Supplementary Information

Chitosan/tripolyphosphate nanoformulation carrying paraquat: insights of its enhanced herbicidal activity

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S1. Encapsulation efficiency

The amount of paraquat herbicide encapsulated in the nanostructures was determined by the ultrafiltration/centrifugation method. [1] The suspension of nanoherbicide was centrifugated in Amicon Ultra 30 kDa regenerated cellulose ultrafiltration units, and the paraquat in the filtrate was then quantified using its absorbance band at 257 nm. There were no adsorption of paraquat in the centrifugal ultrafiltration devices. The total amounts of herbicide in the NP suspensions were determined after dilution in methanol to disintegrate the particles, followed by filtration through a 0.22 µm membrane. Paraquat was then quantified using a UV-vis spectrometer (UV-5200 spectrophotometer, Global Trade Technology). The limit of determination (LOD) and limit of quantification (LOQ) were calculated using the PQ analytical curve following the recommendations of the International Union of Pure and Applied Chemistry (IUPAC, 1987). The LOD and LOQ are 0.019389 µg/L and 0.0646297 µg/L, respectively. From the result obtained, the encapsulation efficiency (EE%) was calculated according to Eq. 1.

$$EE(\%) = \frac{m_{total} - m_{free}}{m_{total}} x\ 100 \qquad [Eq.\ 1]$$

S2. Scanning electron microscopy and atomic force microscopy conditions

The scanning electron microscopy (SEM) was operated at 20 kV with a spot size of 3.0−4.0 mm and a working distance (WD) of 9.5 mm. The silicon substrate was bonded to the sample holder with carbon tape, followed by the deposition of a 5 nm thick gold film, under an argon atmosphere. Also, the atomic force microcopy (AFM) analysis were performed using an Agilent 5500 *AFM,* operated in contactless mode with the PPP-CONTPt-20 (Nanosensors™)

cantilevers. Topographic images were captured at 512 × 512 pixels' resolution, with a scan size of 0.5 μm × 0.5 μm, at 25 °C and analysed using Gwyddion 2.49 software.

S3. In vitro release kinetic study

The paraquat release kinetic profile from nanoparticles was determined based on release test using two compartments (one donor and the other acceptor) separated by a cellulose membrane with a 10 kDa molecular exclusion pore. [1] In the donor compartment, 1 mL of the CS/TPP:PQ was added, and in the recipient compartment, 100 mL of deionized water (pH 7). The sink dilution condition was maintained throughout the experiment. Thus, aliquots were collected at intervals of time from 0 to 800 min and analysed by UV− visible spectroscopy. The concentration values were transformed in percentages and plotted as a function of time using GraphPad Prisma 8.0 software. The Korsmeyer−Peppas mathematical model was applied to obtain the potential release mechanism of the PQ on CS/TPP nanoparticles. [2]

Table S1. Physicochemical characterization of FNR.

Uniprot ID	PS51384
Protein	FNR
Sequence length	369
M.wt.	411188.47
pI	8.67
Total number of negatively charged residues (ASP + GLU)	46
Total number of positively charged residues (ARG + LYS)	51
Extinction coefficient ($M^{-1}cm^{-1}$)	55,350 – 55,600
Instability Index	31.64
Aliphatic index	72.95
GRAVY	-0.388

