

# Synthesis and characterization of gelatin/montmorillonite/hydroxyapatite nanocomposites: preliminary biocompatibility tests

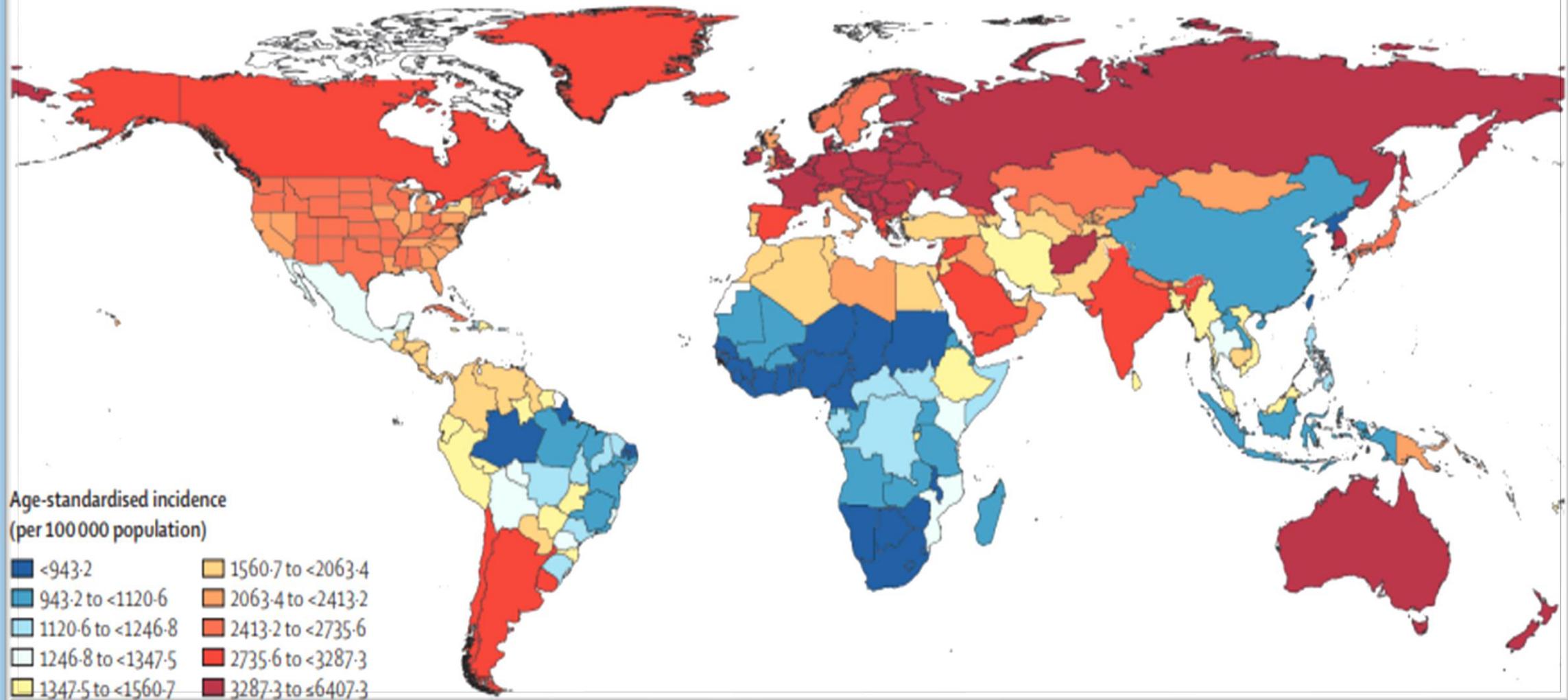


<sup>1</sup>Maby M. Martínez, <sup>1</sup>Lotero, M, <sup>1</sup>Rosero-Moreano. M, <sup>2</sup>Jiménez, F. N. G, <sup>2</sup>Giraldo, L. R,  
<sup>2</sup>Hincapié, D. F, <sup>3</sup>Rodríguez, Y. <sup>3</sup>A Lellesch, L.

<sup>1</sup>Universidad de Caldas, <sup>2</sup>Universidad Autónoma de Manizales, <sup>3</sup>Productora de Gelatina Progel – S.A.S



A Females



The pooled cost for treatment in hospital for a hip fracture was estimated to be US\$10075, and total health and social care costs for one hip fracture after 12 months amounted to a global mean of \$43669

