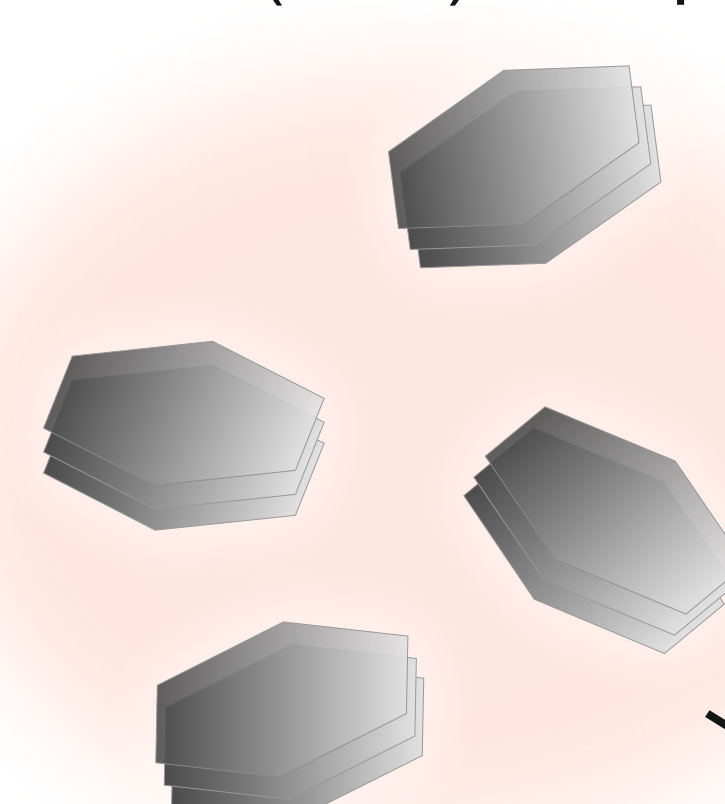


Introduction

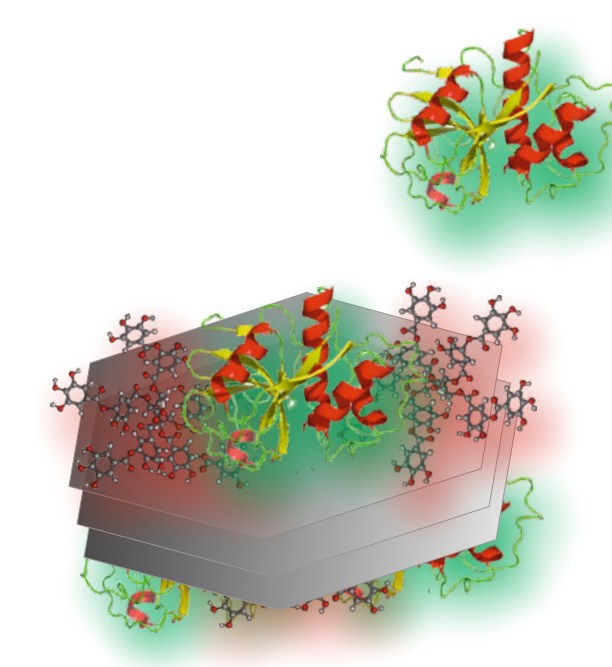
The synchronized operation of antioxidants and enzymes is necessary for the proper functioning of living organisms and for various industrial processes. Antioxidants prevent or interrupt harmful oxidation reactions caused by reactive oxygen species (ROS) and thus prevent oxidative stress.¹ Enzymes accelerate biochemical reactions through their specific (e.g. proteolytic) catalytic functions.² However, these biomolecules can be susceptible to several environmental factors (e.g. sensitivity to light, temperature, pH, low water solubility), which can then lead to the loss of their activity under inadequate conditions. These drawbacks can usually be overcome by their immobilization using appropriate methods. Layered double hydroxides (LDHs) are biocompatible, chemically and mechanically stable materials that are therefore suitable carrier materials.³ The aim of our study was the immobilization of tannic acid (TA) and papain (PPN) on LDH nanoparticles to enhance their stability and facilitate their combined use, which is beneficial e.g. in the food industry.

