Electronic Supplementary Information

Red-green-blue fluorescent hollow carbon nanoparticles isolated from chromatographic fractions for cellular imaging

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Measurement of quantum yield

The quantum yield (Φ_S) of the as-prepared HC-NP sample was determined by comparing the integrated photoluminescence (PL) intensities and optical density (OD) of the as-prepared HC-NP with the reference quinine sulfate (Φ_R= 0.54) in 0.10 M H_2SO_4 (refractive index, η = 1.33) while the as-prepared HC-NP was dissolved in distilled water (η = 1.33) at different concentrations. A Varian Cary 300 scan UV-vis absorption spectrophotometer was used to determine the OD (absorbance) of the solutions at 375 nm. A Photon Technology International QM4 spectrofluorometer was used to record their PL spectra with an excitation wavelength of 375 nm. The integrated PL intensity was the area under the PL curve in the wavelength range400–650 nm. Then a graph of integrated PL intensity against absorbance was plotted. The Φ_S of the as-prepared HC-NP was calculated using the equation:

$$\Phi_S = \Phi_R(Grad_S / Grad_R)(\eta^2_S / \eta^2_R)$$

where the subscripts *S* and *R* refer to the sample HC-NP and reference, respectively. *Grad* is the gradient from the plot of integrated PL intensity against OD. In order to minimise the self-absorption effect, the OD in the 10-mm path-length fluorescence cuvette was kept under 0.10 at the excitation wavelength.[1,2]

The Φ_S of HC-NP fractions were determined by using single-point measurement. The OD of quinine sulfate and HC-NP solutions were taken at 350 nm. The PL spectra at 380–650 nm with an excitation wavelength of 350 nm were recorded. The integrated PL intensity (*I*) was the area under the PL curve. The Φ_S of the HC-NP fractions were calculated using the equation:

$$\Phi_S = \Phi_R(I_S / I_R)(OD_R/OD_S)(\eta^2_S / \eta^2_R)$$

where the subscripts *S* and *R* refer to the sample fraction and reference, respectively.

References

1 P.-C. Hsu and H.-T. Chang, *Chem. Commun.*, 2012, **48**, 3984-3986.

2 X. Zhai, P. Zhang, C. Liu, T. Bai, W. Li, L. Dai and W. Liu, *Chem. Commun.*, 2012, **48**, 7955-7957.

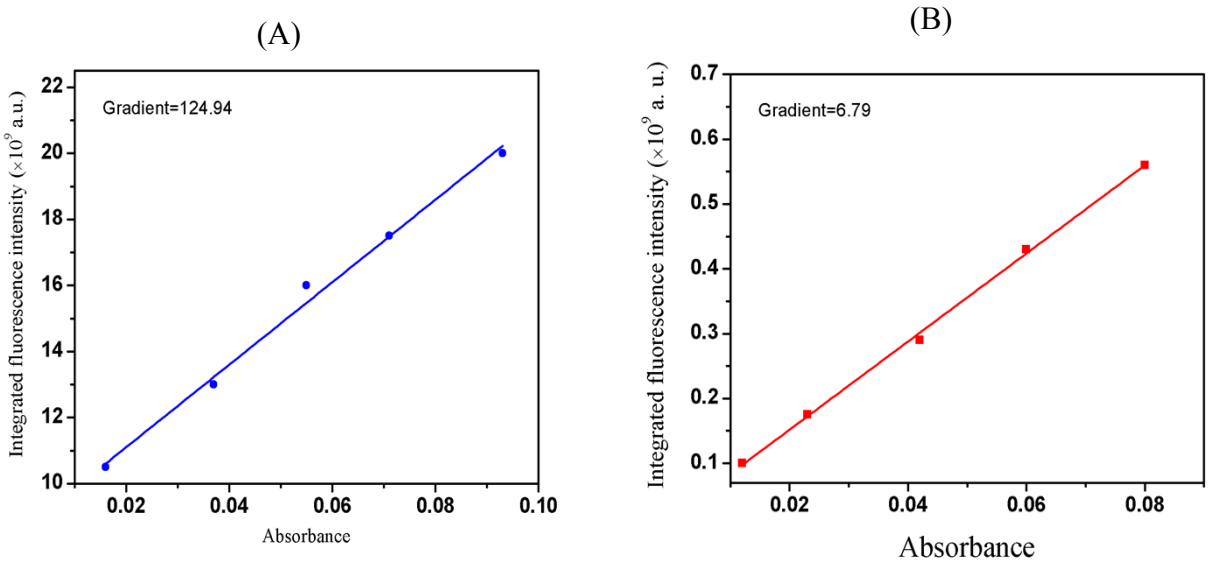


Fig. S1 Plots of integrated PL intensity against absorbance of (A) quinine sulfate and (B) as-synthesised HC-NP product.