## Autograft

Take from a healthy bone of the same person



## Allograft

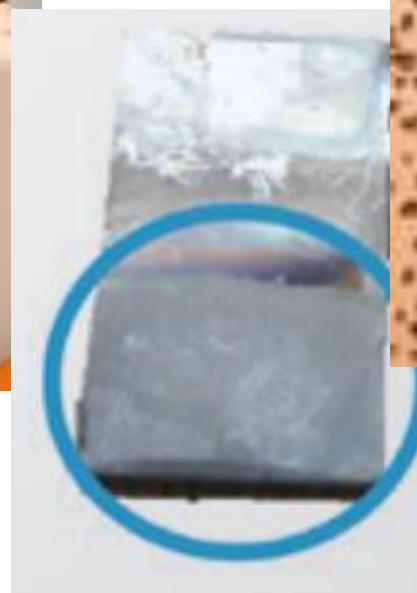
Frozen donated bone

## Ceramic



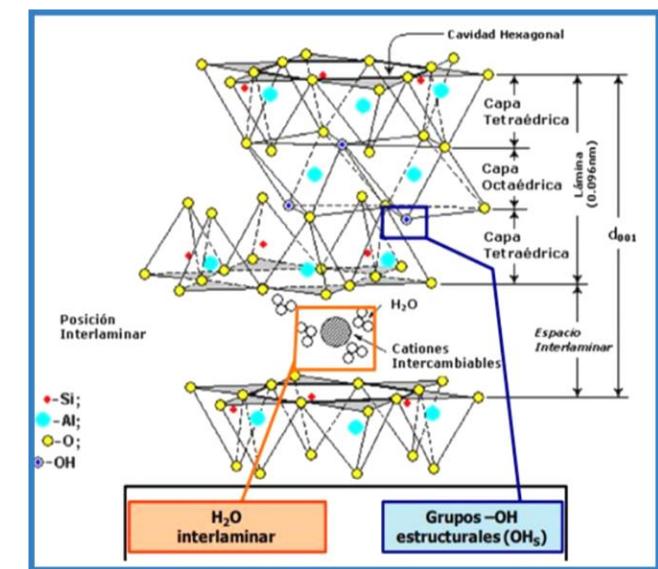
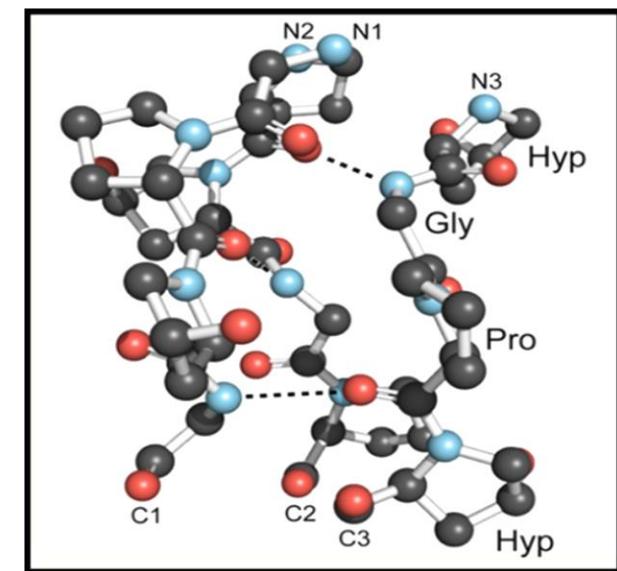
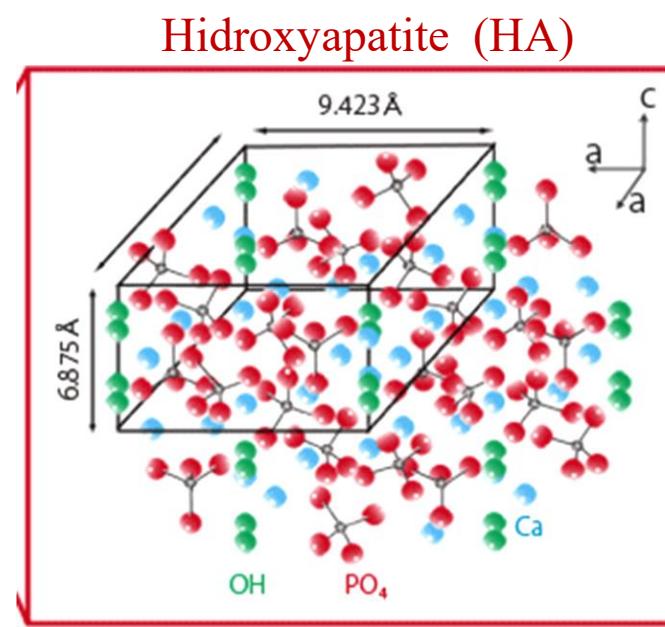
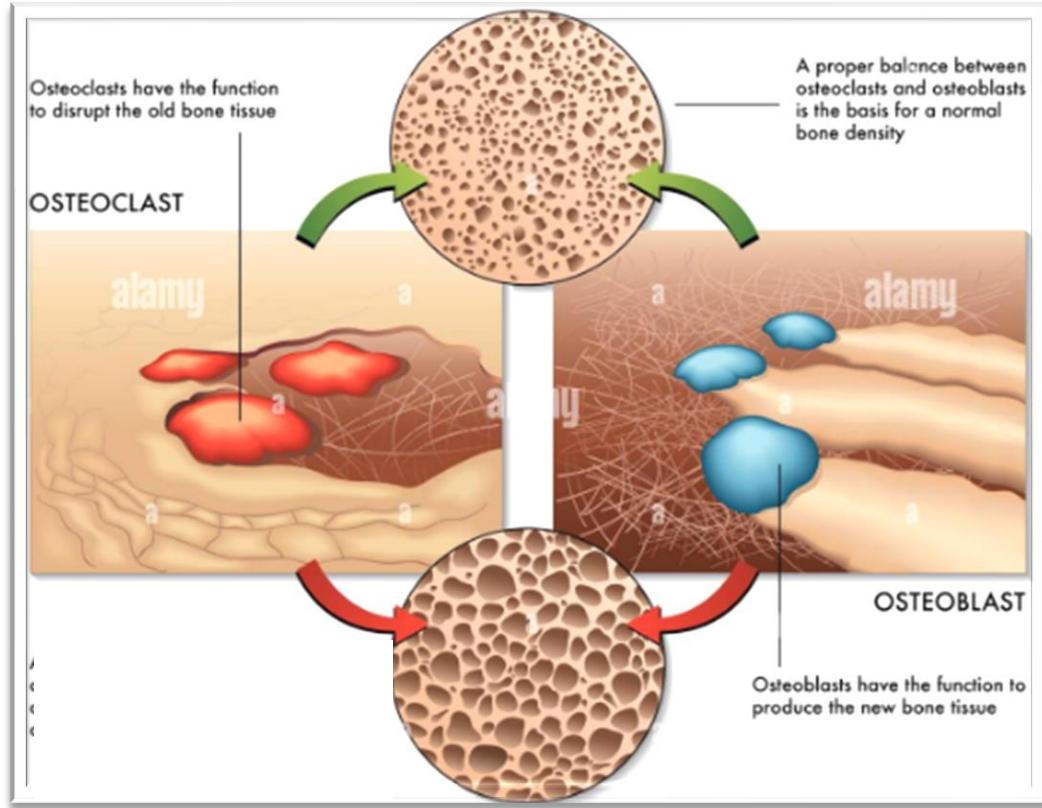
## Biomaterial

### Metal



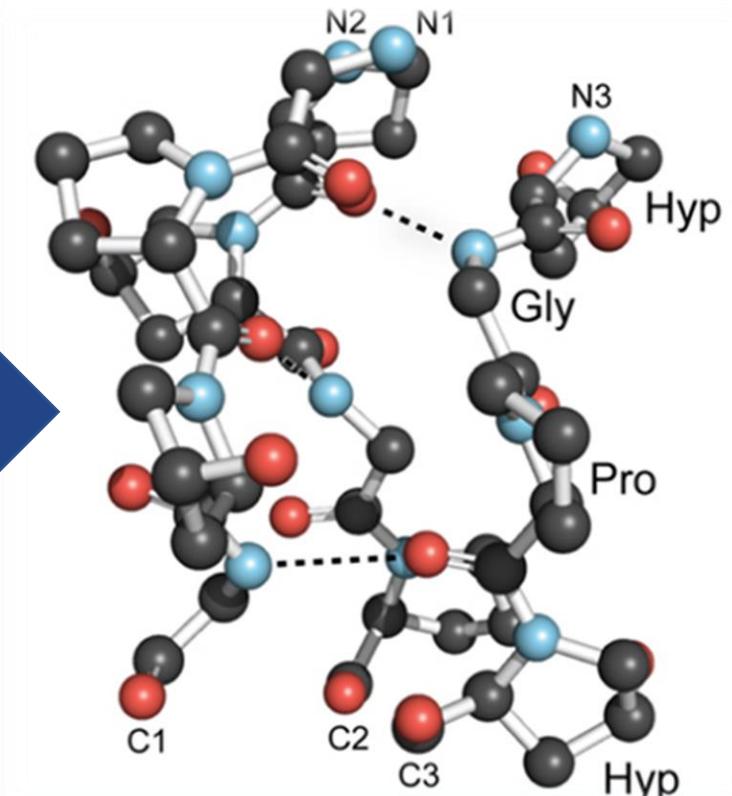
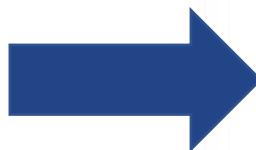
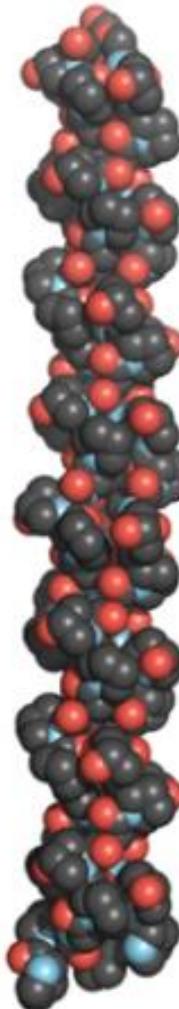
## Nanocomposites