Objectives

1) Preparation of layered double hydroxide (LDH) nanoparticles



2) Immobilization of tannic acid (TA) and papain (PPN)



Results

1) Immobilization of TA and PPN on LDH surface by electrostatic interactions

TA and PPN were immobilized on LDH nanoparticles based on electrostatic interactions. Adsorption of TA resulted in charge reversal of the originally positively charged LDH particles, while PPN was immobilized on the negatively charged LDH/TA nanocomposites. The optimal TA and PPN dose was selected based on electrophoretic- and dynamic light scattering measurements. The optimal dose was 200 mg/g for both biomolecules.

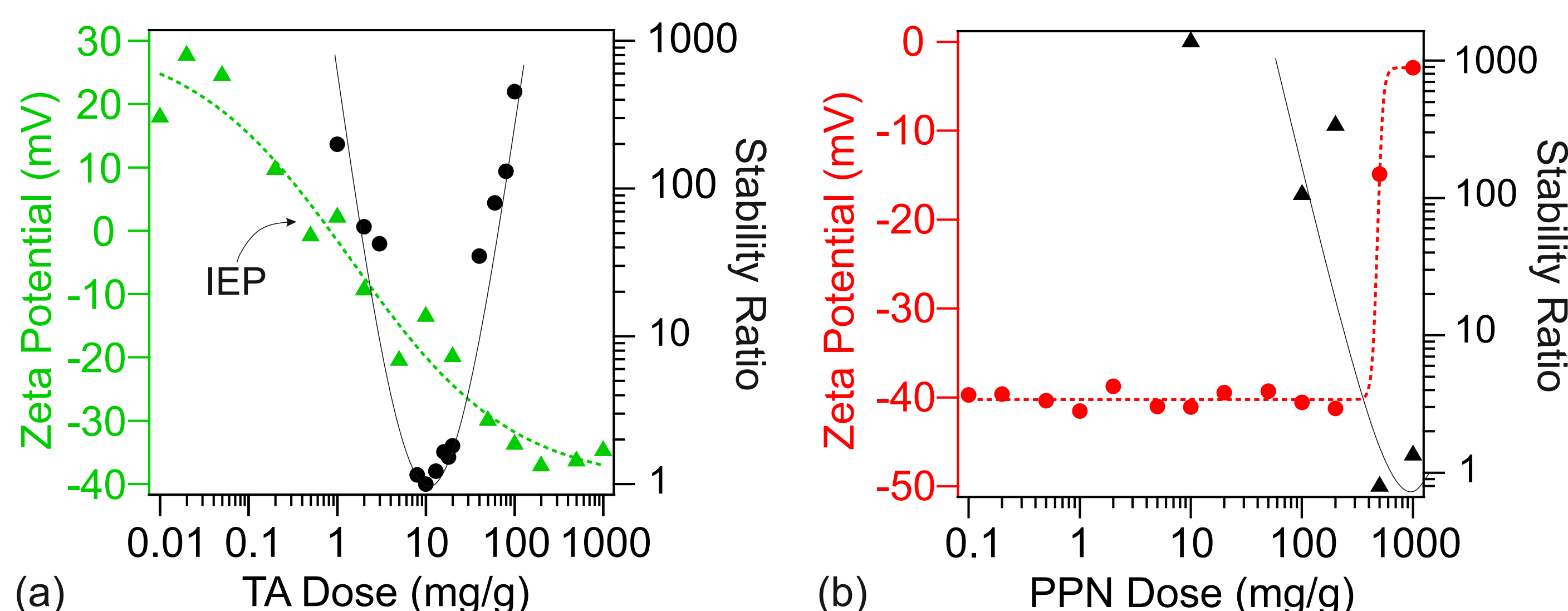


Figure 1. Change in zeta potential after immobilization of TA (a) and PPN (b) on LDH surface.

2) Confirmation of the adsorption of the biomolecules

The adsorption of the biomolecules was confirmed by Raman microscopy and by the Bradford assay. The characteristic peaks of TA and PPN in the Raman spectrum of LDH/TA/PPN and the results of the Bradford test confirmed the successful immobilization of TA and PPN on the surface of the nanoparticles.