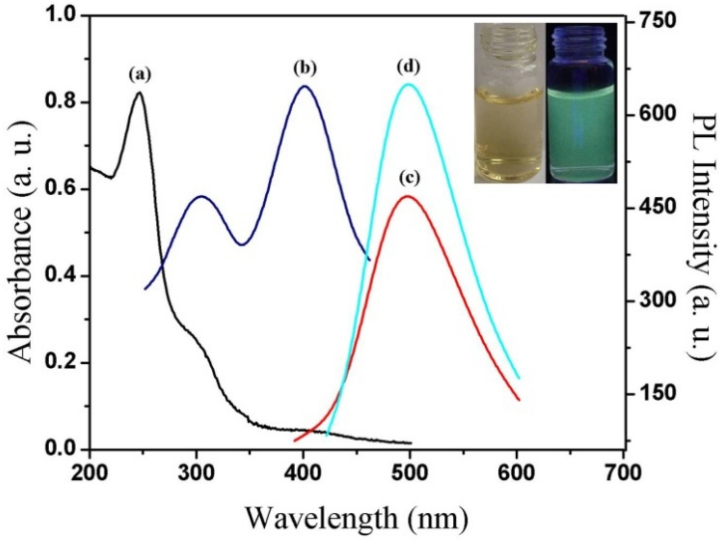


Fig. S2 (a) UV-vis absorption spectrum of the as-synthesised HC-NP product aqueous solution, (b) fluorescence excitation spectrum monitored at emission wavelength of 498 nm, (c) and (d) fluorescence emission spectra monitored at excitation wavelengths of 300 and 400 nm, respectively of the as-synthesised HC-NP product aqueous solution. The inset displays the photographic images of the as-synthesised HC-NP aqueous solution under daylight (left) and an UV (365 nm) lamp irradiation (right). The concentration of HC-NP is 1.0 mg/mL.

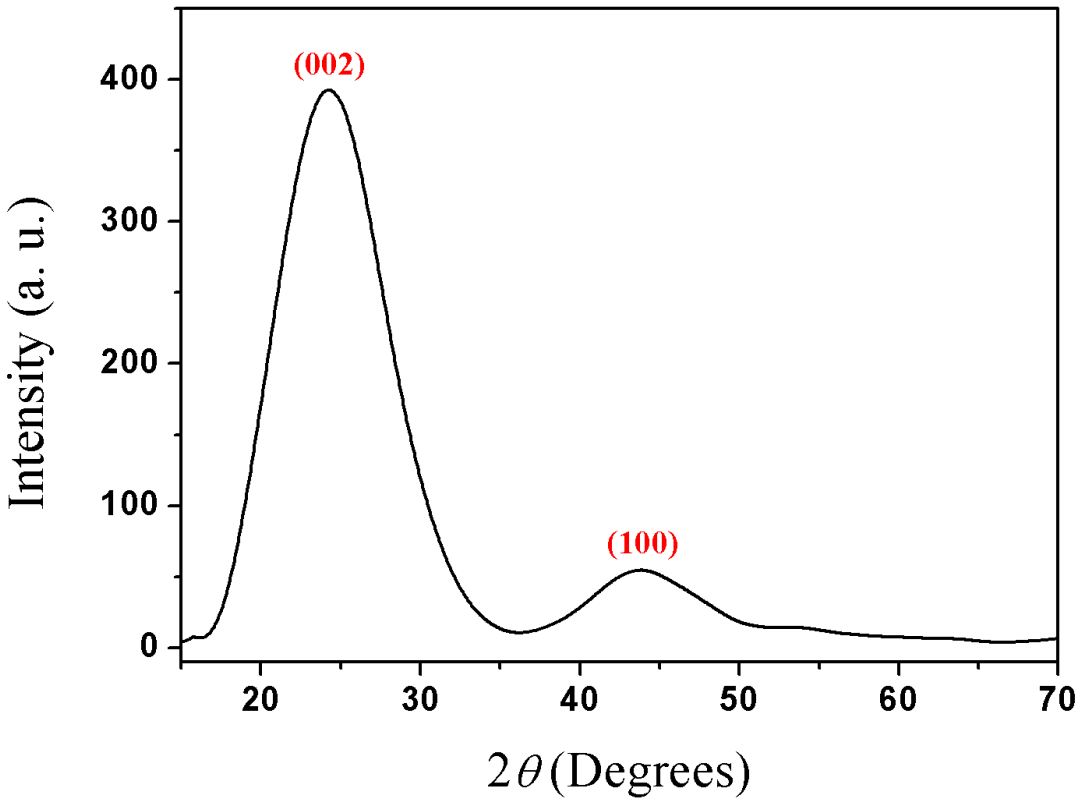


Fig. S3 XRD pattern of the as-synthesised HC-NP product.