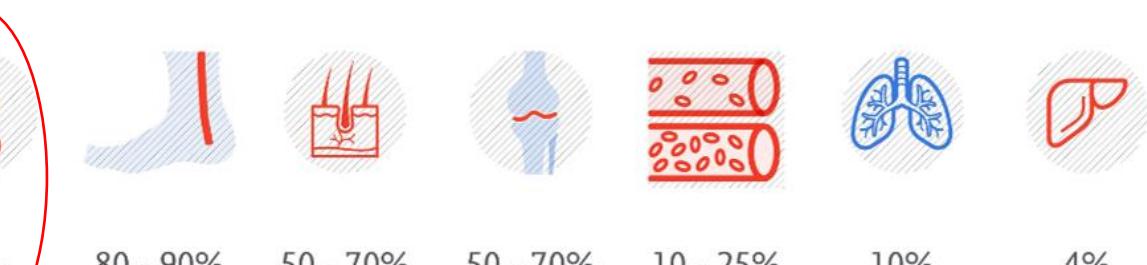


Montmorillonite (MMT)

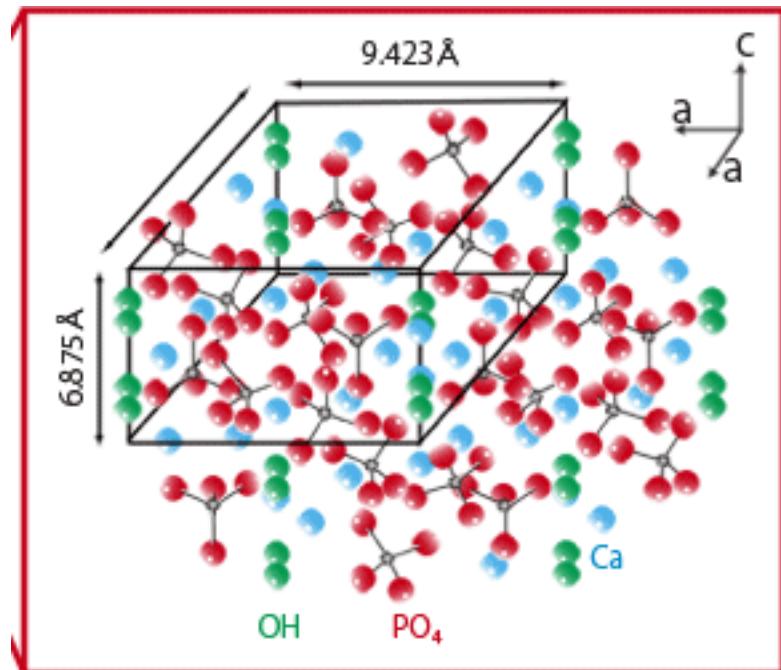
## Gelatin eases bone cell adhesion and proliferation



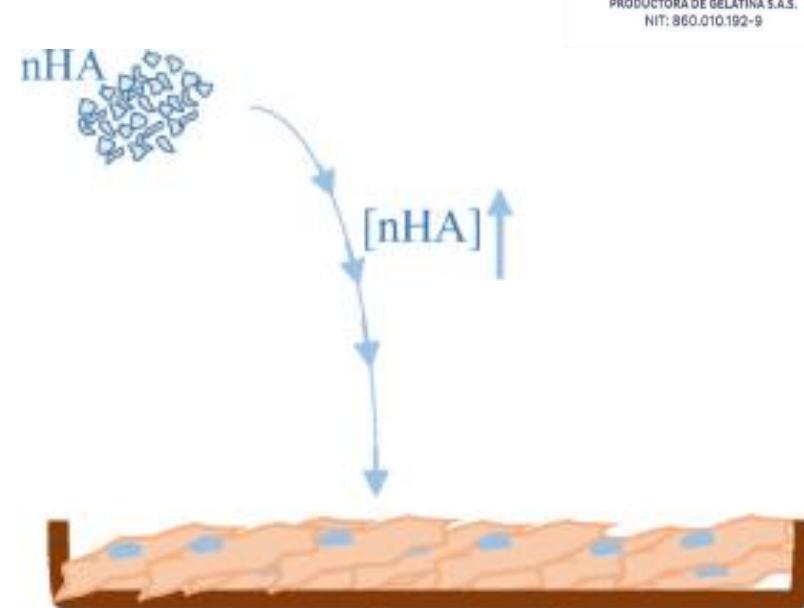
approximate collagen content in different tissues (percentage of dry matter)