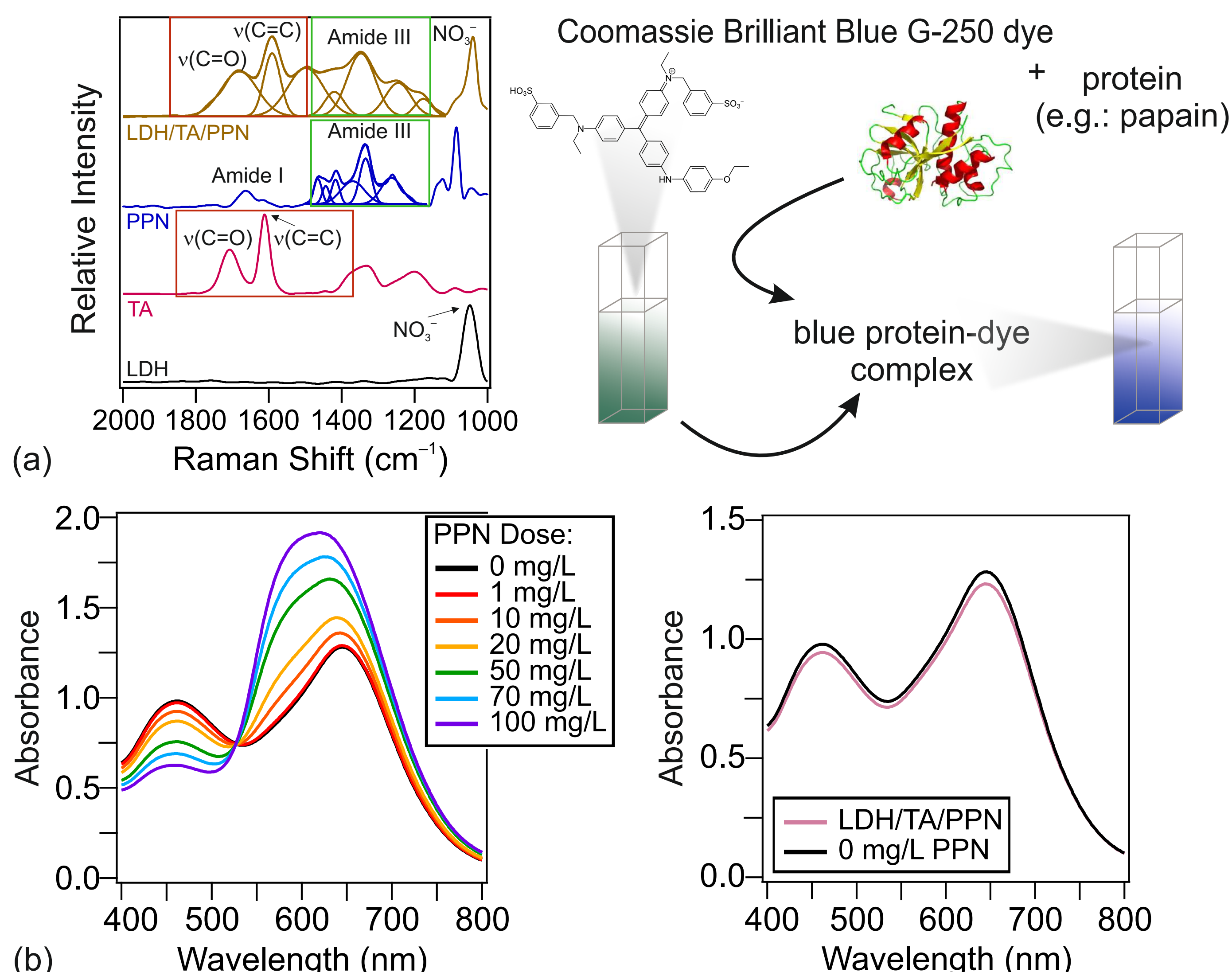


Figure 2. Raman spectra of LDH, TA, PPN and LDH/TA/PPN systems (a). Absorbance spectra of Bradford assays for standard solutions of PPN and for the supernatant of centrifuged LDH/TA/PPN system (b).

Conclusion

In conclusion, TA and PPN were immobilized on LDH nanoparticles, thus conferring antioxidant and proteolytic activity to the LDH/TA/PPN composite. Moreover, the resulting nanocomposite possessed high colloidal stability, which makes the composite a promising material for use in industrial processes where the simultaneous application of the two biomolecules in heterogeneous systems would be advantageous.