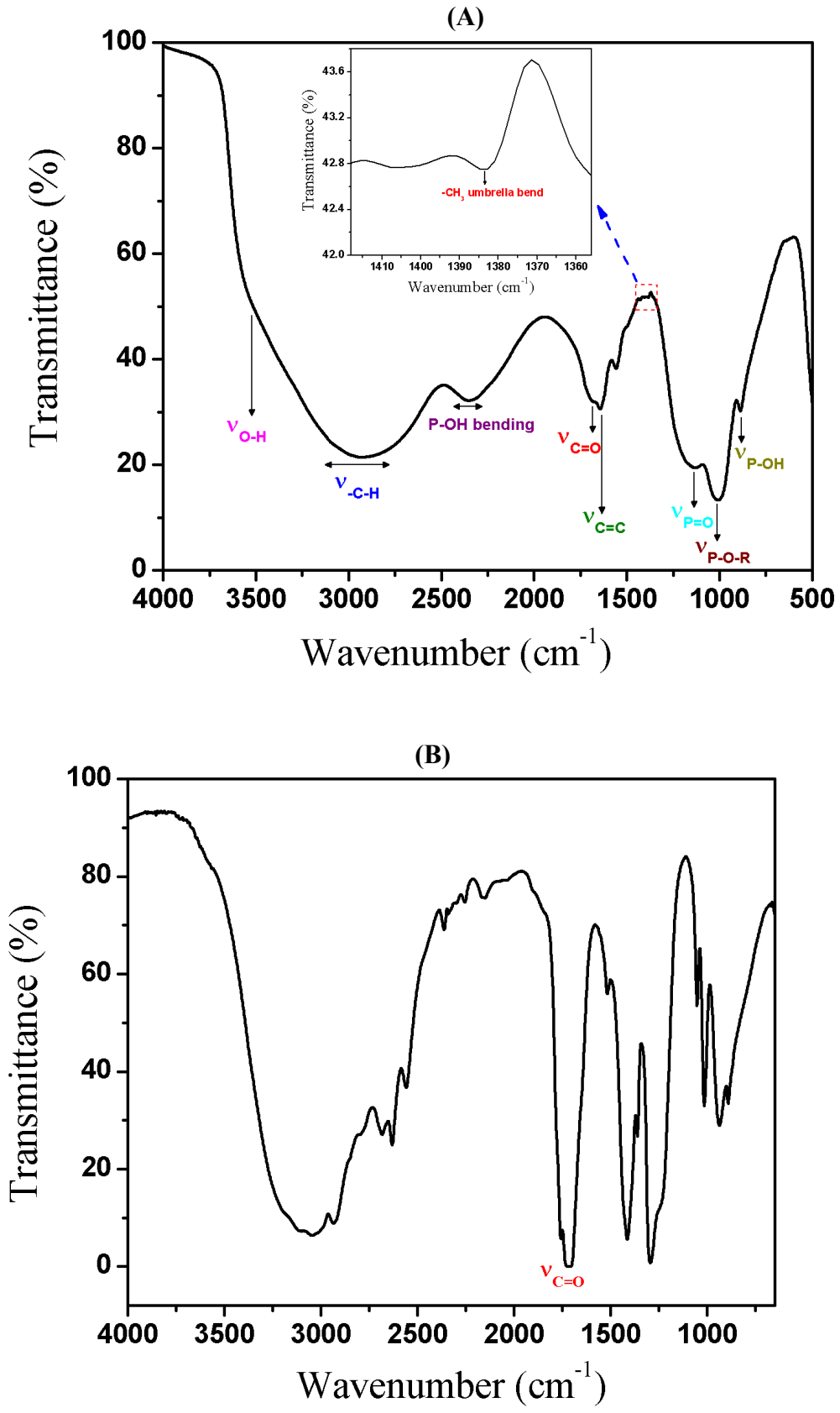


Fig. S4 FTIR spectra of (A) HC-NP mixture and (B) glacial acetic acid. The inset displays the -CH_3 umbrella bend of HC-NP mixture.

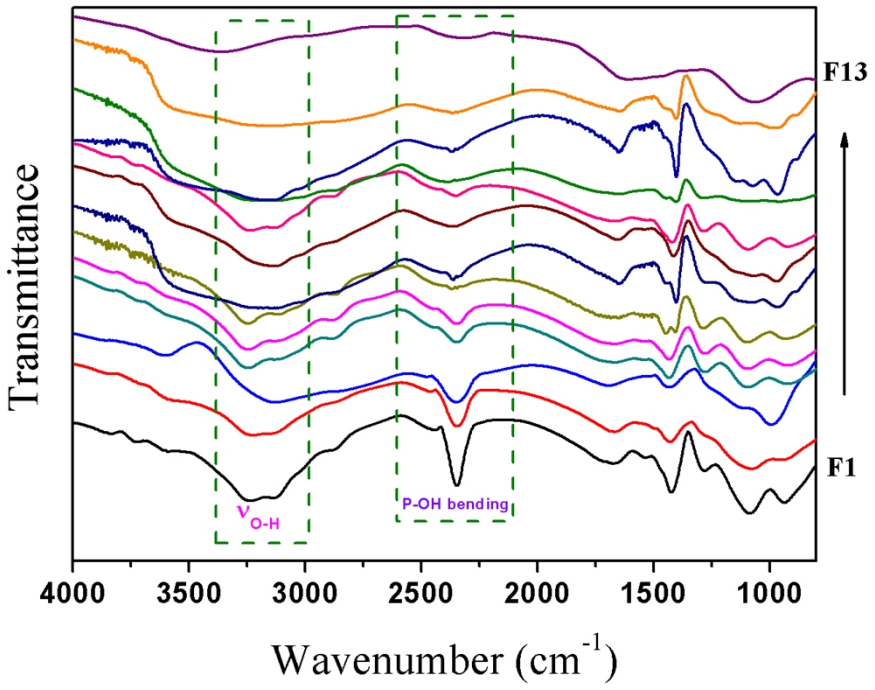


Fig. S5 FTIR spectra of F1–F13: Fractions 1–13 from bottom to top. The spectra are offset for ease of comparison. The square boxes highlight the changes in the absorption intensities of the O–H stretching and P–OH bending of HC-NP fractions.

