

Supplementary Materials

Design and Characterization of Inulin Conjugate for Improved Intracellular and Targeted Delivery of Pyrazinoic Acid to Monocytes

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Supplementary Materials: *Supplementary Data for PCA paper*

NMR spectra of the starting MPI and conjugate

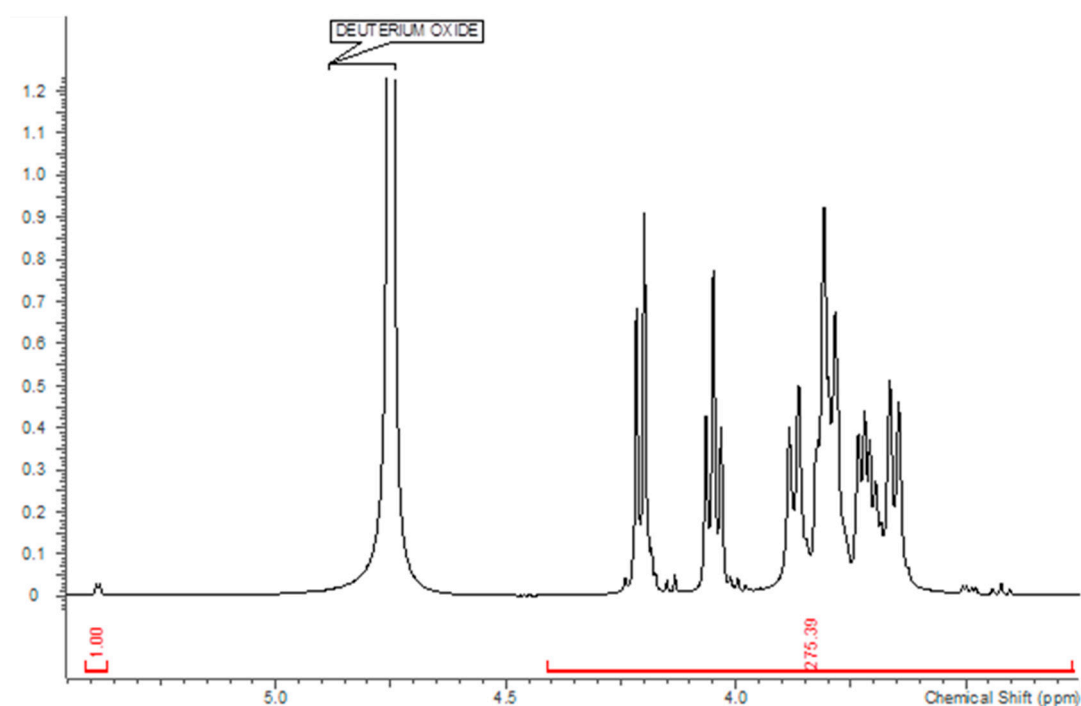
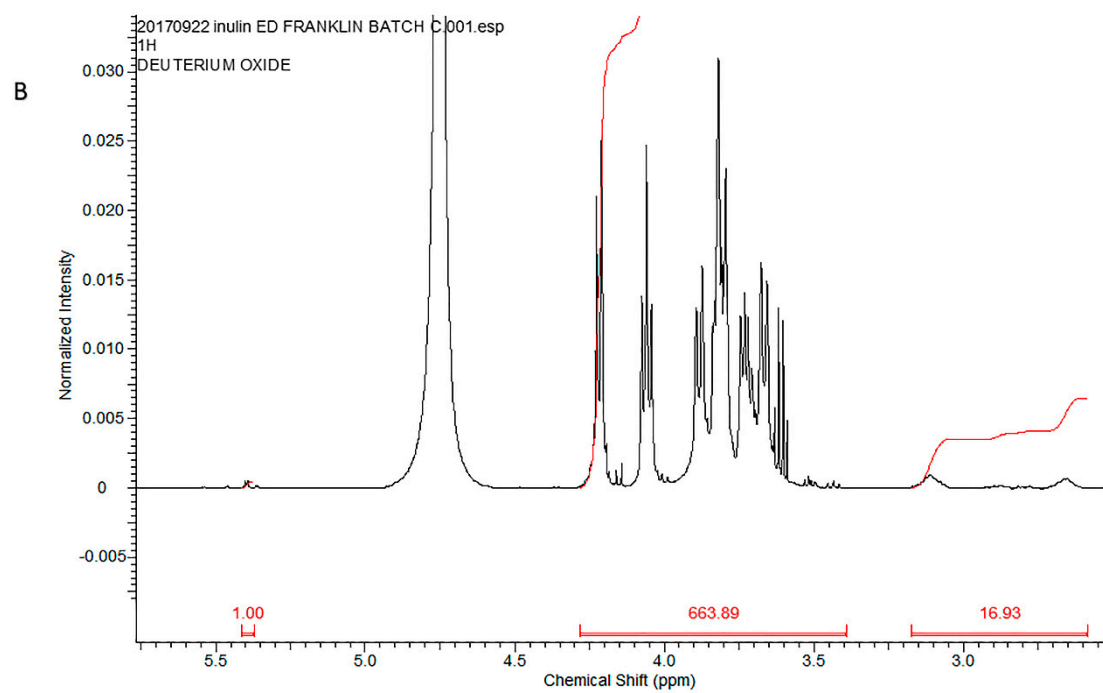
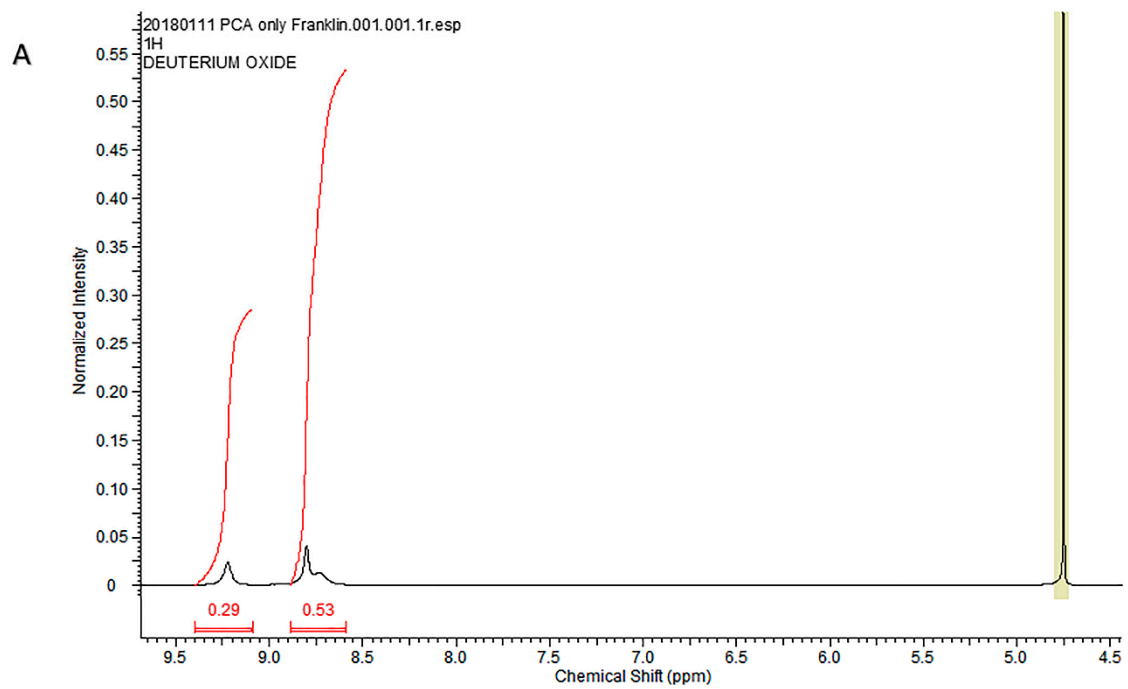


Figure S1. Raw MPI without any drug attachment.



