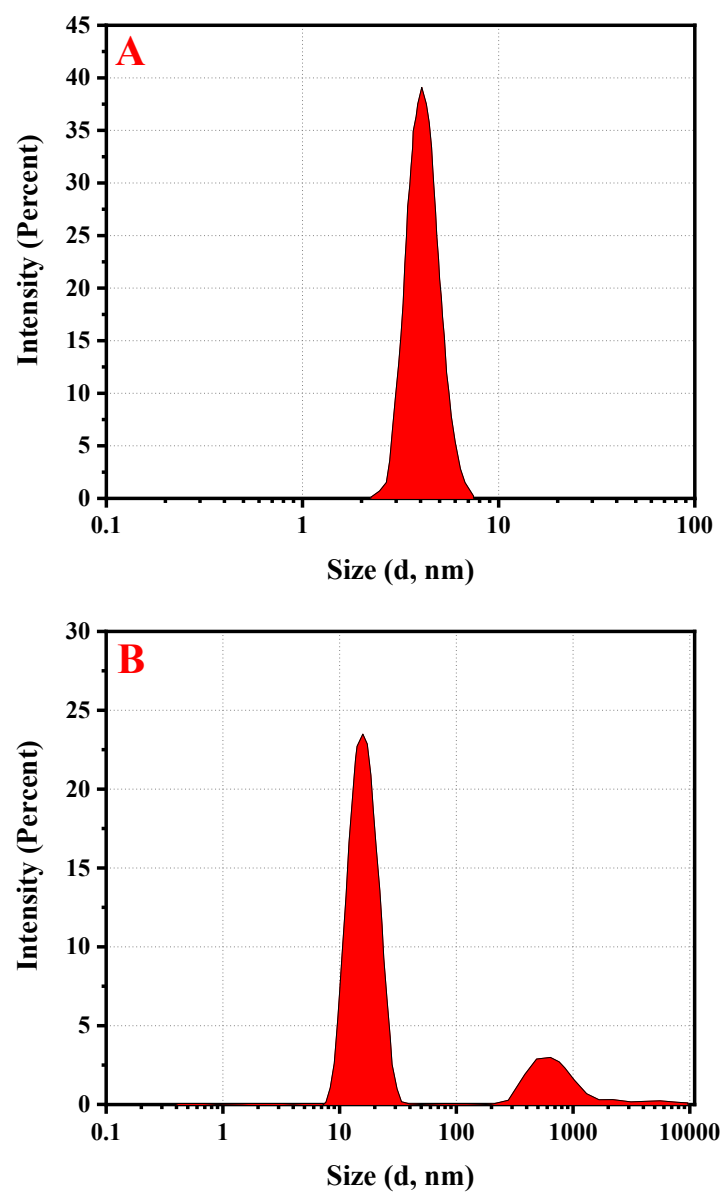


**Fig. S4.** The effect of different endogenous compounds, ions, co-administered pharmaceutical compounds on the ratio of fluorescence intensity ( $F_0/F$ ) of NIR-CDs.

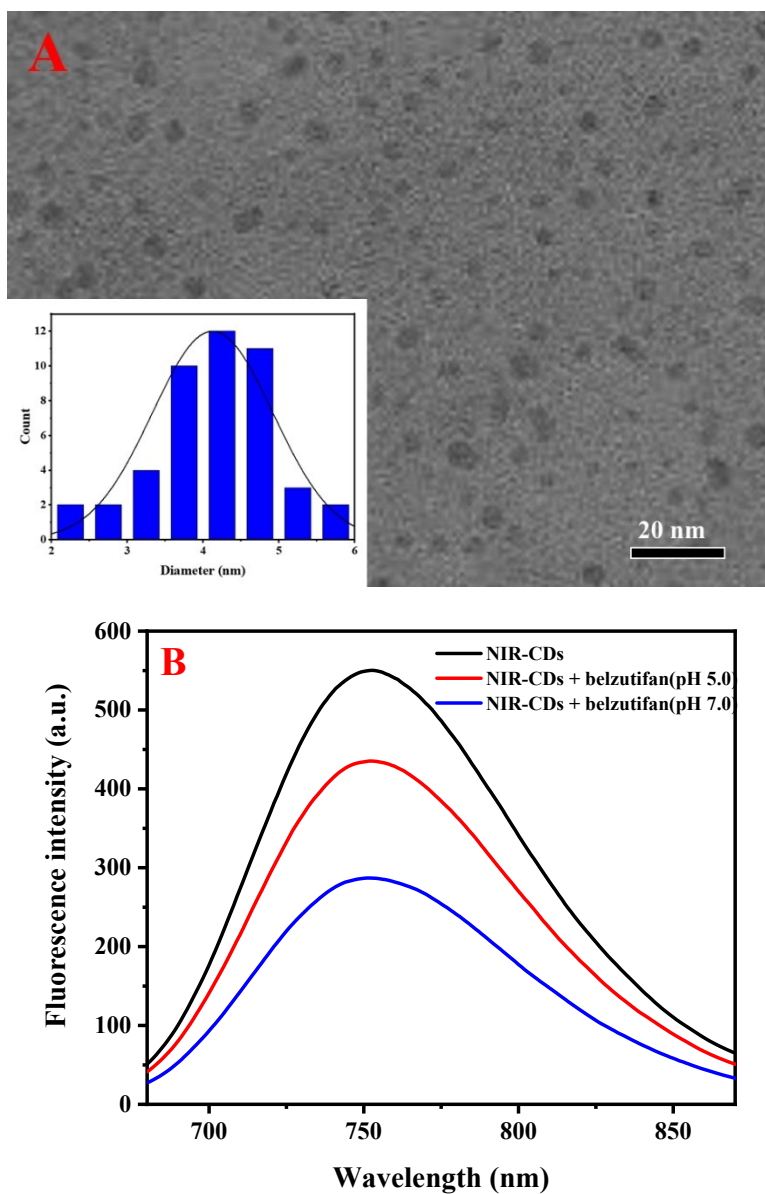


**Fig. S5.** (A) Fluorescence lifetime of NIR-CDs before and after addition of belzutifan, (B) Relationship between %E and concentration of belzutifan.





**Fig. S6.** Change in size of NIR-CDs in DLS on addition of belzutifan.



**Fig. S7.** TEM image of NIR-CDs after addition of belzutifan (pH 5.0), (B) Fluorescence intensity of NIR-CDs, NIR-CDs/belzutifan (pH 5.0) and NIR-CDs/belzutifan (pH 7.0).