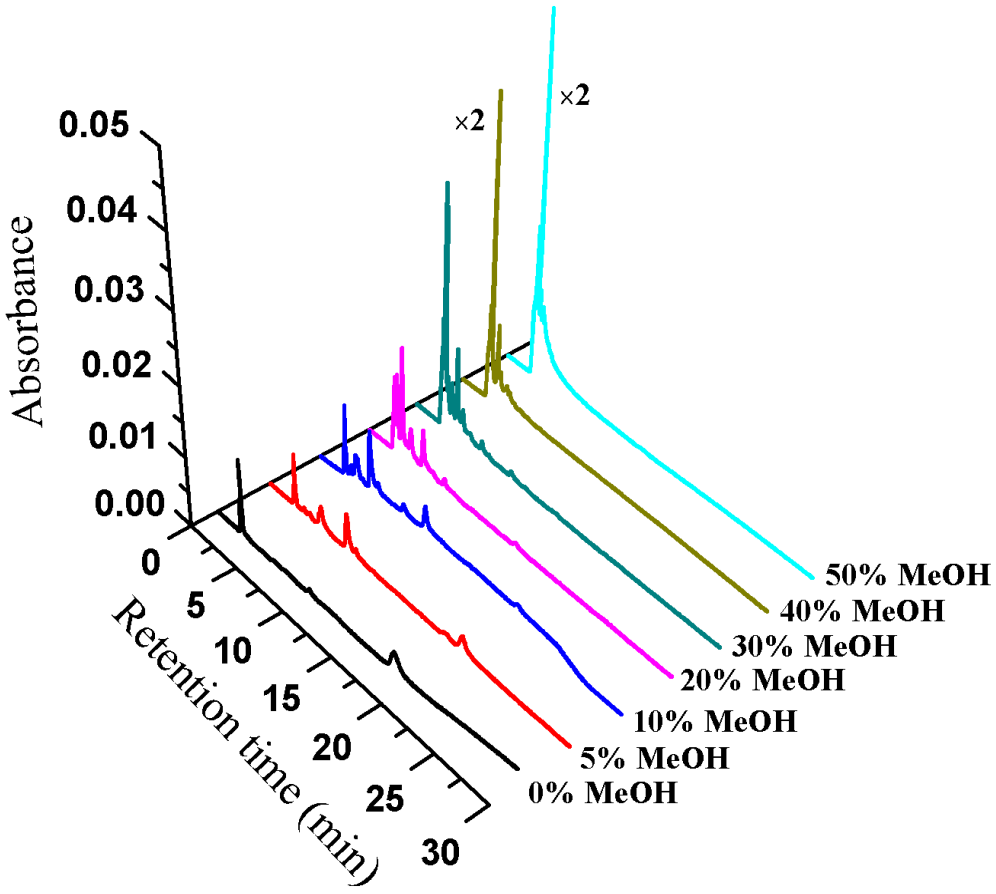
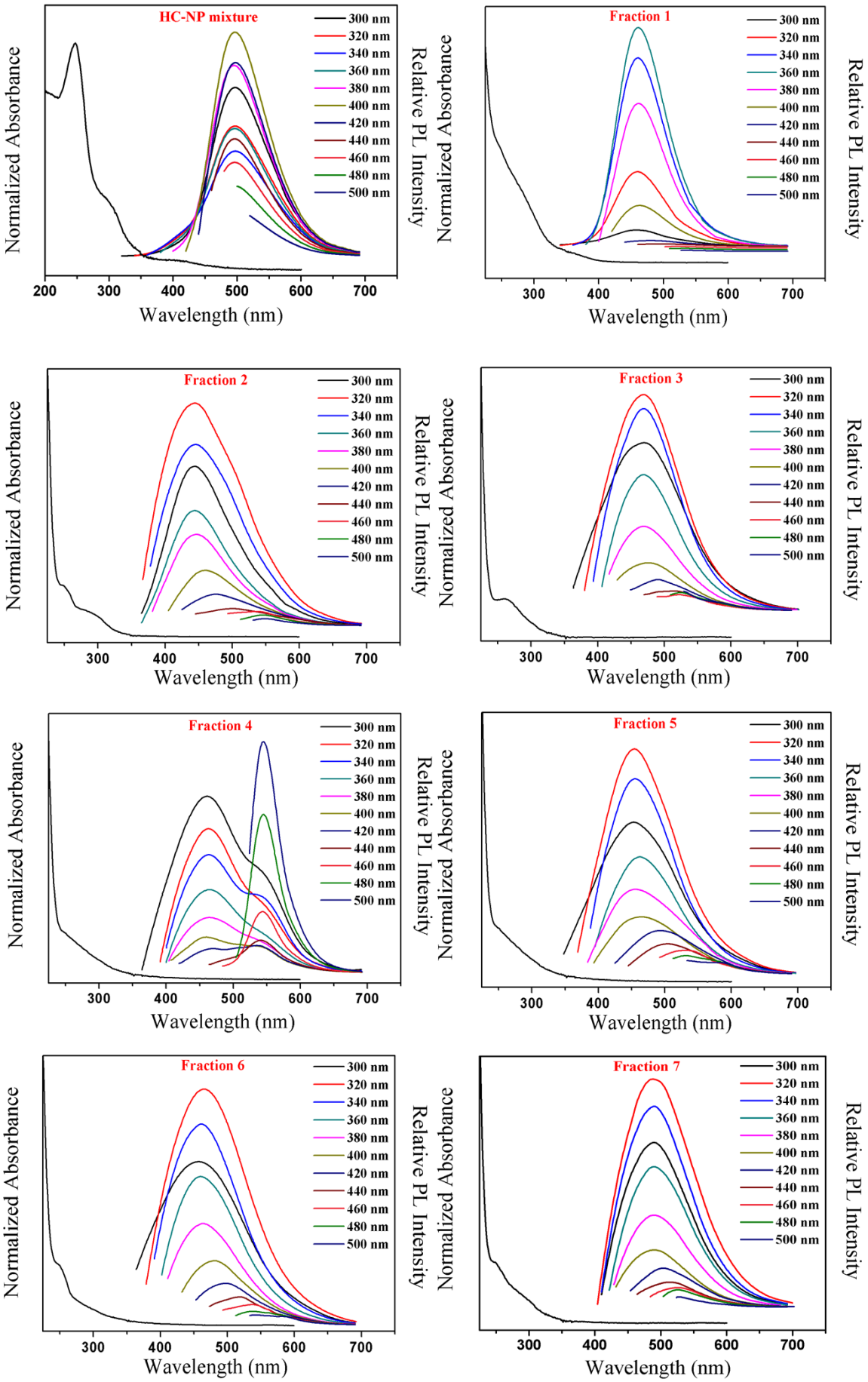


Fig. S6 Effect of methanol (MeOH % v/v) content on HPLC separation of an aqueous solution of HC-NP mixture (1.0 mg/mL). All elutions are isocratic at the flow rate of 0.80 mL/min. Chromatograms are monitored at 300 nm.