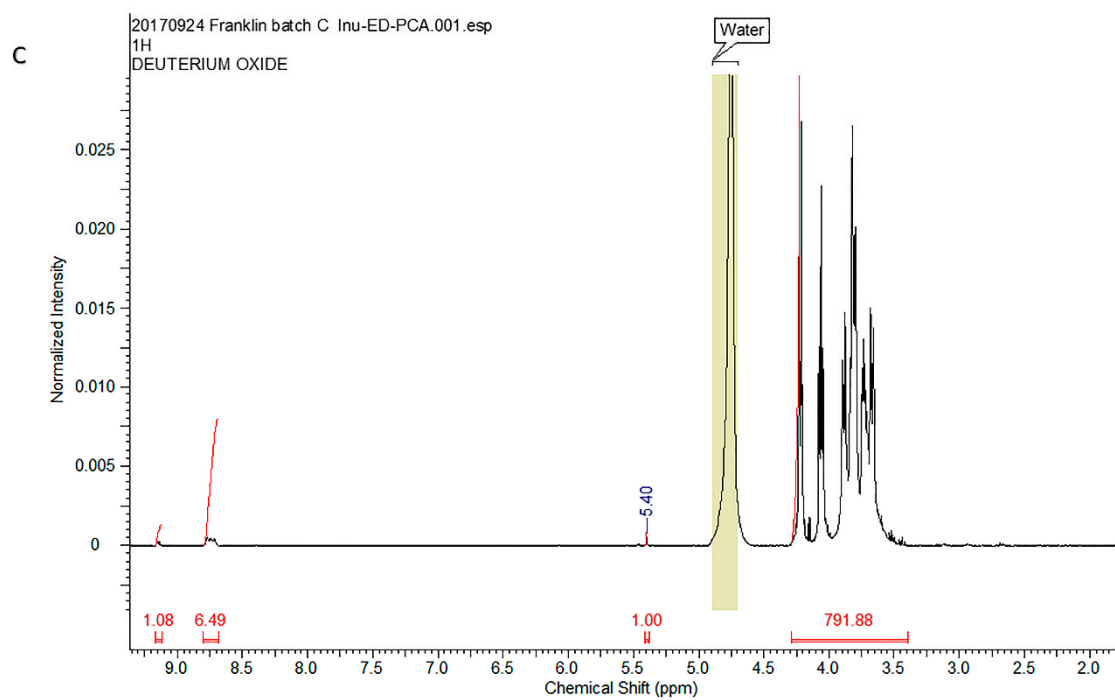


Figure S2. (A–C) showing *NMR spectra* of PCA, amine modified MPI and the conjugate.