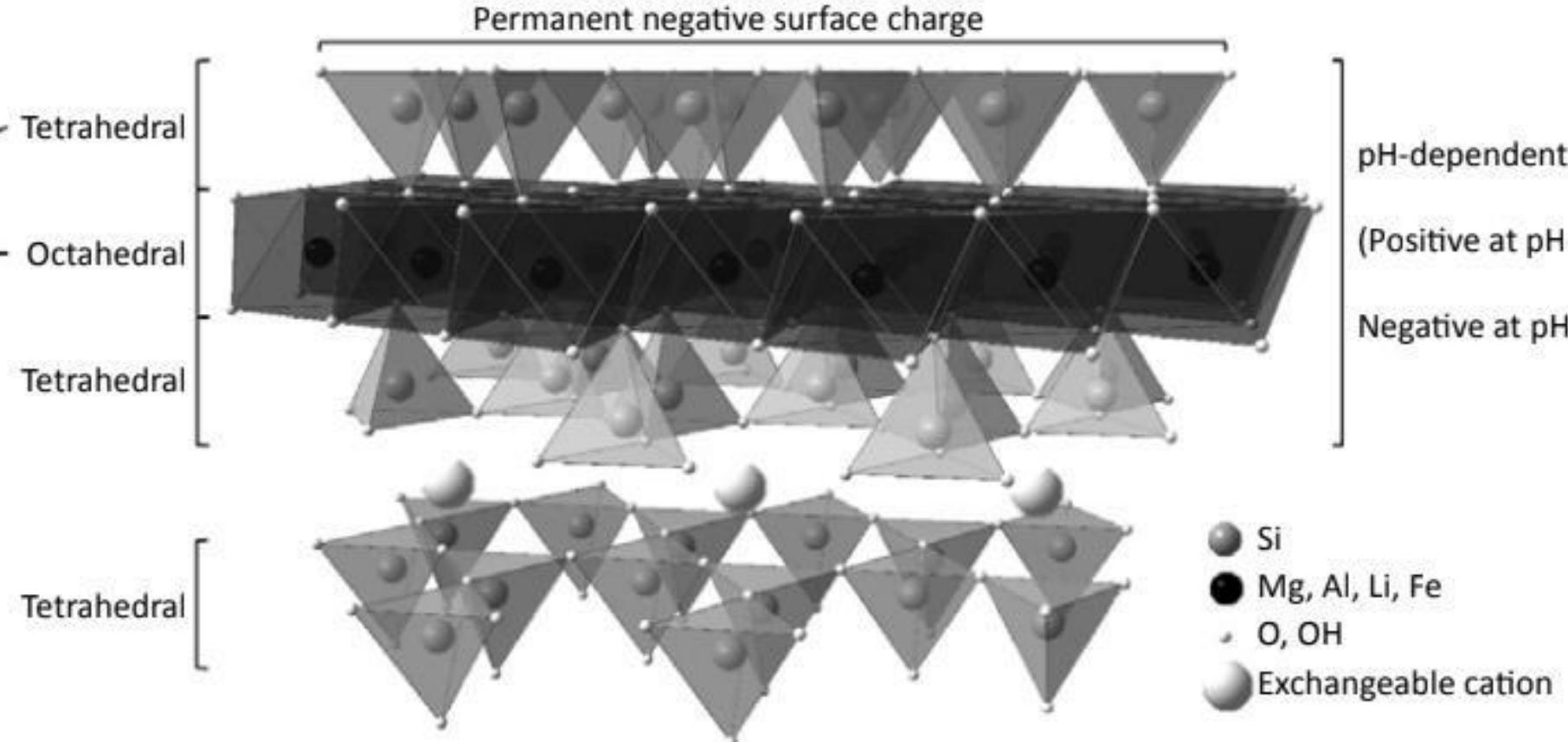
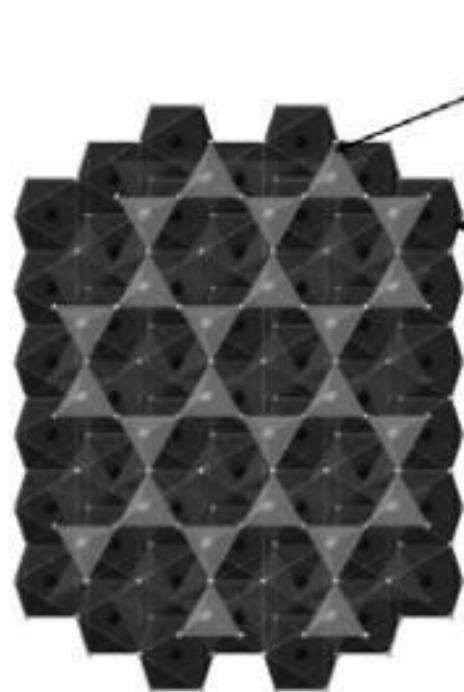
## Hydroxyapatite



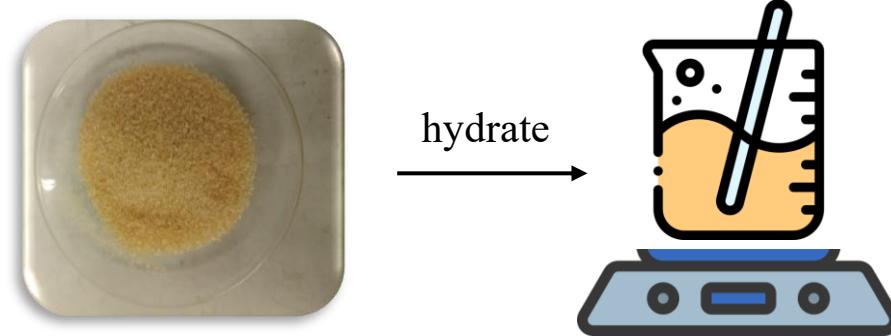
Approximately, 20 nm wide and 80 nm long



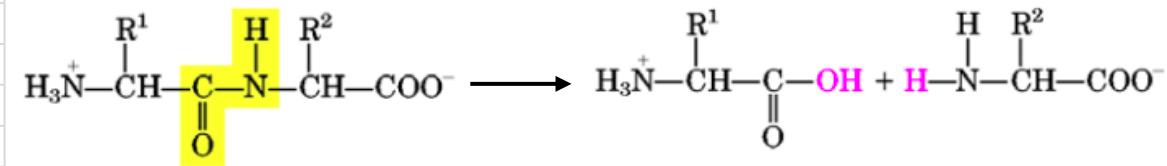
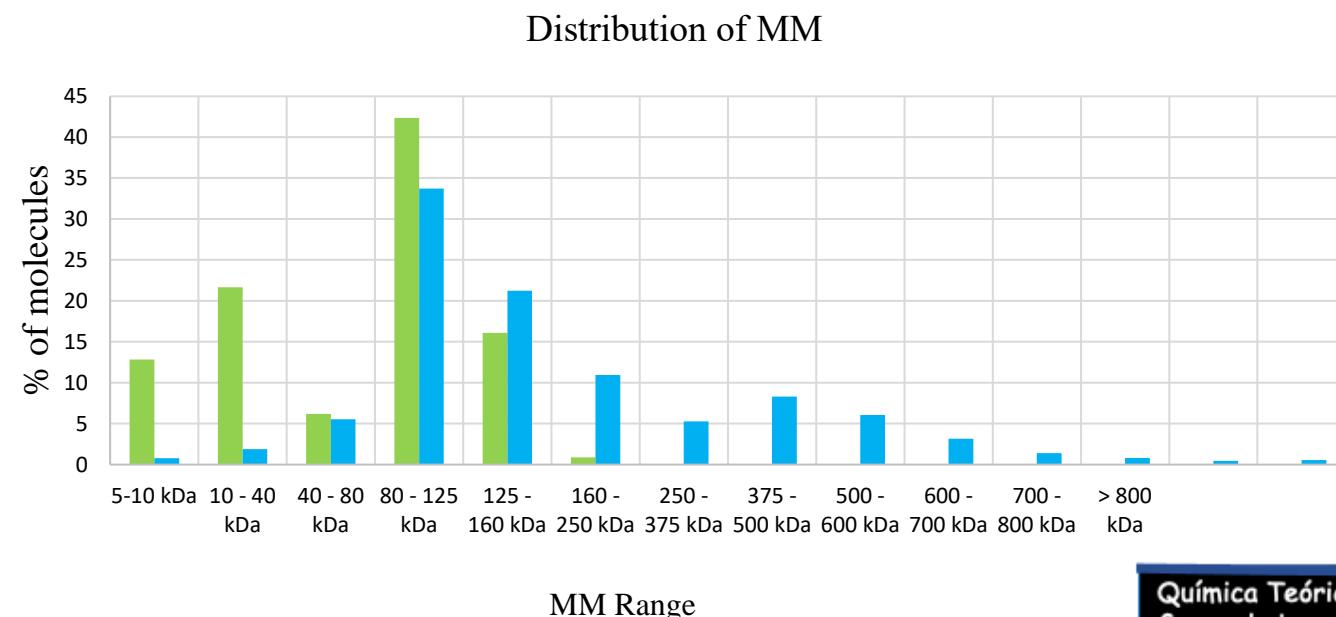
## Montmorillonite