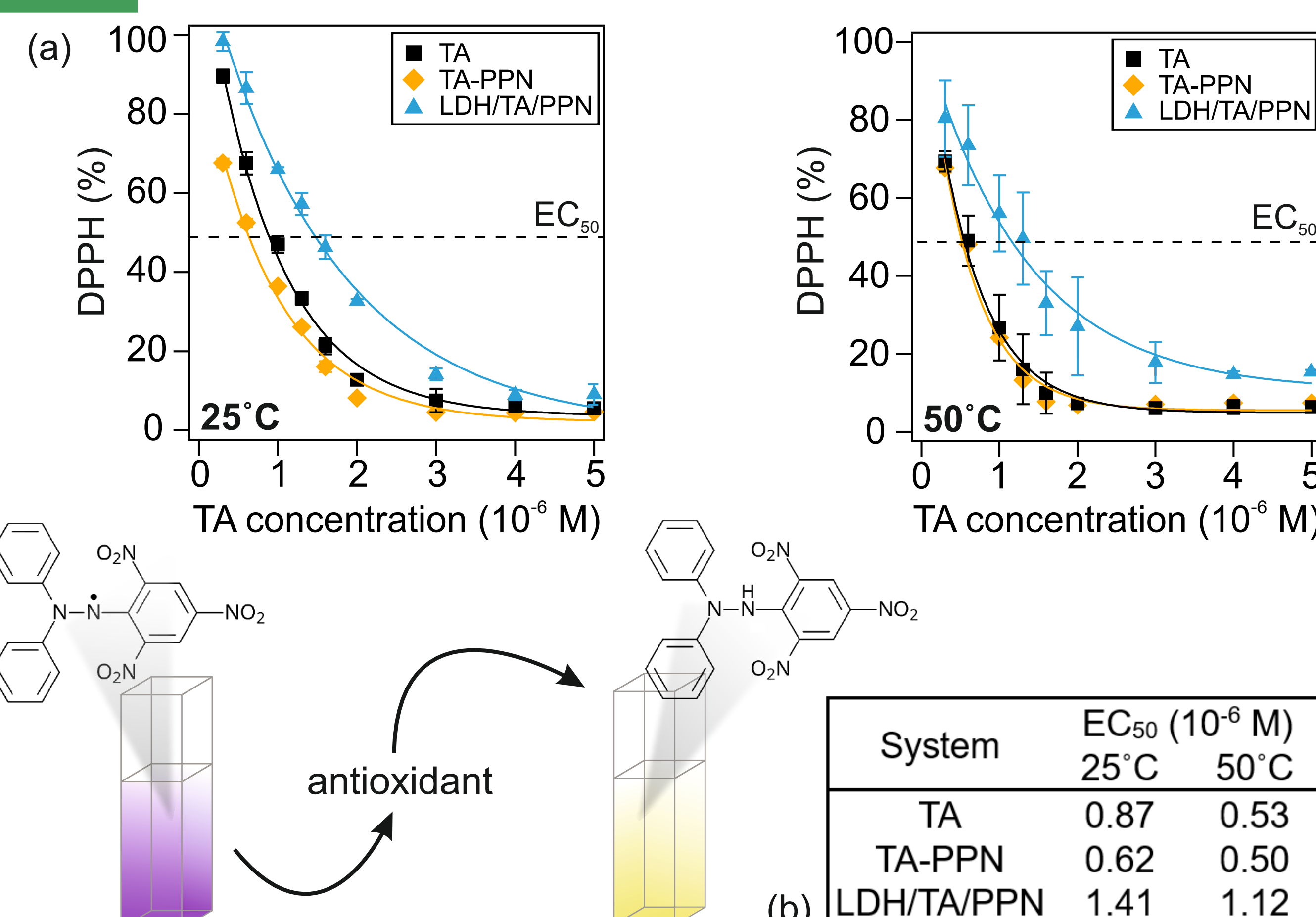


Figure 3. Radical scavenging activity of TA, TA-PPN mixture and LDH/TA/PPN at 25 and 50°C (a). Table of EC₅₀ values for these systems (b).

