DESIGN OF ENZYME IMMOBILIZATION SYSTEM FOR CHITIN BIOCONVERSION

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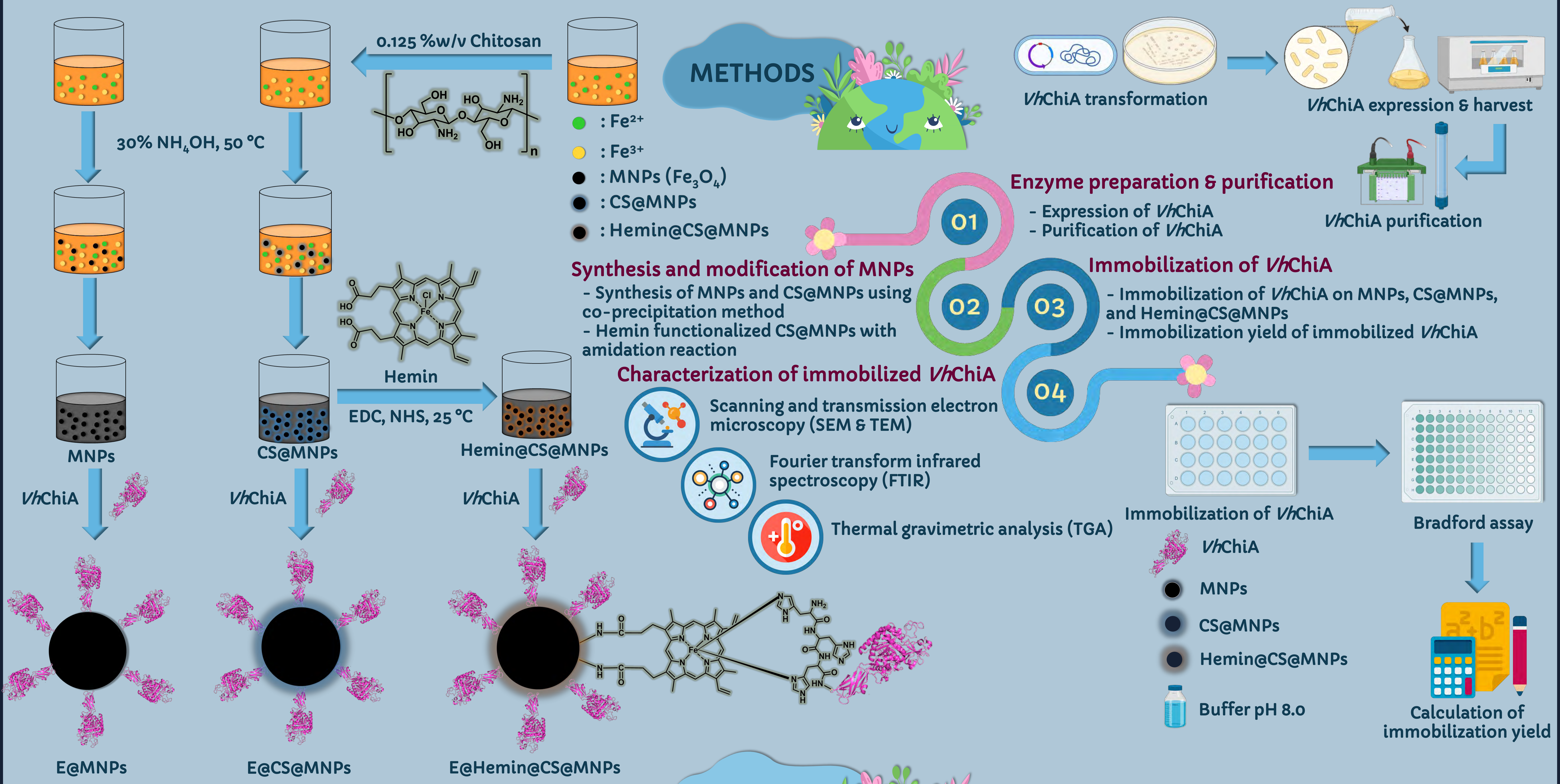
ABSTRACT