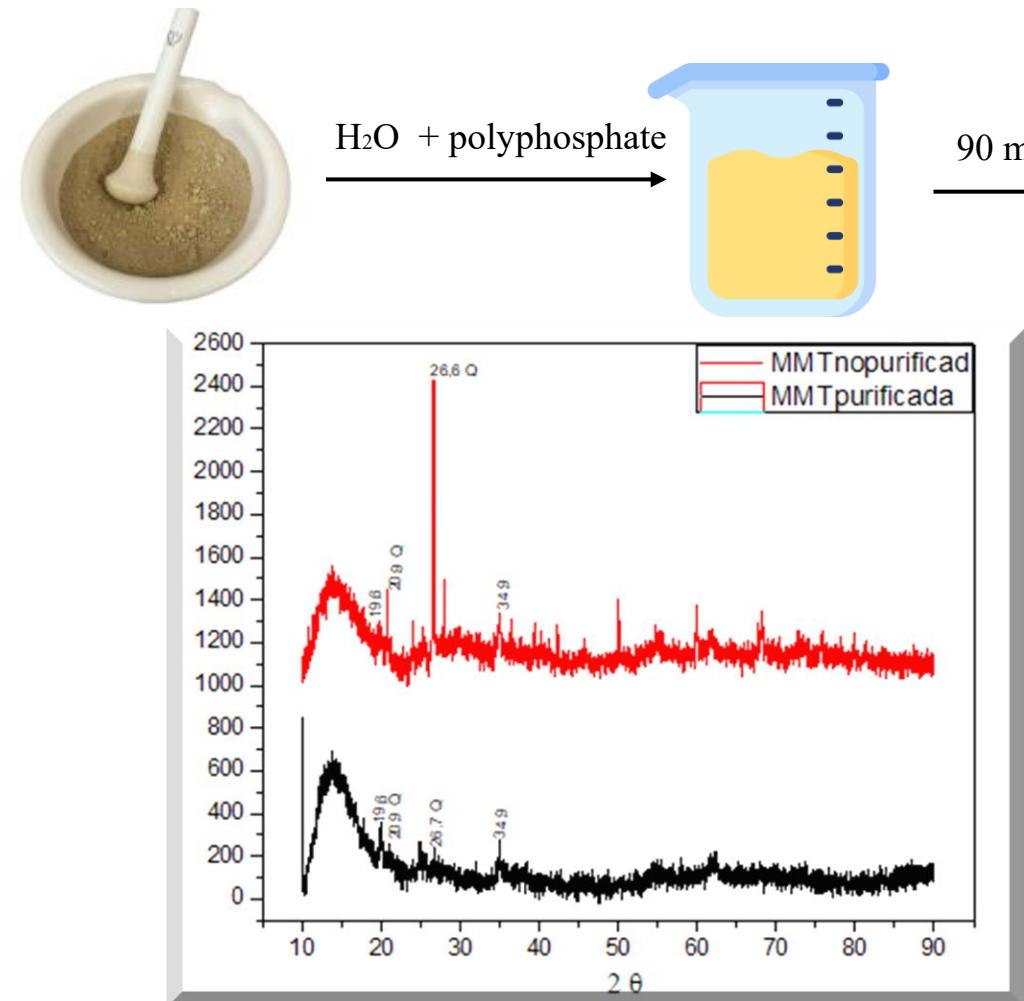


# Materials and Methods

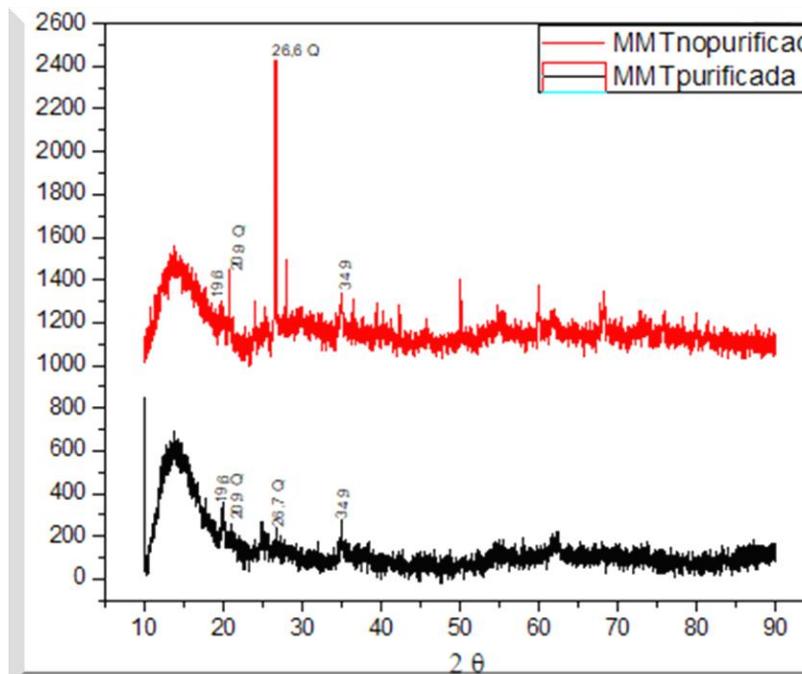


## Gelatin hydrolysis

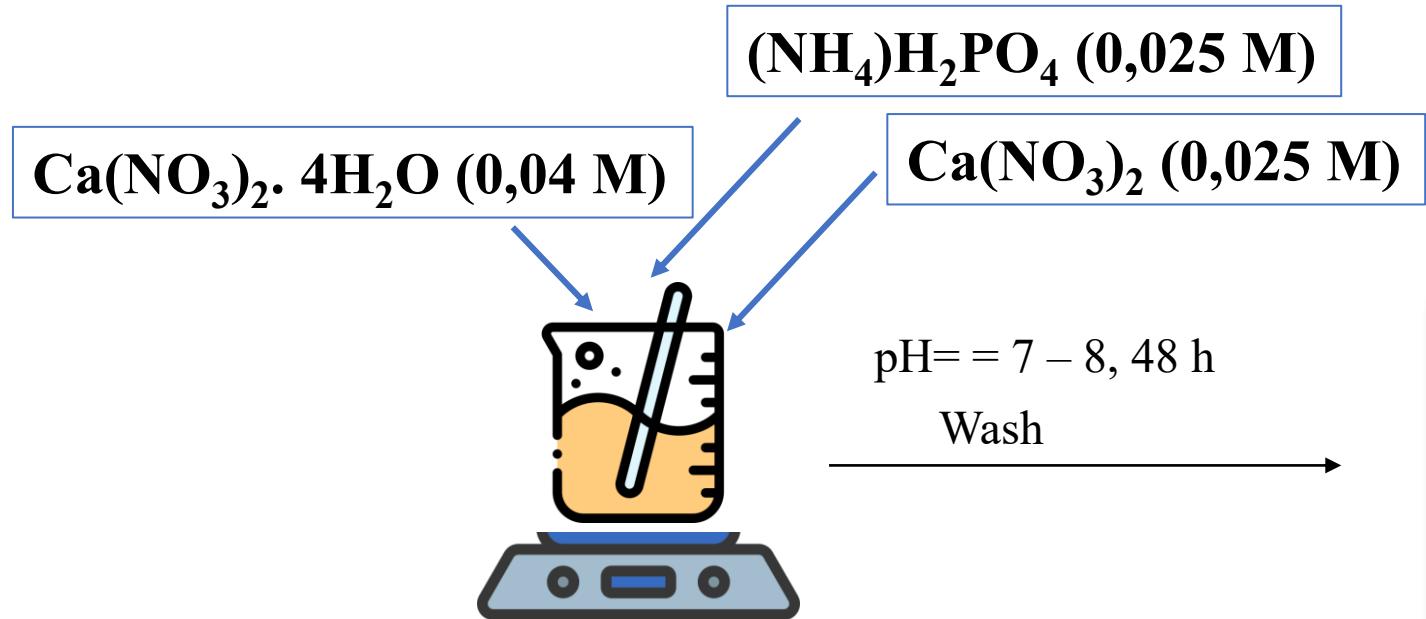




