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Supporting Information

Development and Characterization of Chitosan-Polyvinylpyrrolidone Nanoparticles for Antimicrobial Drug Delivery Applications

Pablo Sebastián Espinel [1,2], Lilian Spencer [3] , Fernando Albericio [4,5]*and Hortensia Rodríguez [2,]*

[1] Departamento de Ciencias Exactas, Universidad de las Fuerzas Armadas ESPE, Av. Gral. Rumiñahui s/n, Sangolquí 171103, Ecuador
[2] Yachay Tech Medicinal Chemistry Research Group (MedChem-YT), School of Chemical Science and Engineering, Yachay Tech, Yachay City of Knowledge Hacienda San José s/n y Proyecto Yachay, Urcuquí 100119, Ecuador; pespinel@yachaytech.edu.ec; and hmrodriguez@yachaytech.edu.ec
[3] School of Biological Science and Engineering, Yachay Tech, Yachay City of Knowledge, Hacienda San José s/n y Proyecto Yachay,Urcuqui 100119, Ecuador; lspencer@yachaytech.edu.ec
[4] Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4001, South Africa; albericio@ukzn.ac.za
[5] Department of Organic Chemistry, University of Barcelona,Barcelona, Spain; albericio@ub.edu

* Correspondence: hmrodriguez@yachaytech.edu.ec (HR); albericio@ukzn.ac.za (FA)

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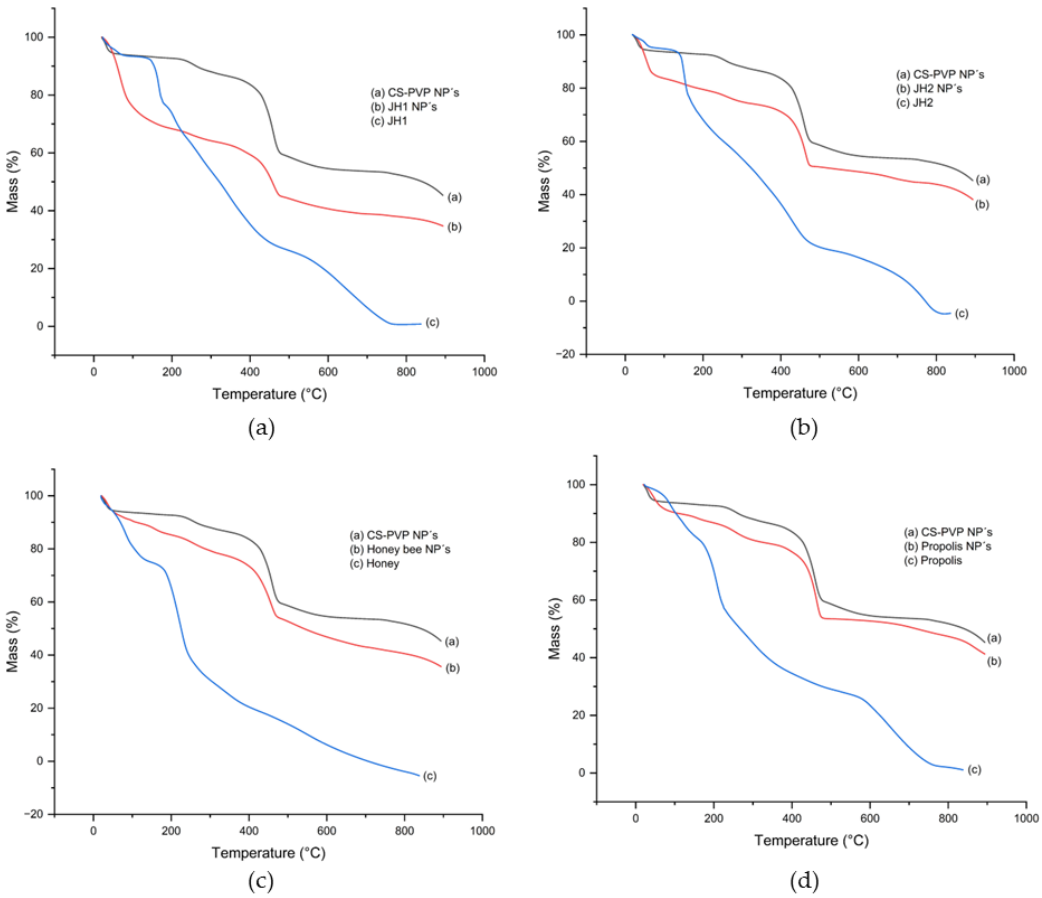


Figure SI-A. Comparison of thermal stability between unloaded CS-PVP NPs, loaded CS-PVP NPs and pure cargos for: (a) JH1; (b) JH2; (c) honey and (d) propolis.