FTIR

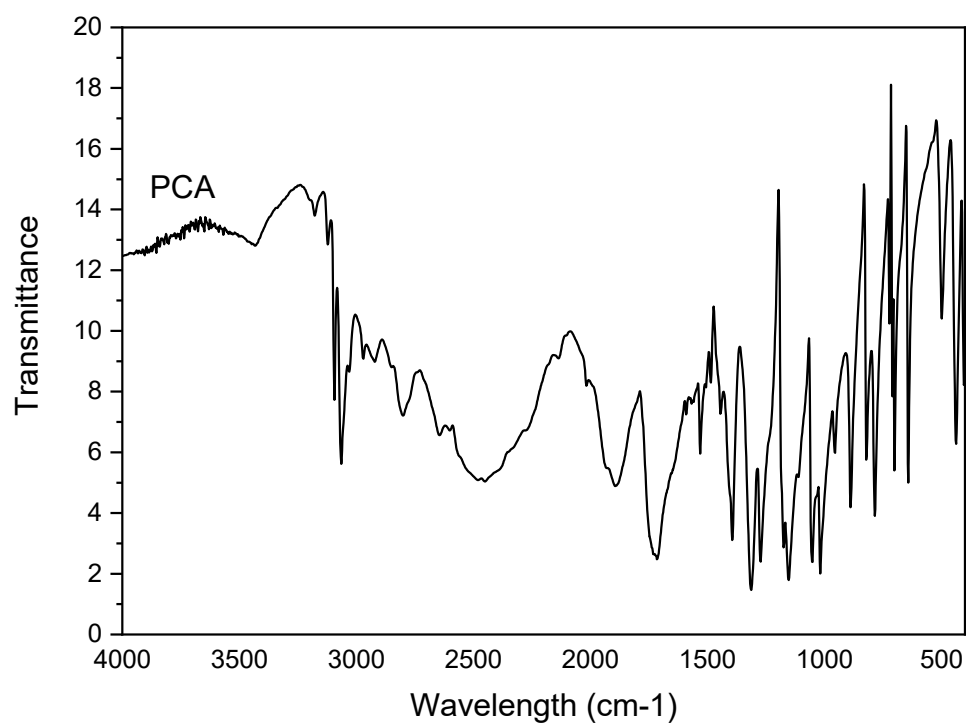


Figure S3 showing the FTIR spectra of PCA.

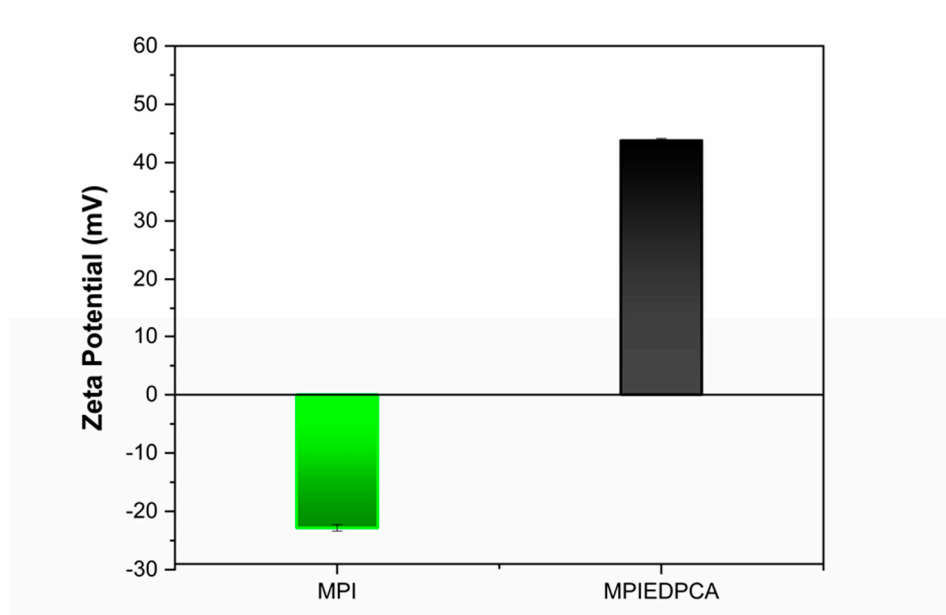


Figure S4 shows change in zeta potential due to modification.

Experiment on ester and ionic bond

- No ester and ionic bond formed

To ensure that there is no formation of ester and ionic bond between PCA and inulin 2 further experiments were conducted. First unmodified inulin particles was added to PCA activated with EDC/NHS reaction to see if any PCA attachments will occur with inulin OH (Inulin-PCA) – producing esters. (Figures S5 and S6) Second experiment involves using amine modified inulin with PCA without EDC/NHS (Inulin-ED+ PCA) which can produces ionic bond. The ^1H NMR result in both experiment didn't show any PCA attachment