Chitooligosaccharides (COS) produced by the enzymatic hydrolysis of chitin are of significant interest; their high value indicates that they have interesting bioactivities such as anticancer, antifungal, and anti-inflammatory properties, making them a viable pharmaceutical product. Utilizing of immobilized enzyme for COS production is interesting, as long as the enzyme is stable enough for industrial application. In this study, chitinase A from the marine bacterium *Vibrio harveyi* (*Vh*ChiA) was expressed and purified by a Ni-NTA column. Chitosan coated magnetic nanoparticles (CS@MNPs) were synthesized by in situ co-precipitation method and were then functionalized with hemin through amidation reaction. VhChiA was immobilized on to three different types of magnetic nanoparticles including uncoated MNPs, CS@MNPs, and Hemin@CS@MNPs. Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and thermal gravimetric analysis (TGA) were used to illustrate the MNPs and immobilized *Vh*ChiA. Among of three types of magnetic nanoparticles, CS@MNPs provided the highest immobilization yield around 95.96±3.45%, followed by Hemin@CS@MNPs, and uncoated MNPs which gave 87.70±1.95% and 29±4.99%, respectively.

METHODS



RESULTS

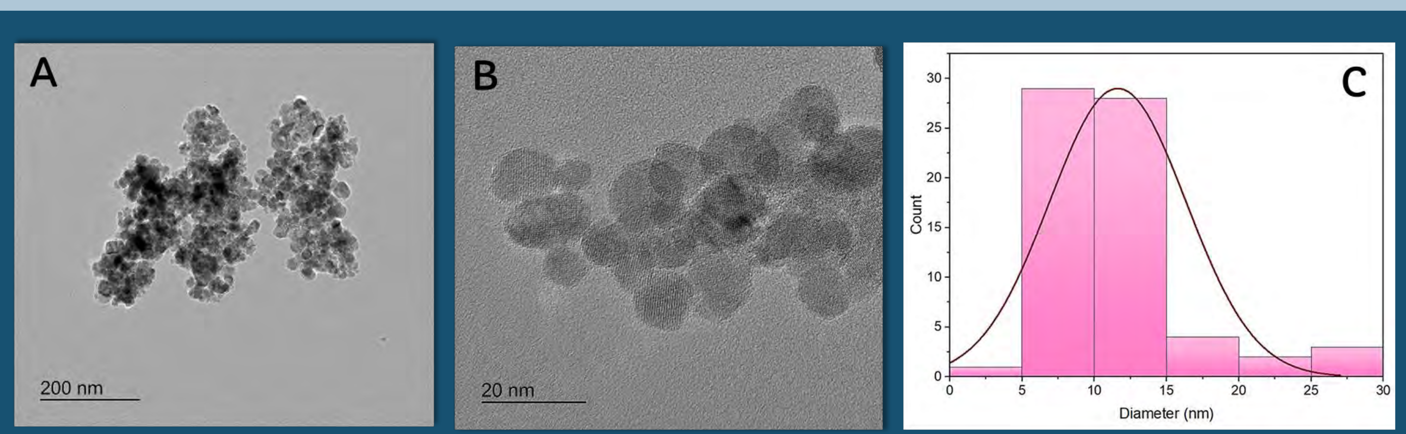


Figure 1 TEM micrographs of nanoparticles prepared by coprecipitation method with 0.125 % (w/v) of chitosan (A-B). Nanoparticle diameter histograms of CS@MNPs (C).