HC-NP mixture
Fraction 1
Fraction 2
Fraction 3
Fraction 4
Fraction 5
Fraction 6
Fraction 7
300 nm
320 nm
340 nm
360 nm
380 nm
400 nm
420 nm
440 nm
460 nm
480 nm
500 nm
Normalized Absorbance
Relative PL Intensity
Wavelength (nm)
200
300
400
500
600
700

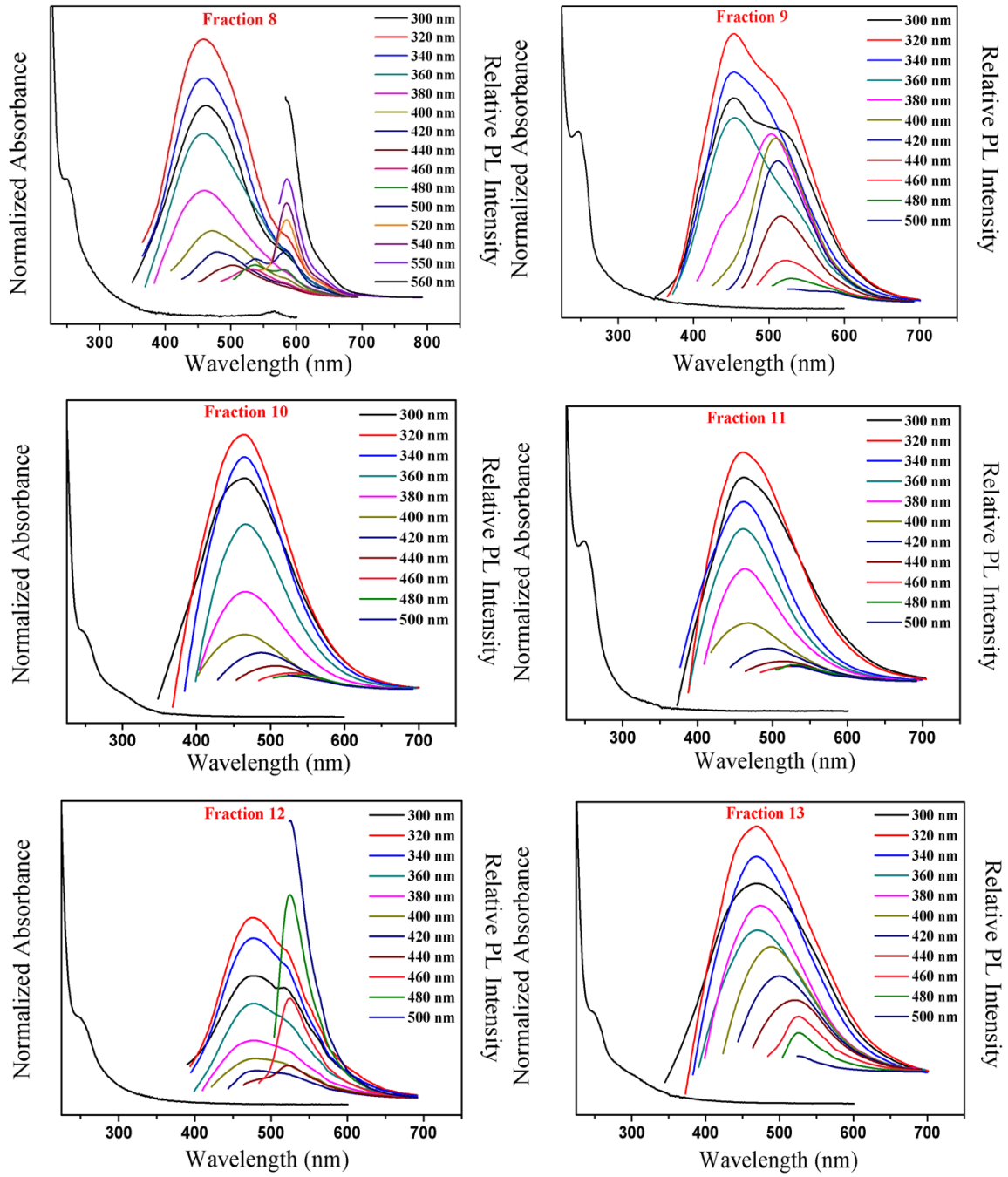


Fig. S7 UV-vis absorption and PL spectra at different excitation wavelengths of the as-prepared HC-NP product and Fractions 1–13.