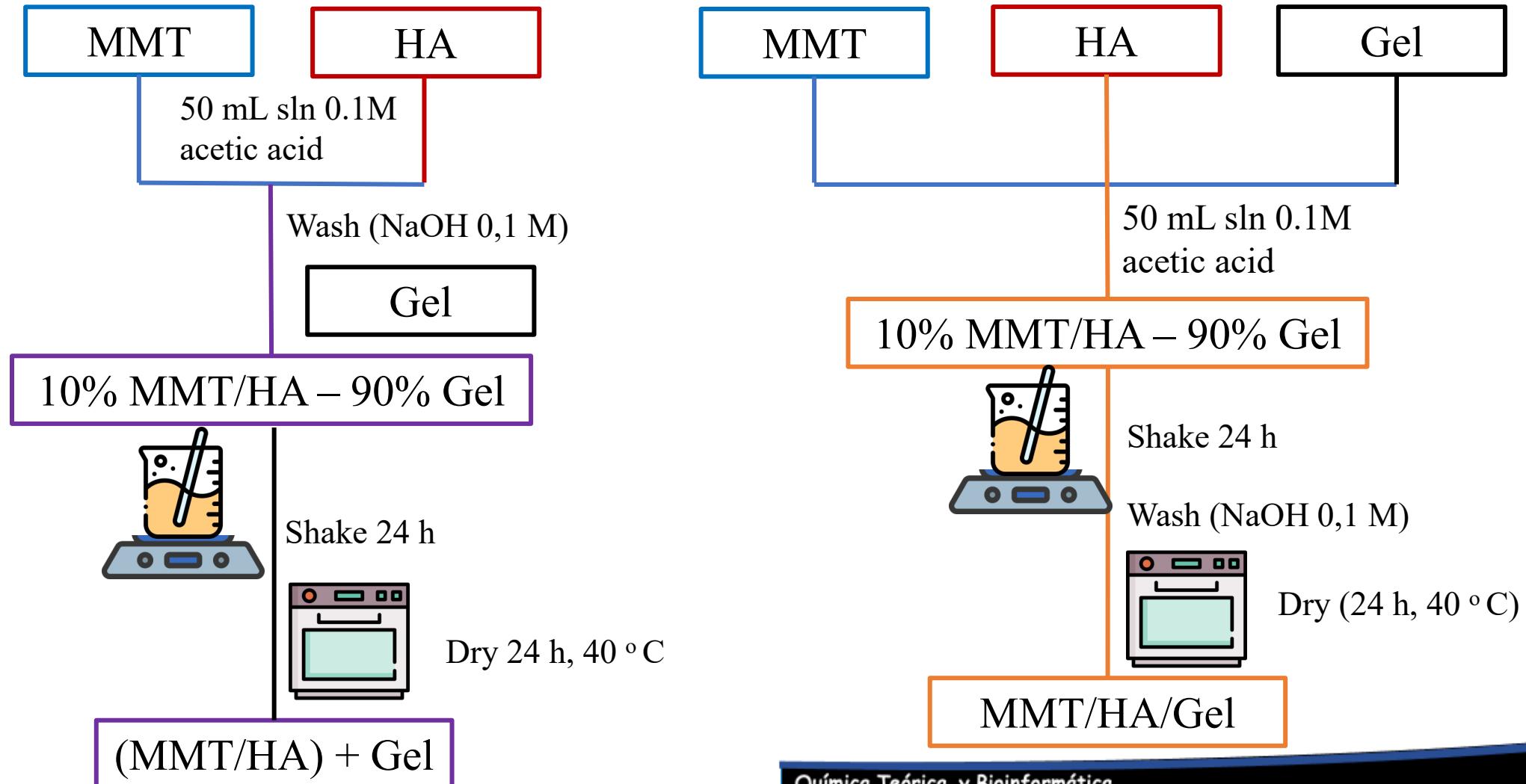
## Purification of Montmorillonite



## Synthesis of Hydroxyapatite

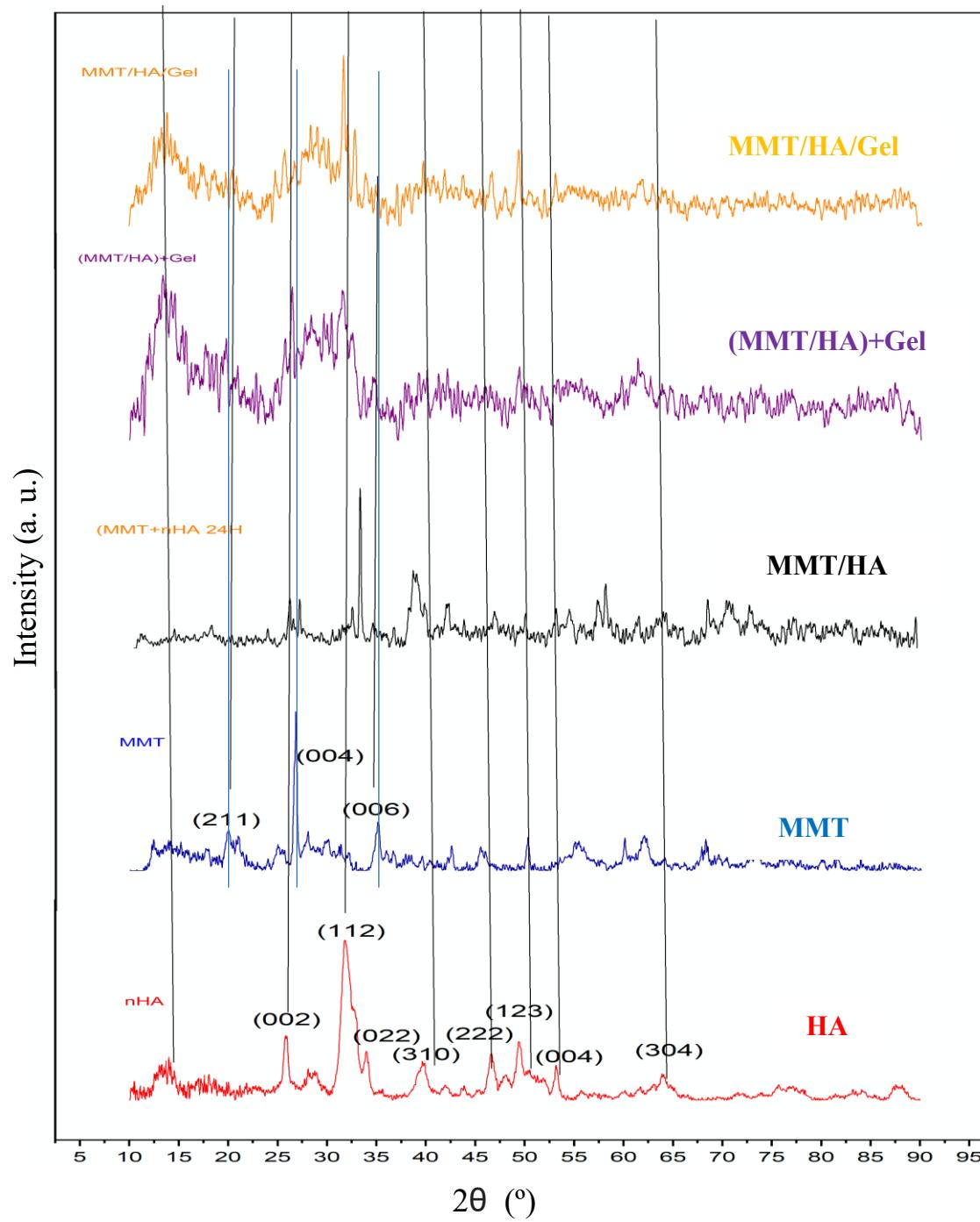