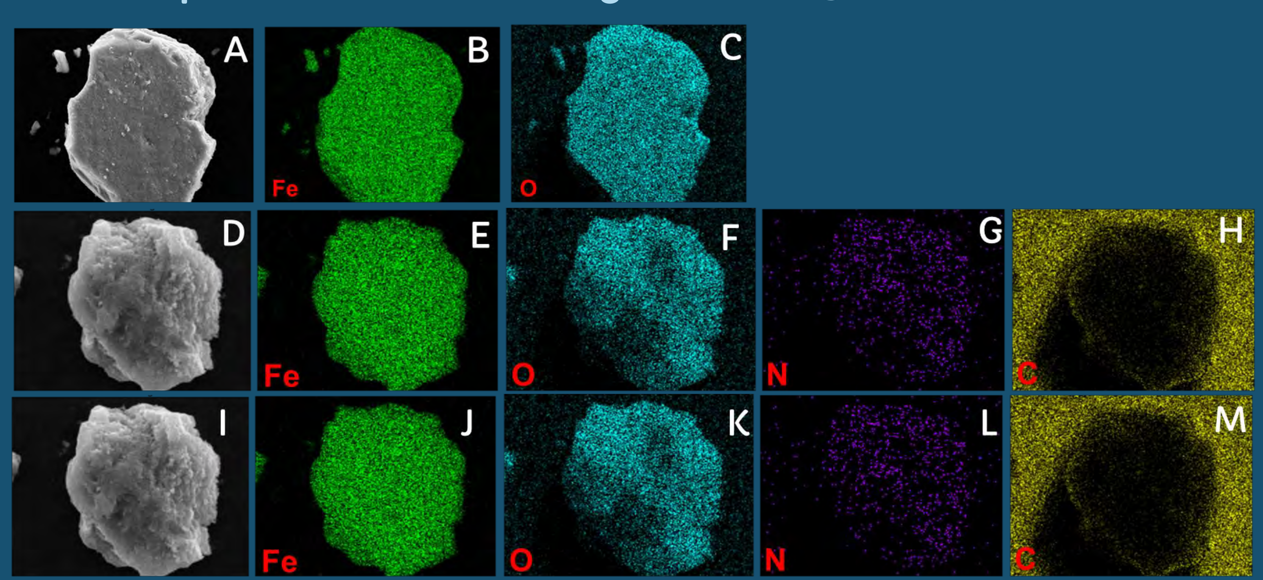


Figure 2 SEM-EDS images of all nanoparticles: MNPs (A), CS@MNPs (D), Hemin@CS@MNPs (I). Elemental mapping images of MNPs: Fe element (B), O element (C), CS@MNPs: Fe element (E), O element (F), N element (G), C element (H), Hemin@CS@MNPs: Fe element (J), O element (K), N element (L), C element (M).

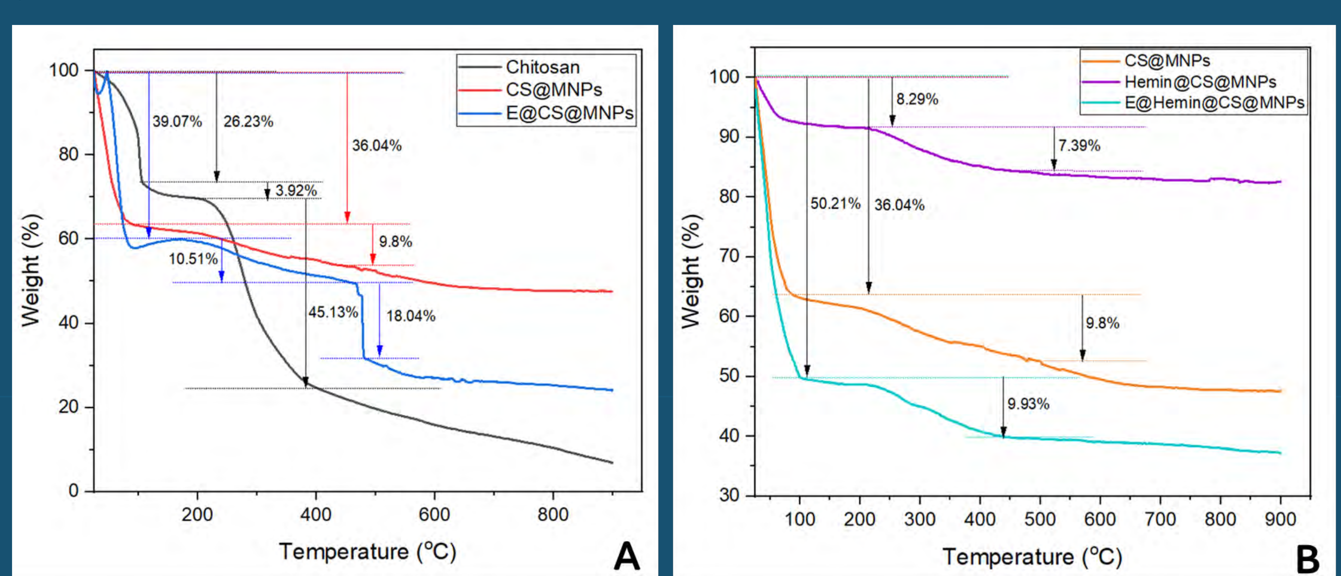


Figure 3 Thermal degradation curves of nanoparticles prepared by co-precipitation method: the down arrows indicate the %weight loss of each component in nanoparticles. . TGA analysis of CS@MNPs (red line), E@CS@MNPs (blue line), and pure chitosan (black line) (A). TGA analysis of Hemin@CS@MNPs (purple line), E@Hemin@CS@MNPs (blue-green line), and CS@MNPs (orange line) (B).