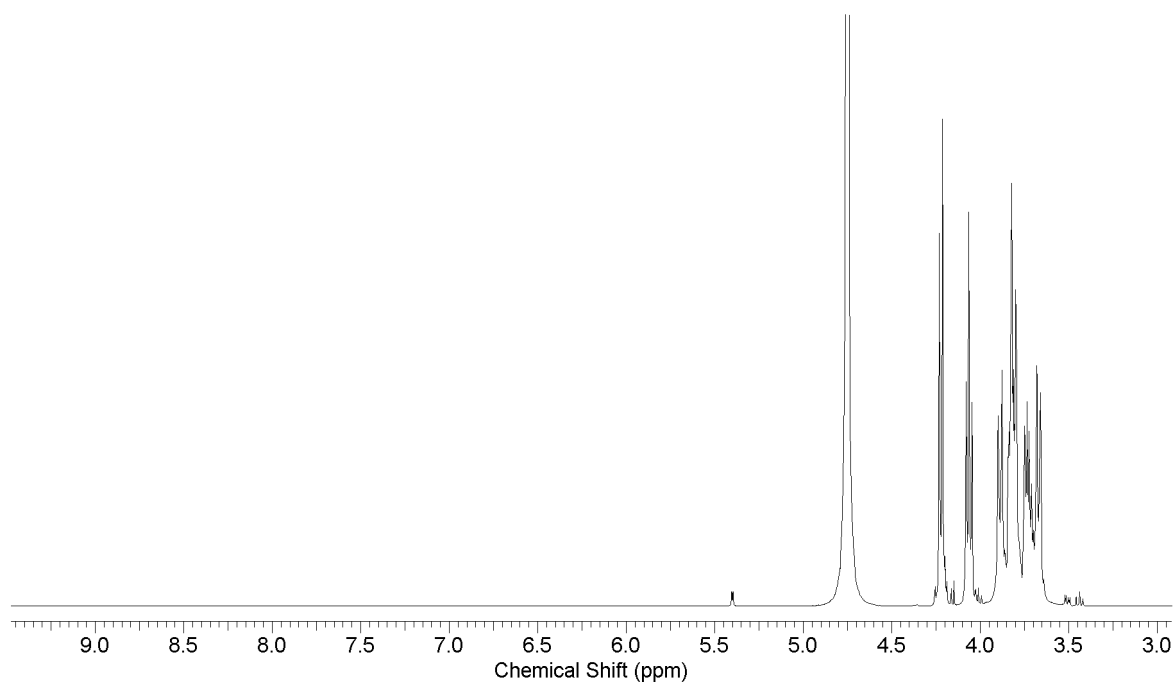


Figure S5 showing the ^1H -NMR spectra of reaction product of unmodified inulin particles and PCA activated with EDC/NHS. Figure S3 shows No PCA attachment via ester bond formation.