## Synthetic procedures

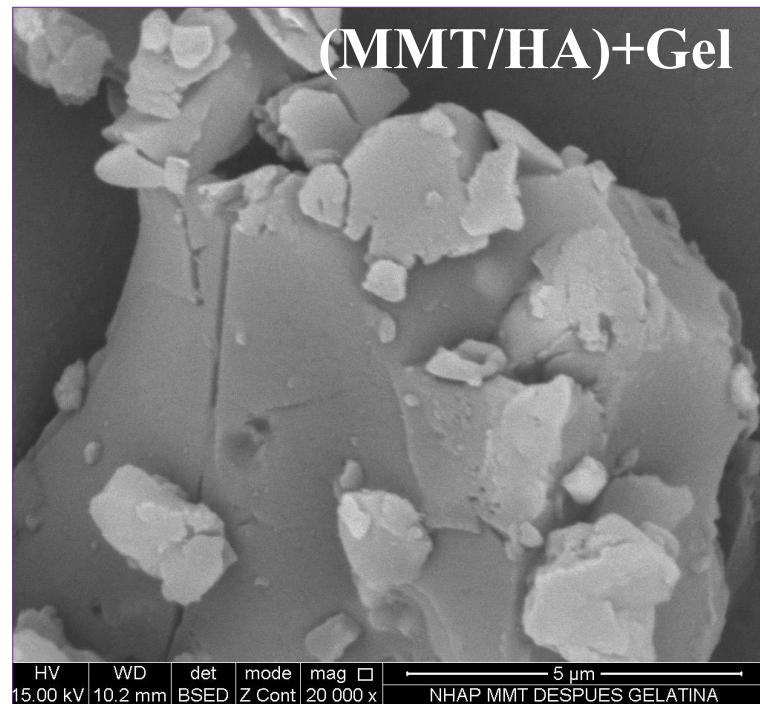
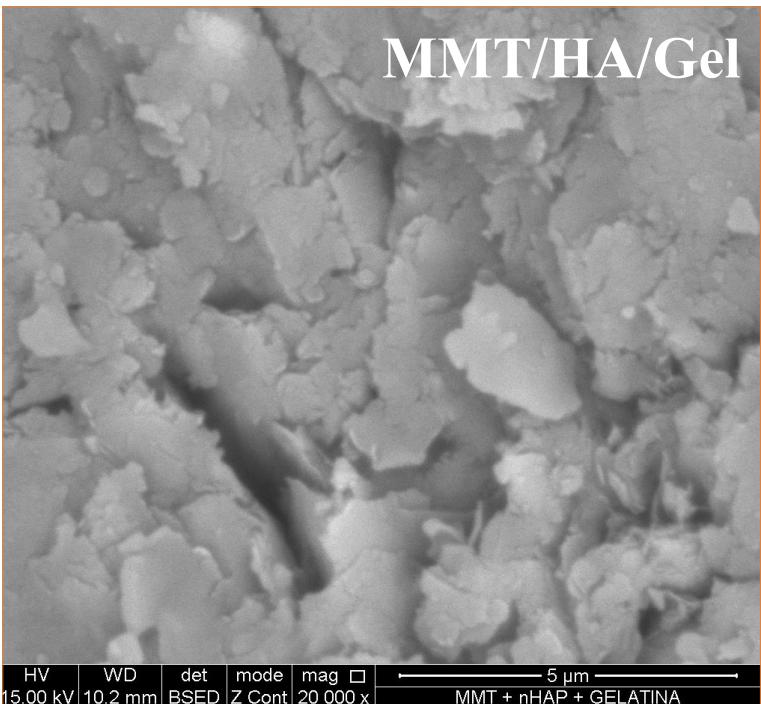
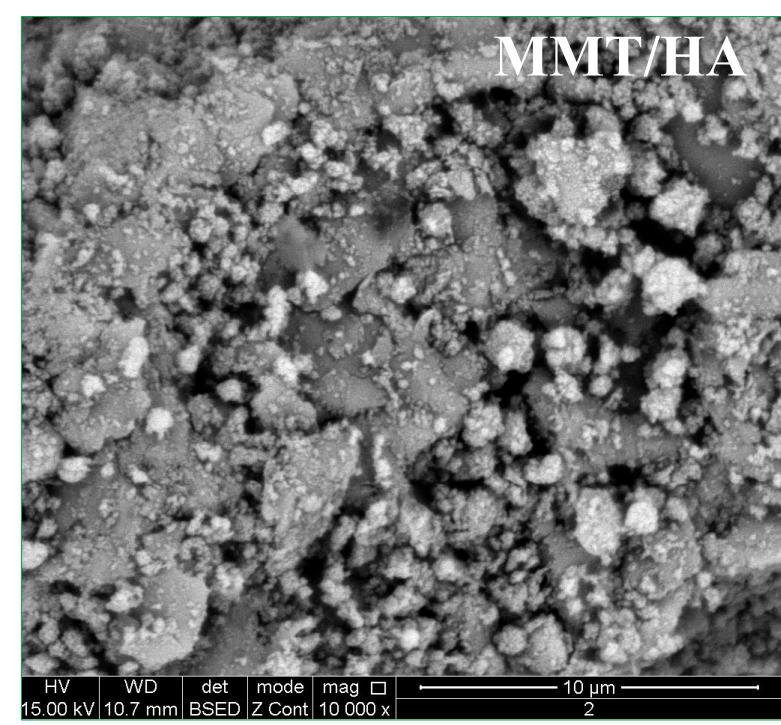