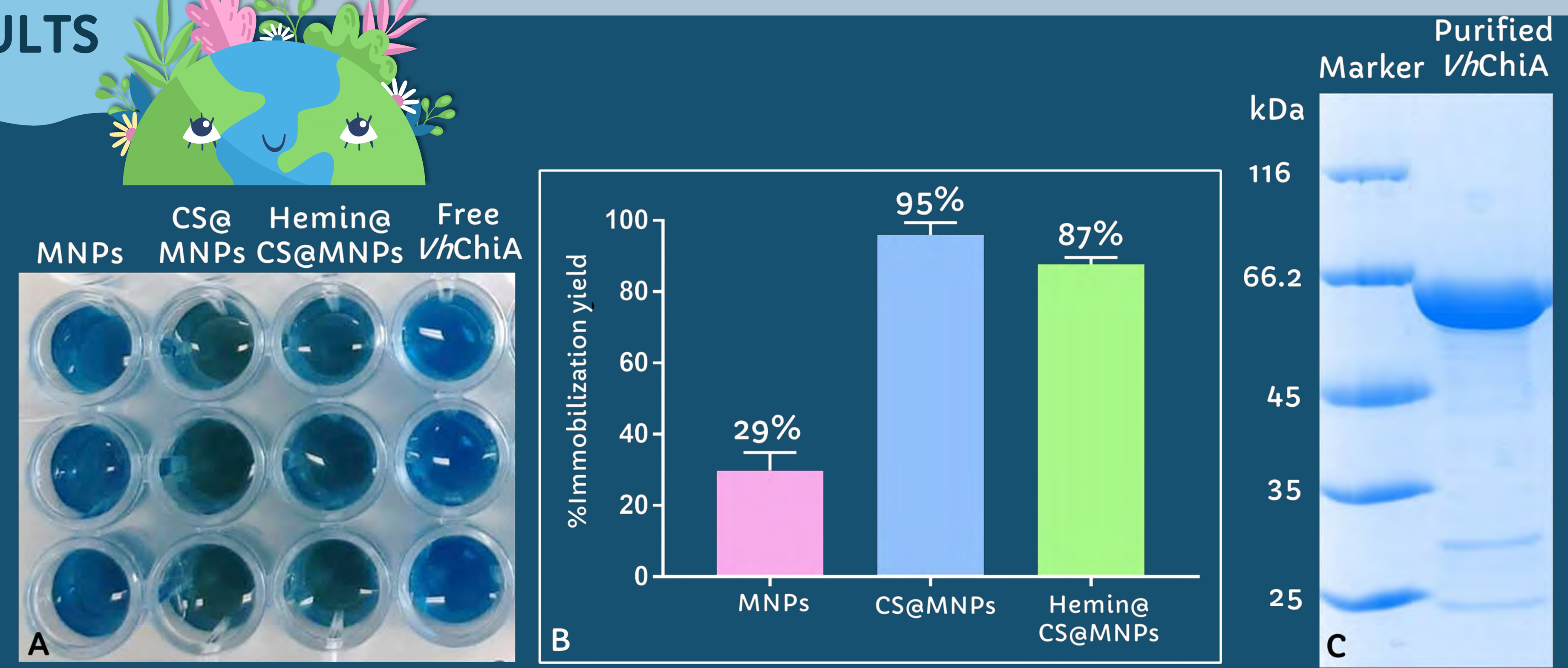


Figure 4 Bradford assay of free *Vh*ChiA and immobilized *Vh*ChiA on different carrier types (MNPs, CS@MNPs, and Hemin@CS@MNPs) (A). The bar graph represents an average %immobilization yield of immobilized *Vh*ChiA on different carrier types (MNPs, CS@MNPs, and Hemin@CS@MNPs) from 3 replicate experiments, and error bar shows SD (B). SDS-PAGE analysis of purified *Vh*ChiA (C).