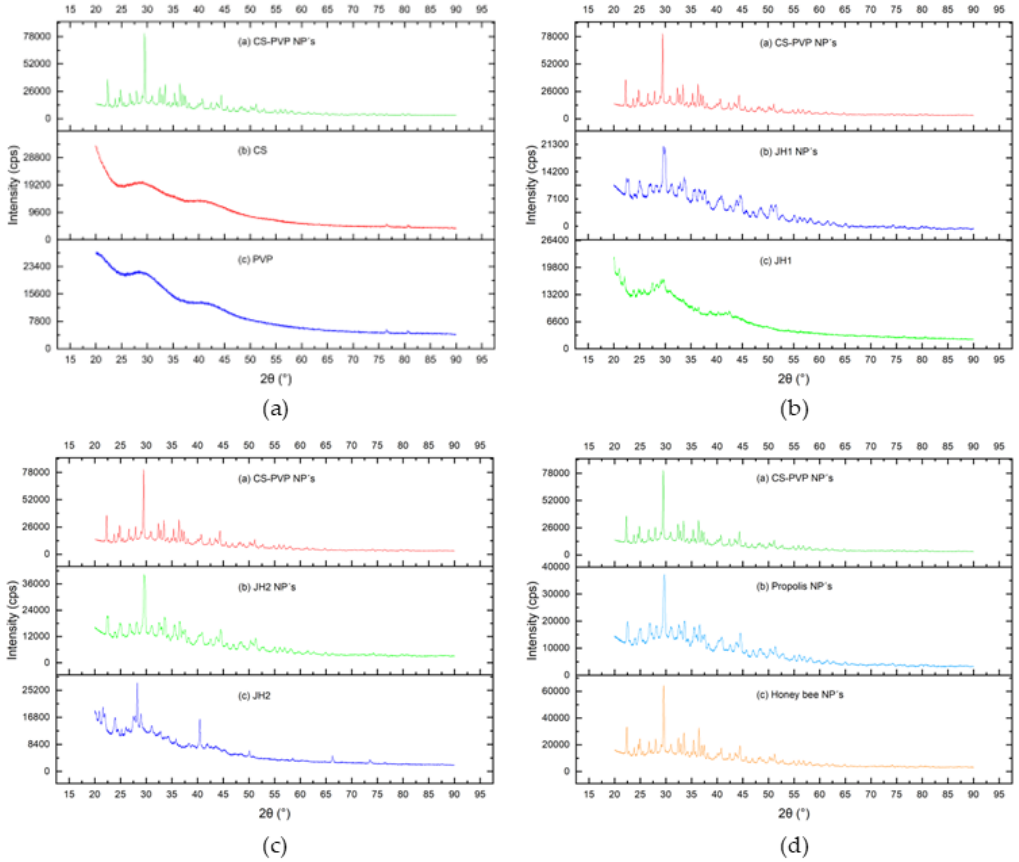


Figure SI-B. Comparison of XRD patterns against CS-PVP NPs: (a) CS and PVP; (b) JH1 NPs and JH1; (c) JH2 NPs and JH2; (d) honey NPs and propolis NPs.

Antimicrobial Activity test

To test the prepared nanoparticles' antimicrobial capacity, Escherichia Coli ATCC 25922 strains and *Staphylococcus Aureus* 25923 were reactivated and standardized with nutrient agar (N.A.) N9405 (Sigma-Aldrich, United States) in Incubator 01-C (J.P. SELECTA, s.a., Spain) at 35°C for 24 hours. All necessary materials, supplies, and water were sterilized in autoclave 01-B (J.P. SELECTA, s.a., Spain). The inoculation of the bacteria was carried out in 5 ml of nutrient broth (N.B.) N7519 (Sigma-Aldrich, United States) and Sheaker SHKE4450 (Thermo Scientific, United States) was used to incubate at 35°C with shaking at 120 rpm for 6 hours, then the inoculated was brought to 100 ml with nutrient broth and left under the same incubation and shaking conditions overnight (approximately 18 hours). Next, 2 ml of the last inoculum was diluted to 250 ml with nutrient broth, maintaining an incubation temperature of 35°C with shaking at 120 rpm and Spectrophotometer 4211/50 (Zuzi Technologies, China) was used to measure absorbance every 20 minutes until reaching an approximate value of 0.12 at 600 nm. Serial dilutions of 9 ml of sterile 0.9% NaCl (Merck, Germany) solution were made to calculate colony-forming units. Inside laminar flow cabinet AV 100 (Azbil Telstar Technologies S. L. U., Spain) the bacteria were seeded on Mueller Hinton NCM0023A agar (NEOGEN, United States) previously gelled. Finally, the disk dispersion method was used to evaluate the antimicrobial activity. The control drugs were ampicillin (1g, Vitalis Laboratories) for *E. coli*, vancomycin (1g, Caplin Point Laboratories Ltd.) for *S. aureus*, and hydroxychloroquine (DIMARD 200, Siegfried Laboratories) to compare the antimicrobial capacity. Inhibition zones were measured in millimeters (mm) after 24 hours of incubation at 35 °C with an electronic digital caliper. The experiment was repeated 2 times in duplicate.