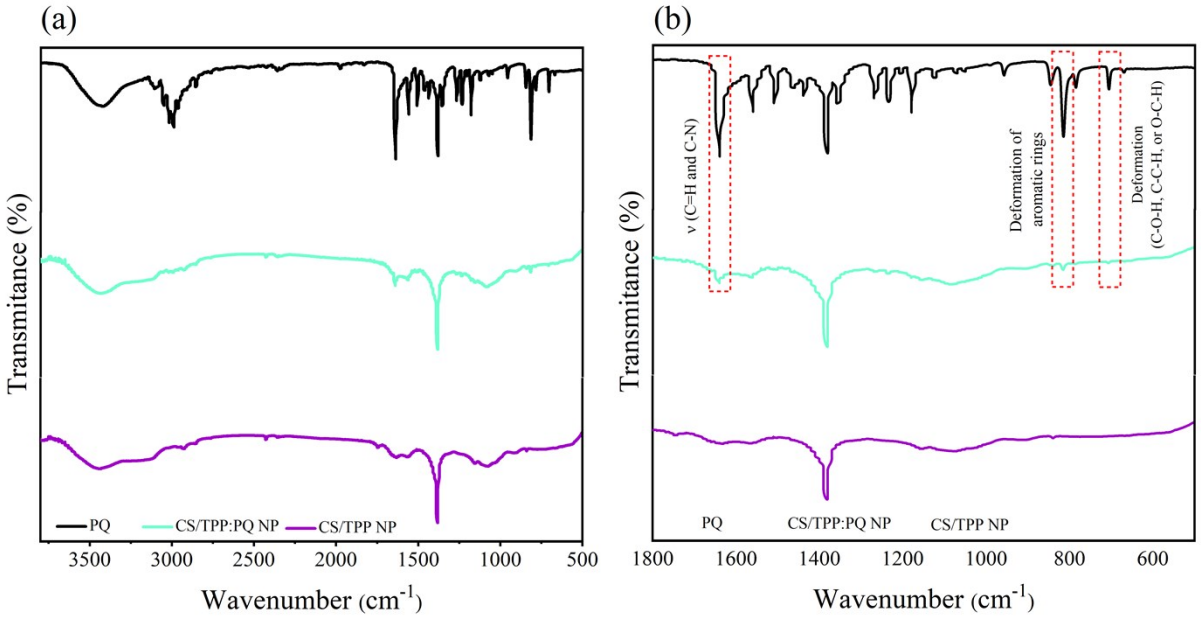

Figure S1. Fourier transform infrared (FTIR) spectrum of paraquat (PQ), chitosan/tripolyphosphate nanoparticles with (CS/TPP:PQ) and chitosan/tripolyphosphate nanoparticles without paraquat (CS/TPP): (a) in a range from 4000 to 500 cm^{-1}, and (b) in a range from 1800 to 600 cm^{-1}

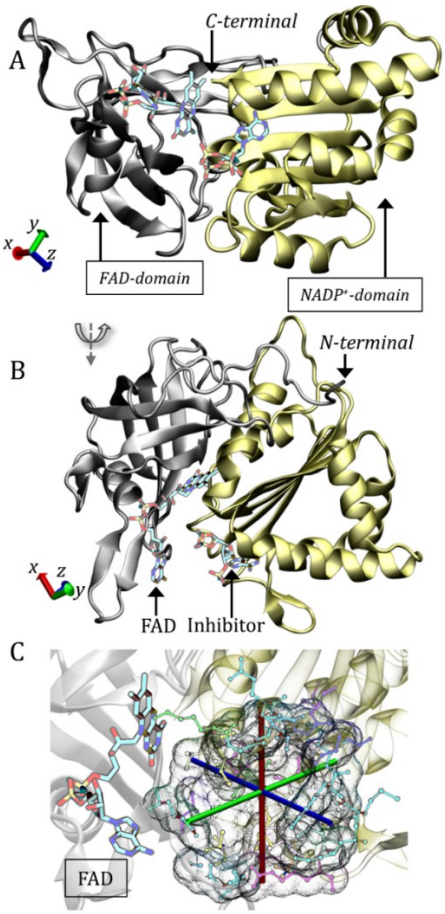

Figure S2. Details of the three-dimensional structure of the ferredoxin-$NADP^+$-oxidoreductase (FNR) highlighting in (A) the FAD and $NADP^+$ domains; (B) FAD and the ligand (P-AMP) positions and (C) the protein domain explored for Paraquat-FNR interactions.