3) Determination of the radical scavenging activity of the nanocomposite

The radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay for TA, TA-PPN mixture and LDH/TA/PPN systems. The assay was performed at two different temperatures (25 and 50°C). The composite possessed significant activity at both temperatures despite the immobilization of TA, as determined by calculating EC₅₀.

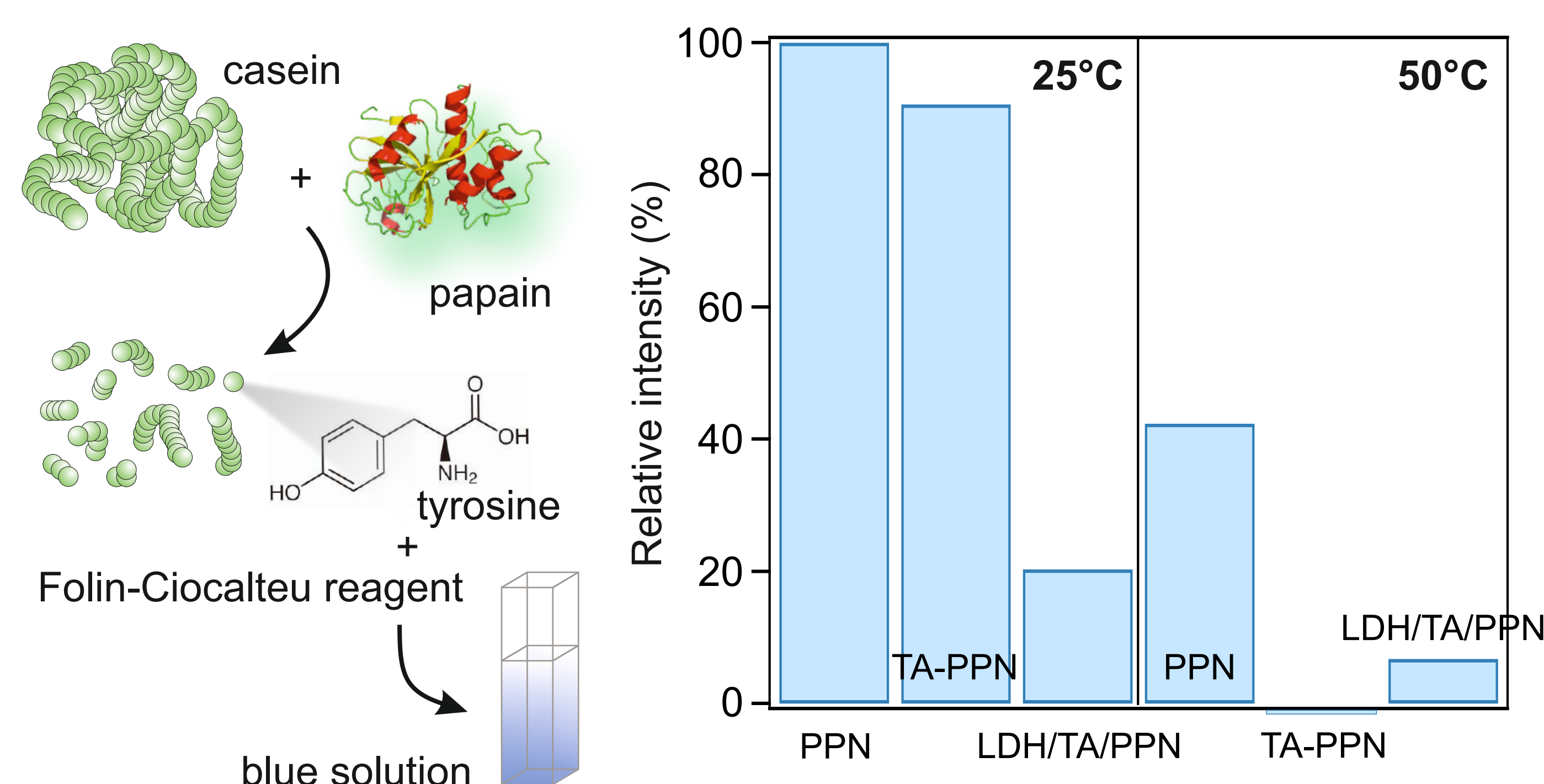


Figure 4. Proteolytic activity of PPN, TA-PPN mixture and LDH/TA/PPN at 25 and 50°C.