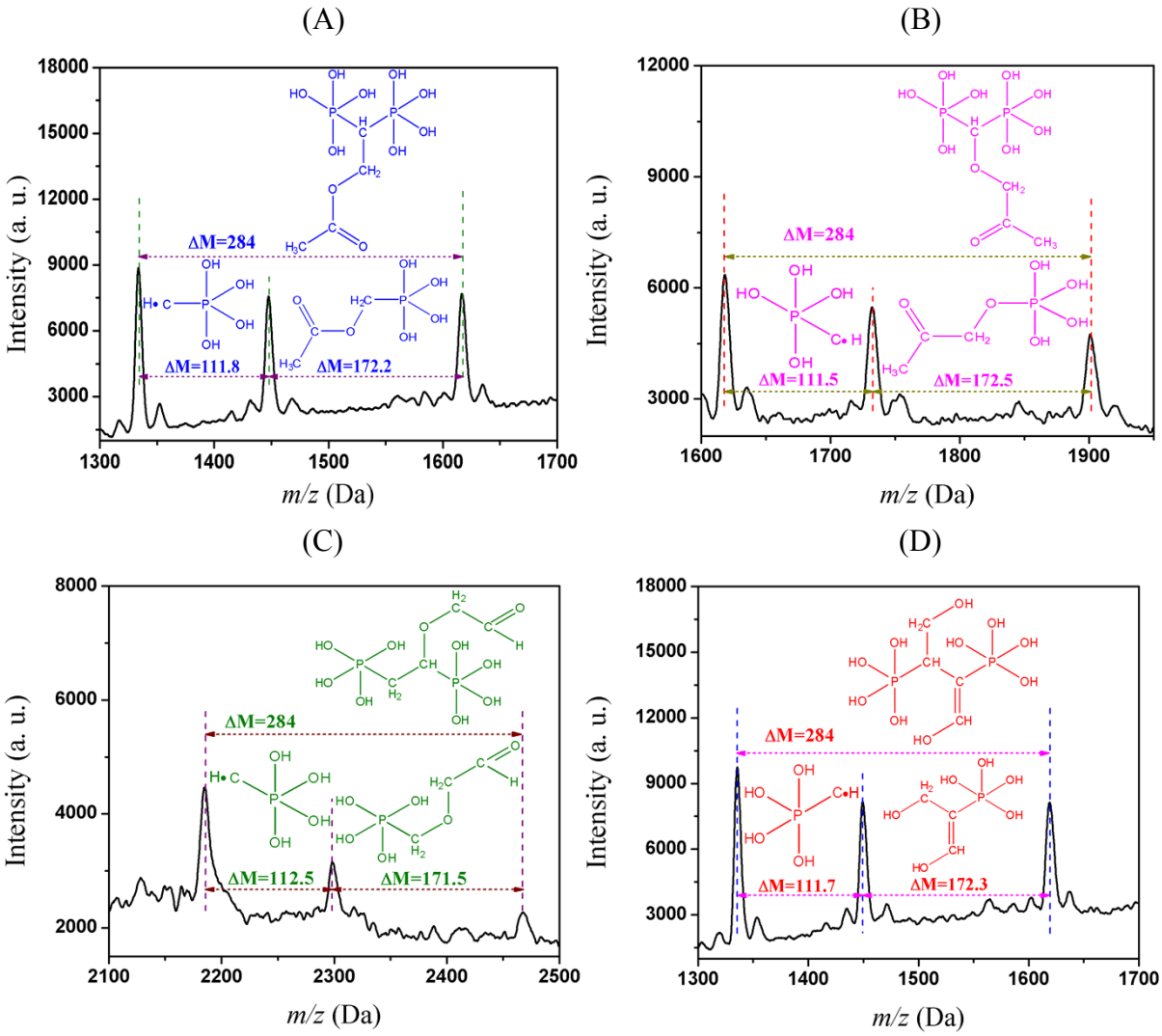
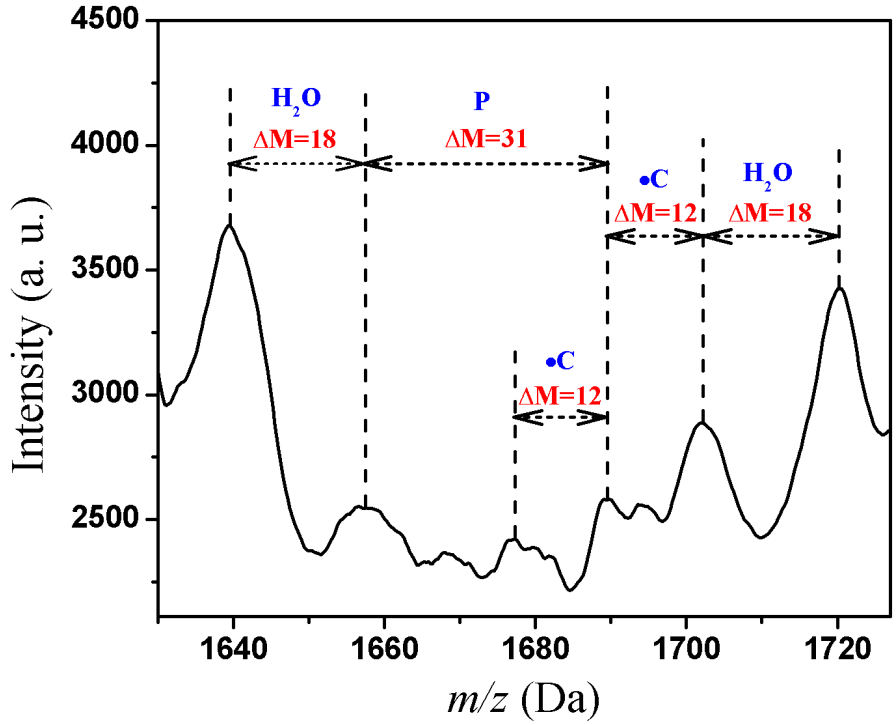


Fig. S8 Expanded mass spectra of (A) Fraction 2 at mass range 1300–1700 Da, (B) Fraction 4 at mass range at 1600–1950 Da, (C) Fraction 8 at mass range 2100–2500 Da, and (D) Fraction 12 at mass range 1300–1700 Da. The insets display the chemical formula of the mass loss of 284 fragment ions.