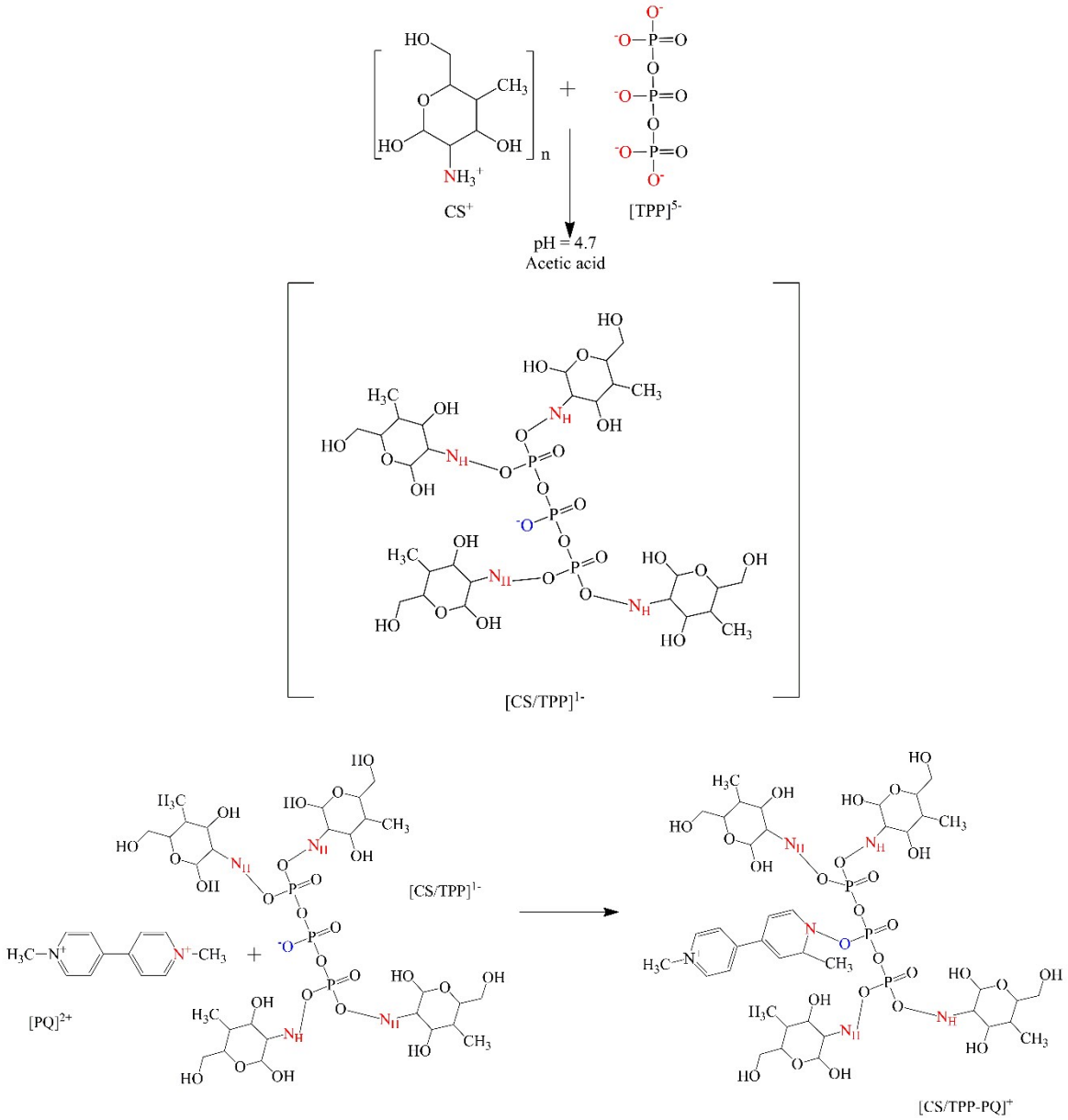

Figure S3. Schematic representation of the suggested mechanism of electrochemical reduction of nanoencapsulated paraquat observed on the surface of glassy carbon electrode.

References

1. R. Grillo, A. E. S. Pereira, C. S. Nishisaka, R. de Lima, K. Oehlke, R. Greiner and L. F. Fraceto, Chitosan/tripolyphosphate nanoparticles loaded with paraquat herbicide:

An environmentally safer alternative for weed control, *J Hazard Mater*, 2014, **278**, 163-171.

2. P. Costa, J. Manuel and S. Lobo, Modeling and comparison of dissolution profiles, *Eur J Pharm Sci*, 2001, **13**, 123-133.