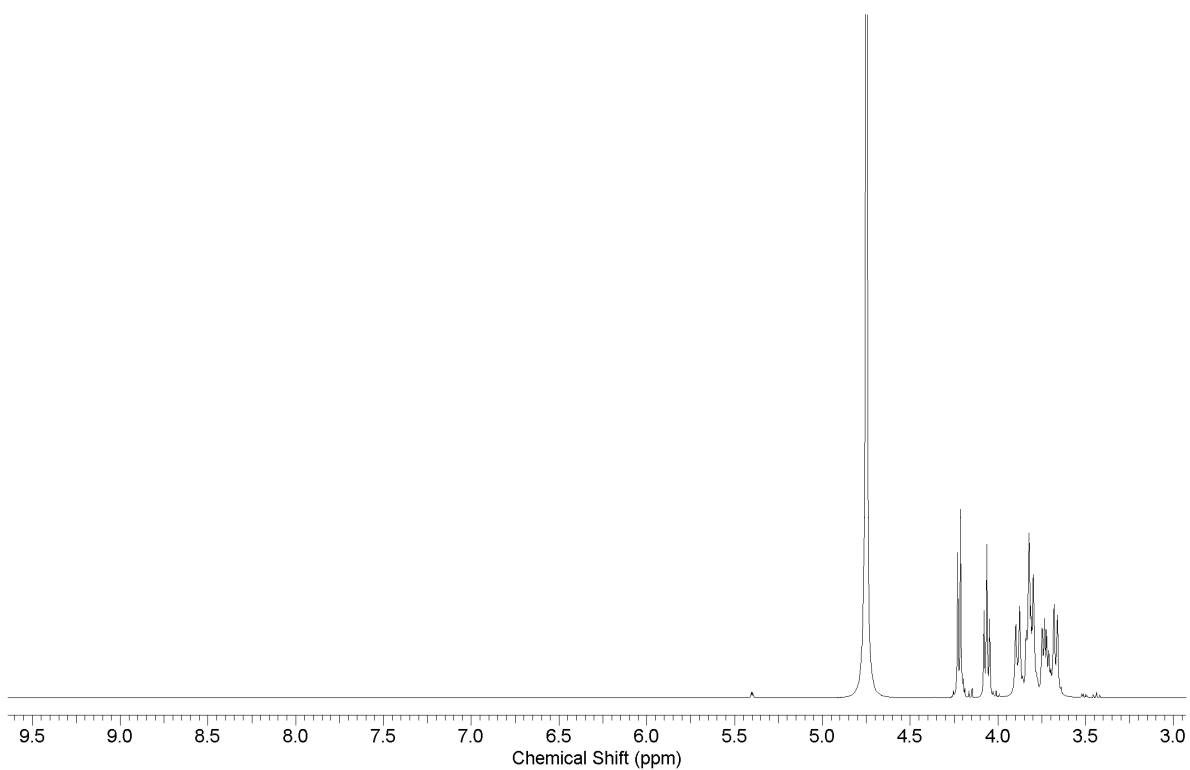


Figure S6 showing the ^1H -NMR spectra of reaction product of unmodified inulin particles and PCA without EDC/NHS. Figure S4 shows No PCA attachment via ester bond formation

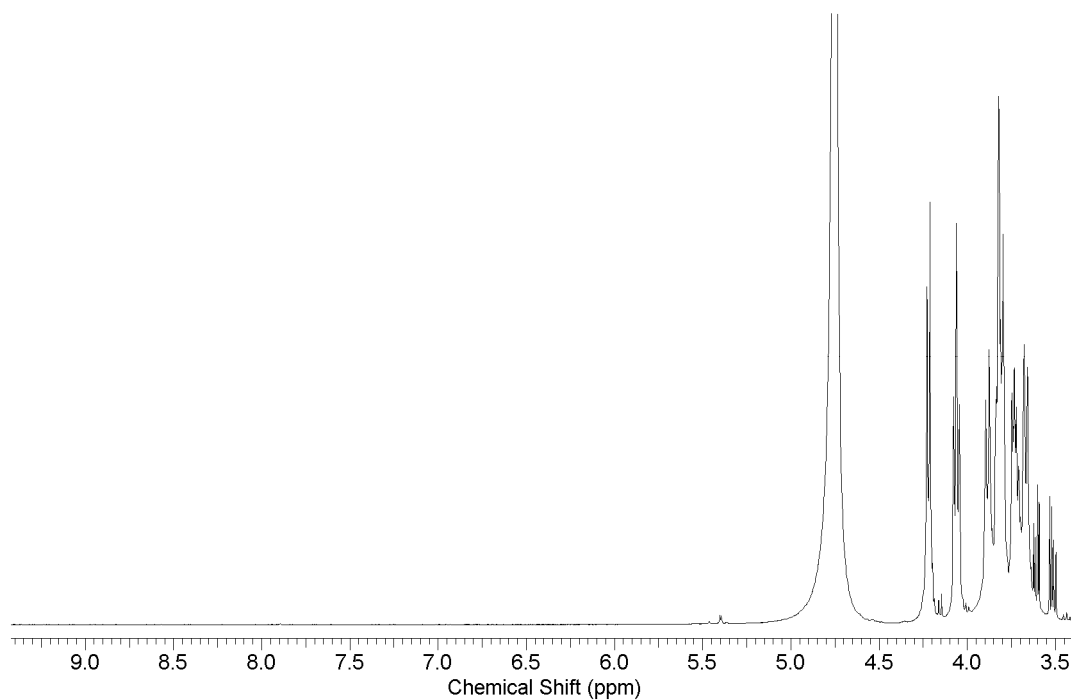


Figure S7 showing the ^1H -NMR spectra of reaction product of amine modified inulin with PCA without EDC/NHS (Inulin-ED+ PCA). Figure S5 shows No PCA attachment via ionic bond.