(A)



(B)

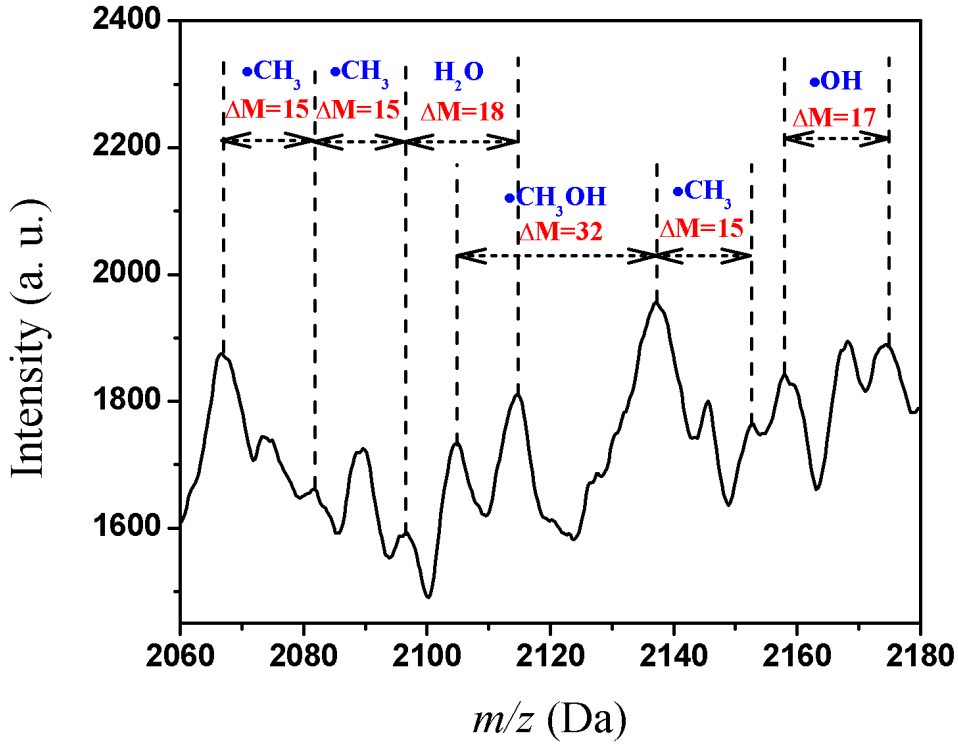


Fig. S9 Expanded mass spectra of Fraction 12 at mass range (A) 1630–1727 and (B) 2060–2180 Da. The loss of these fragment ions are found in all fractions.

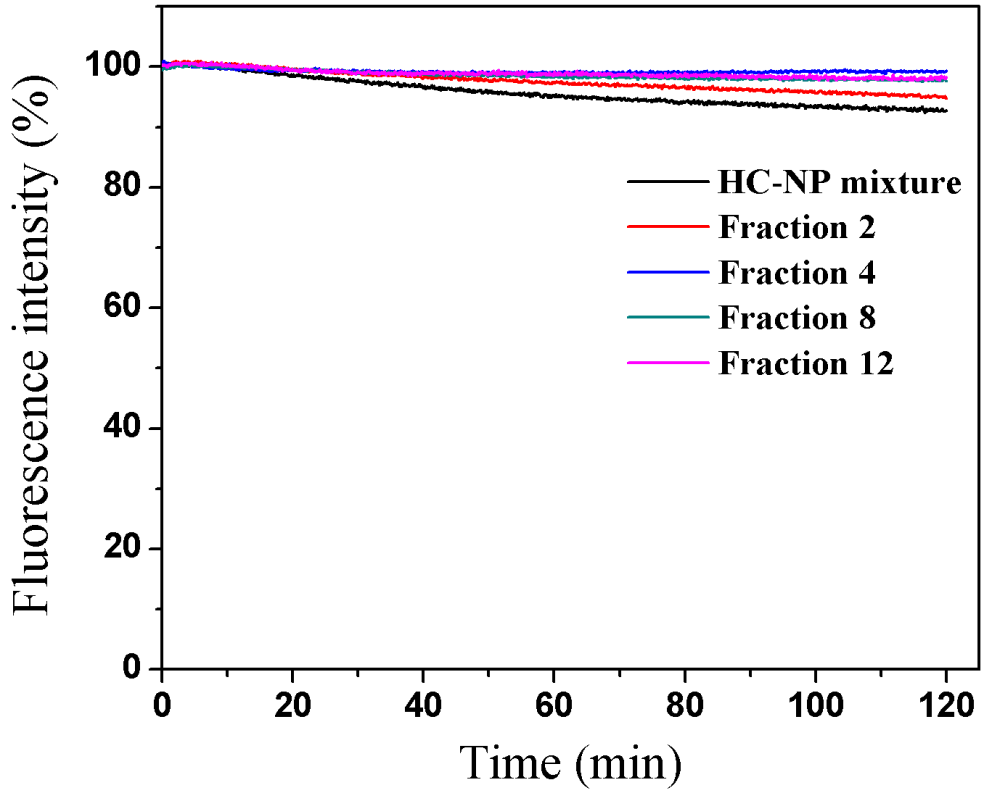


Fig. S10 The time-dependence of fluorescent intensity of the as-prepared HC-NP product and Fractions 2, 4, 8, and 12 at $\lambda_{ex}/\lambda_{em}$ of 320/430 nm.