4) Evaluation of the proteolytic activity of the nanocomposite

The proteolytic activity was tested in a non-specific protease assay for PPN, TA-PPN mixture and LDH/TA/PPN systems. The assay was performed at two different temperatures (25 and 50°C). The composite had significant proteolytic activity after the immobilization of PPN. Moreover, in contrast to the TA/PPN mixture in solution phase, the composite retained higher activity after being treated at 50°C.

References

- ¹A.M. Pisoschi, A. Pop. Eur. J. Med. Chem. 97:55-74 (2015)
- ²J. Fernandez-Lucas, D. Castaneda, D. Hormigo. Trends Food Sci. Technol. 68:91-101 (2017)
- ³A. Szerlauth et al. J. Colloid Interface Sci. 632:260-270 (2023)

Development of multifunctional layered double hydroxide-based nanocomposites

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Introduction

The synchronised operation of antioxidants and enzymes is necessary for the proper functioning of living organisms and for various industrial processes. Antioxidants prevent or interrupt harmful oxidation reactions caused by reactive oxygen species (ROS) and thus anticipate oxidative stress.¹ Enzymes, on the other hand, accelerate biochemical reactions through their specific (e.g. proteolytic) catalytic functions.² However, these biomolecules can be susceptible to several environmental factors (e.g. sensitivity to light, temperature, pH, low water solubility), which can then lead to the loss of their activity under inadequate conditions. These drawbacks can usually be overcome by their immobilisation using different methods. Layered double hydroxides (LDHs) are biocompatible, chemically and mechanically stable materials that are therefore suitable carrier materials.³ The aim of our study was to immobilise tannic acid (TA) and papain (PPN) on LDH nanoparticles in order to enhance their stability and facilitate their combined use.

Experimental

LDH nanoparticles were synthesised by the coprecipitation method in which a metal salt ($\text{Mg}(\text{NO}_3)_2$ and $\text{Al}(\text{NO}_3)_3$) solution was mixed with NaOH solution. After three washing steps, the LDH slurry was dried and the resuspended powder was used for further experiments. The adsorption of the biomolecules (TA, PPN) was monitored by electrophoretic- and dynamic light scattering measurements using a Litesizer 500 device. The adsorption of the biomolecules was confirmed by Raman microscopy (Bruker Senterra II) and by the Bradford assay. To determine the radical scavenging and proteolytic activity of the composite, the DPPH assays and the protease activity assay were performed using a Genesys 10S spectrophotometer.

Results and conclusions

TA and PPN were immobilised on the surface of the nanoparticles by electrostatic interactions. The adsorption of TA resulted in a charge reversal of the originally positively charged LDH particles, while PPN was immobilised on the negatively charged LDH/TA nanocomposites. The characteristic peaks of TA and PPN in the Raman spectrum of LDH/TA/PPN and the results of the Bradford assay confirmed the proper immobilisation of TA and PPN on the surface of the nanoparticles. The composite had significant antioxidant and proteolytic activity after binding of the two molecules. Moreover, in contrast to the TA/PPN mixture in solution phase, the composite retained higher activity after being treated at 50 °C. In conclusion, TA and PPN were successfully immobilised on LDH nanoparticles, which conferred antioxidant and proteolytic activity to the LDH/TA/PPN composite. The resulting nanocomposite possessed high colloidal stability,

References

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1. A.M. Pisoschi, A. Pop. *Eur. J. Med. Chem.* **97**:55-74 (2015)
 2. J. Fernandez-Lucas, D. Castaneda, D. Hormigo. *Trends Food Sci. Technol.* **68**:91-101 (2017)
 3. A. Szerlauth et al. *J. Colloid Interface Sci.* **632**:260-270 (2023)

significant free radical scavenging and proteolytic activity. This makes the composite a promising material for use in industrial processes where the simultaneous application of the two biomolecules would be advantageous.

References

1. A.M. Pisoschi, A. Pop. *Eur. J. Med. Chem.* **97**:55-74 (2015)
2. J. Fernandez-Lucas, D. Castaneda, D. Hormigo. *Trends Food Sci. Technol.* **68**:91-101 (2017)
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