Preparation of ALF and SBF

The artificial lysosomal fluid was prepared from the reference paper [1].

HPLC Method for the determination of PCA loading via HPLC

1000 μL of the inulin – PCA complexes which is equivalent to 10 mg was added to 1000 μL of HCl acid (pH 1.2) in order to break the bond (hydrolysis) and release the attached PCA. After 24 h the sample was centrifuged for 5 min at 4500 rcf. Then the total amount of PCA in the supernatant was evaluated using HPLC after dilution of the test sample to appropriate concentration. Method adapted from Pitarresi et al [2] The calibration curve was performed by using standard PCA in mobile phase ($y = 78953x + 5074$ $R^2 = 0.9999$).

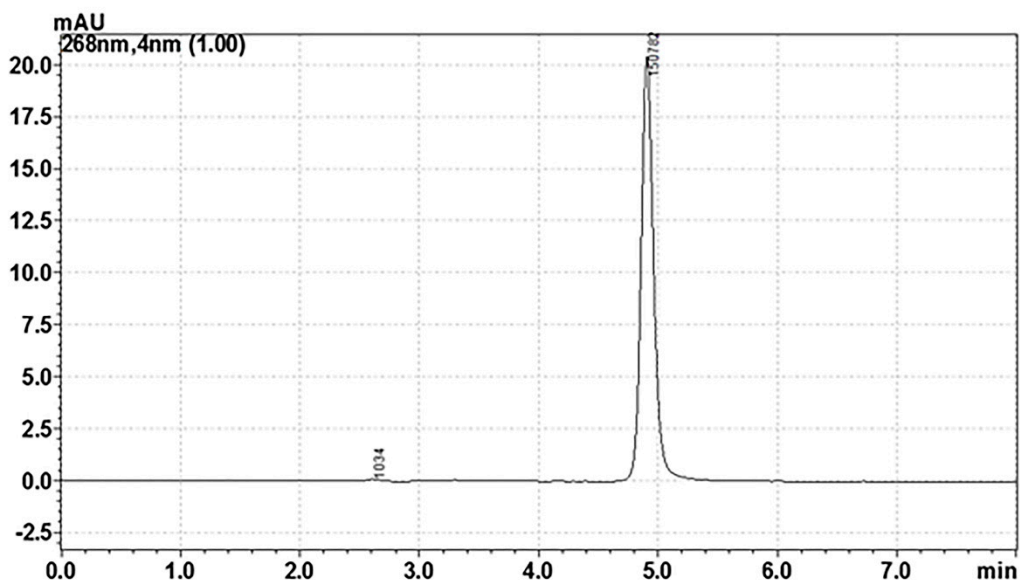
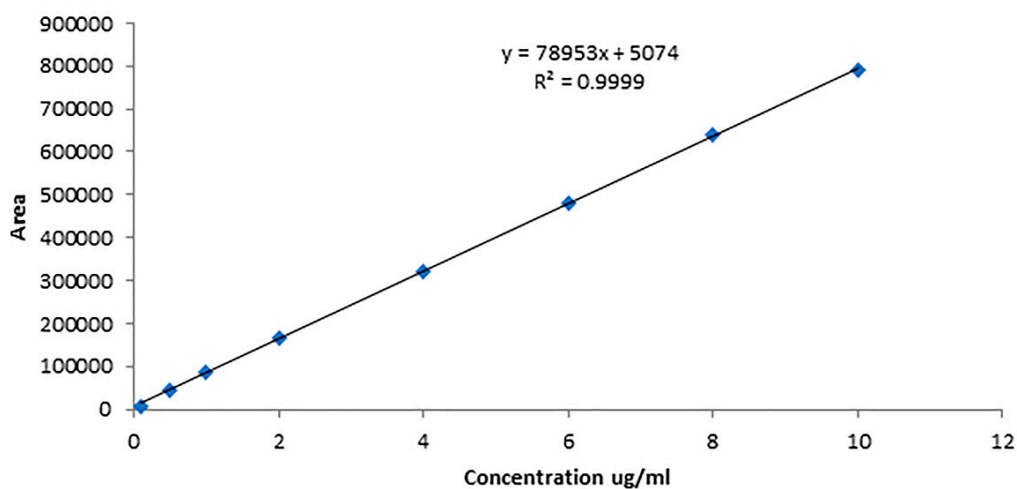


Figure S8. HPLC chromatogram of PCA.