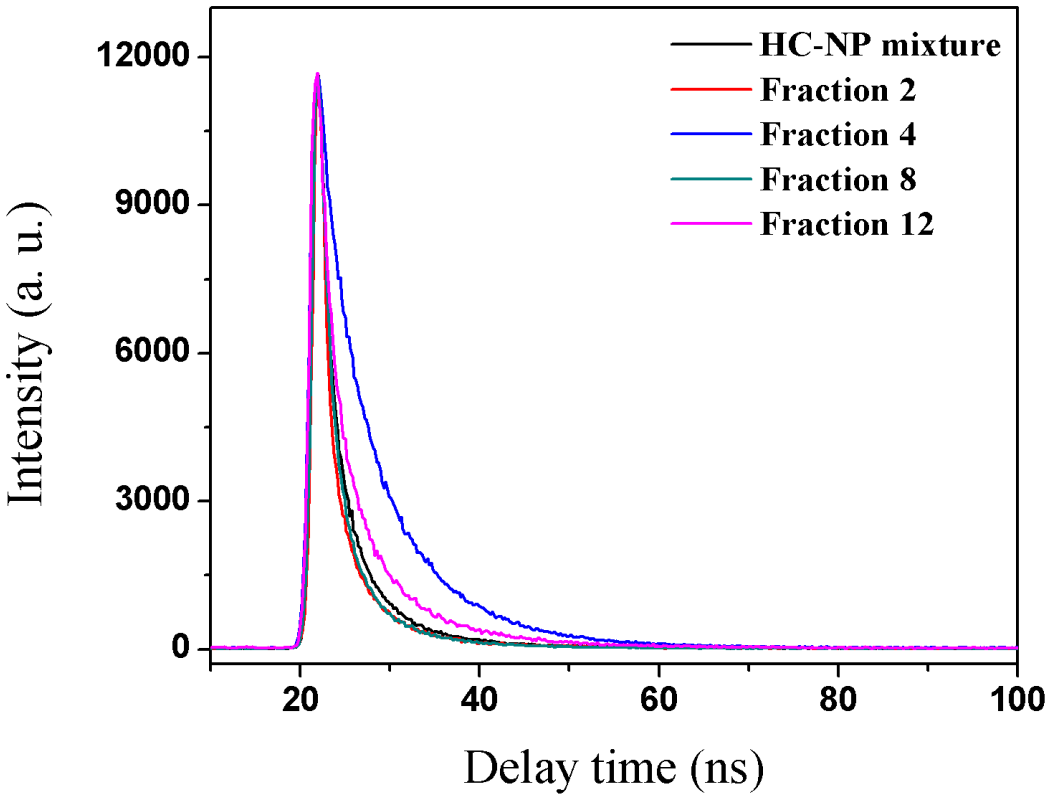


Fig. S11 The time-resolved spectra of the as-prepared HC-NP product and Fractions 2, 4, 8, and 12 at $\lambda_{ex}/\lambda_{em}$ of 405/430 nm.

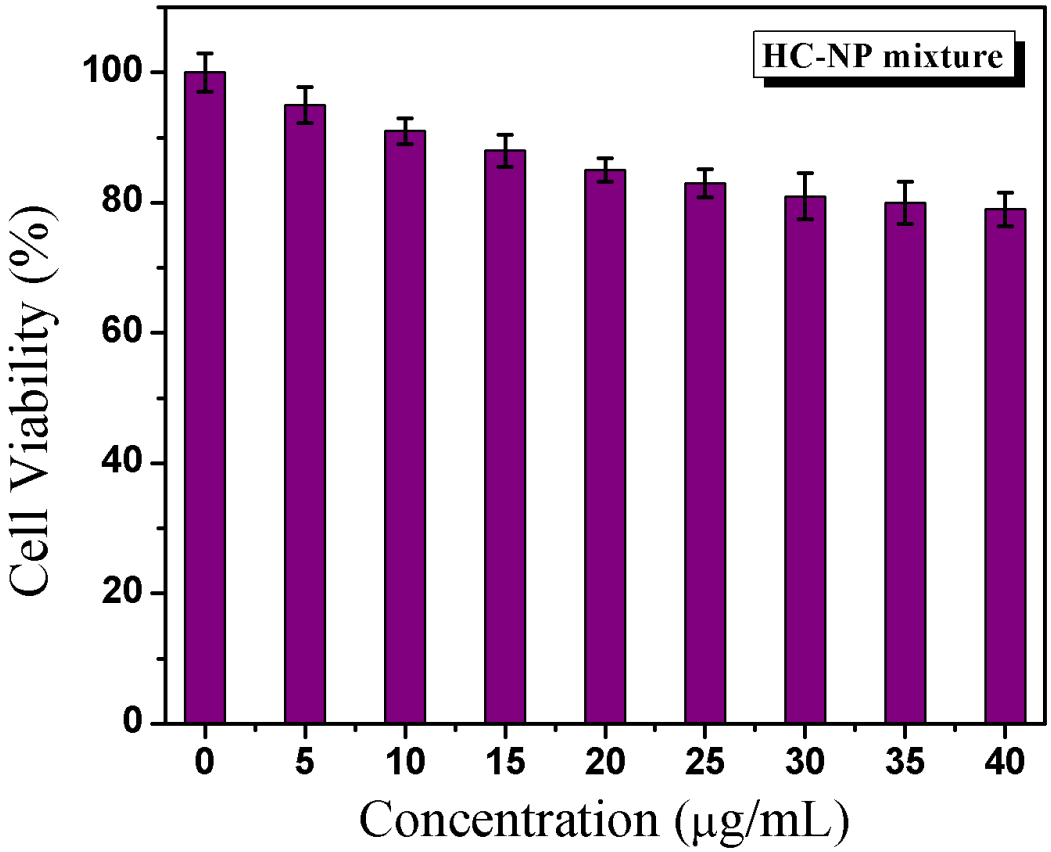


Fig. S12 Cytotoxicity test of the as-prepared HC-NP product on MCF-7 human breast adenocarcinoma cells. The error bars represent the standard deviation of three independent experiments.