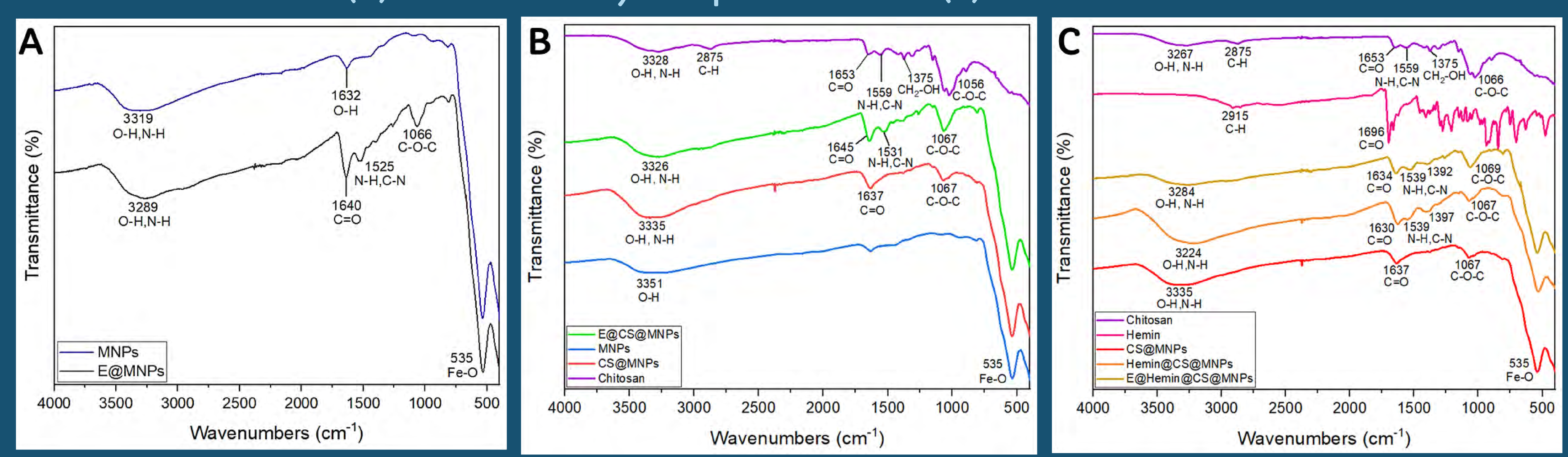


Figure 5 Fourier transform infrared spectroscopy (FTIR) spectra of MNPs (blue line), *Vh*ChiA@MNPs (dark brown line) (A). MNPs (blue line), CS@MNPs (red line), *Vh*ChiA@CS@MNPs (green line), and chitosan (purple line) (B). Hemin@CS@MNPs (orange line), *Vh*ChiA@hemin@CS@MNPs (brown line), hemin (pink line) (C).

ACKNOWLEDGEMENTS

This research was supported by Vidyasirimedhi Institute of Science and Technology (VISTEC) and The Thailand Research Fund (TRF) through The Basic Research Grant (Grant no: BRG610008), and Thailand Science Research and Innovation (TSRI) under the Global Partnership Grant. RT received the full-time PhD Scholarship from VISTEC.

CONCLUSION

In this research, *Vh*ChiA was successfully immobilized on all three types of MNPs in which CS@MNPs provided the highest immobilization yield around 95.96±3.45%, followed by Hemin@CS@MNPs and uncoated MNPs that gave 87.70±1.95% and 87.70±1.95%, respectively. The uncoated MNPs and CS@MNPs grafted the *Vh*ChiA using physical adsorption through electrostatic interaction while Hemin@CS@MNPs immobilized through His-tag affinity interaction between hemin molecule and His-tag of *Vh*ChiA. The TEM and SEM images showed that the nanoparticles have rough spherical morphology with diameter in the range of 5-15 nm and show homogenously elemental distribution of Fe and O in MNPs. The results of the FTIR spectra indicated the enzyme was immobilized on the carrier, the vibration in the range of 1600-1670 and 1500-1570 cm^{-1} was observed in the immobilized *Vh*ChiA which belong to the amide bond of enzyme. Interestingly, the TGA analysis showed 18.04% weight loss in the CS@MNPs which indicated the high content of *Vh*ChiA in the CS@MNPs. The immobilized *Vh*ChiA can expand the use of enzymes in industrial applications in which the enzyme can be easily separated from the reaction and recycled using the external magnetic force.