Calibration curve for pure PCA

Figure S9. showing the calibration curve for the pure PCA standard.

| CONCENTRATION ($\mu\text{g/mL}$) | AREA |
|------------------------------------|--------|
| 0.1 | 7771 |
| 0.5 | 36339 |
| 1 | 74128 |
| 2 | 150782 |
| 4 | 295842 |
| 6 | 446920 |
| 8 | 585645 |
| 10 | 739117 |

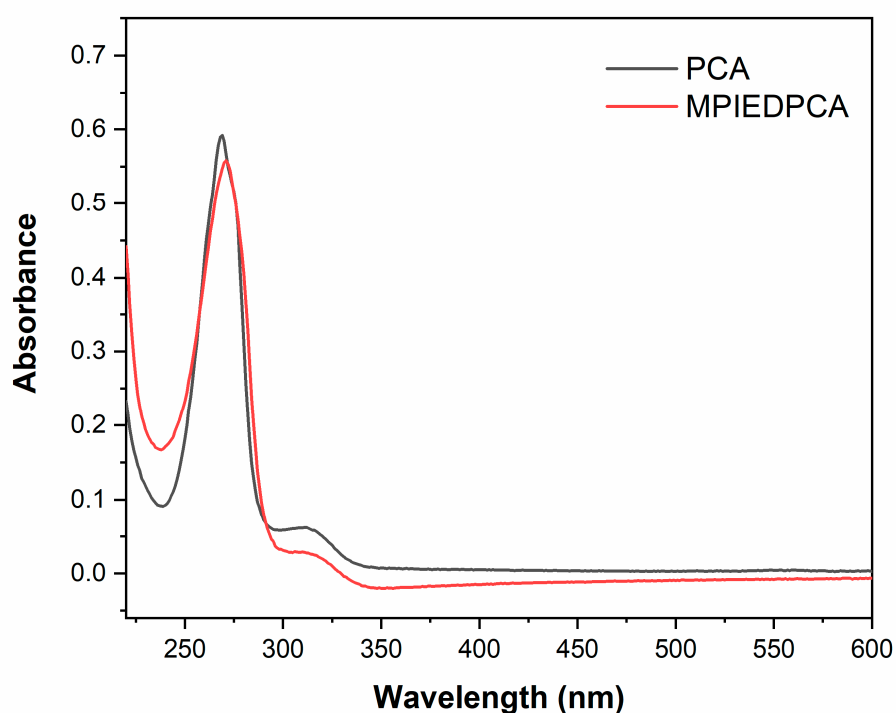


Figure S10. Graph showing that both UV spectrum of both PCA (10 μg) and MPIEDPCA (equivalent amount of PCA). The spectrum is similar when overlaid.

Table S1. kinetic release models for PCA.

| Mechanism | R ² |
|-------------|----------------|
| Zero order | 0.9557 |
| First order | 0.9933 |
| Huguchi | 0.9597 |
| Kors peppas | 0.9225 |
| Hixson | 0.953 |

References for Supplementary Part

1. Marques, M.; Löbenberg, R.; Almukainzi, M. Simulated Biological Fluids with Possible Application in Dissolution Testing. *Dissolution Technol.* **2011**, 15–28, doi:10.14227/DT180311P15.
2. Pitarresi, G.; Tripodo, G.; Cavallaro, G.; Palumbo, F.S.; Giammona, G. Inulin-iron complexes: A potential treatment of iron deficiency anaemia. *Eur. J. Pharm. Biopharm.* **2008**, 68, 267–276.