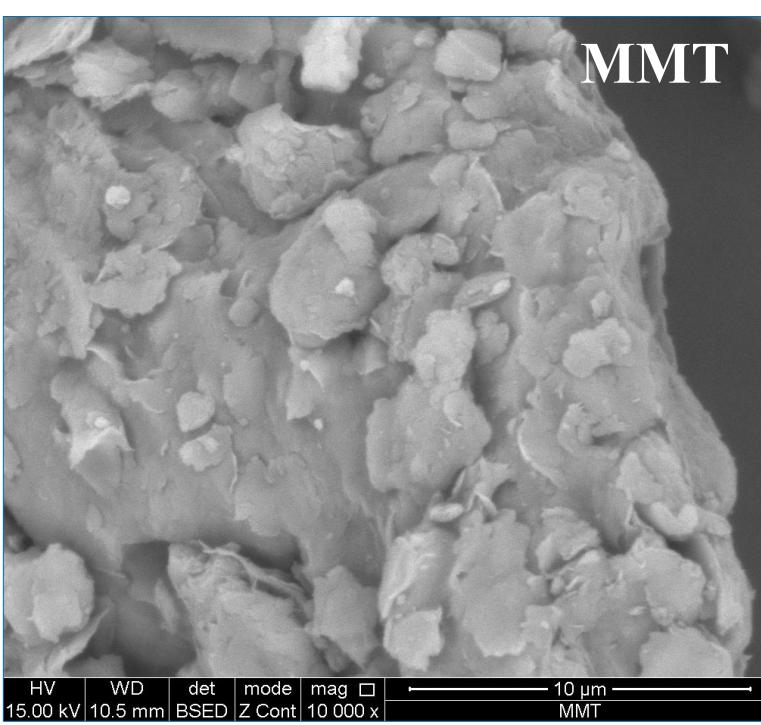
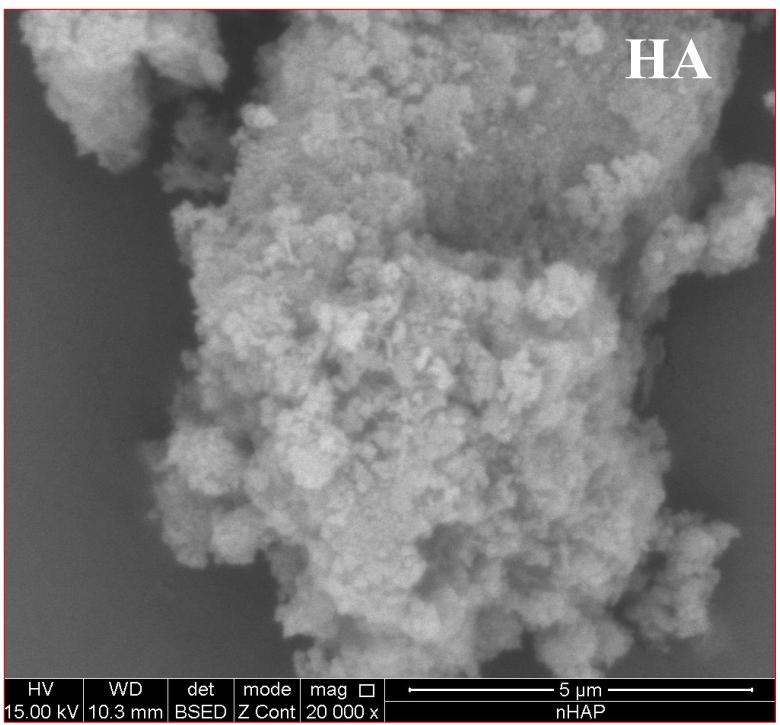


# Results

## X-Ray Diffraction



# Scanning Electron Microscopy

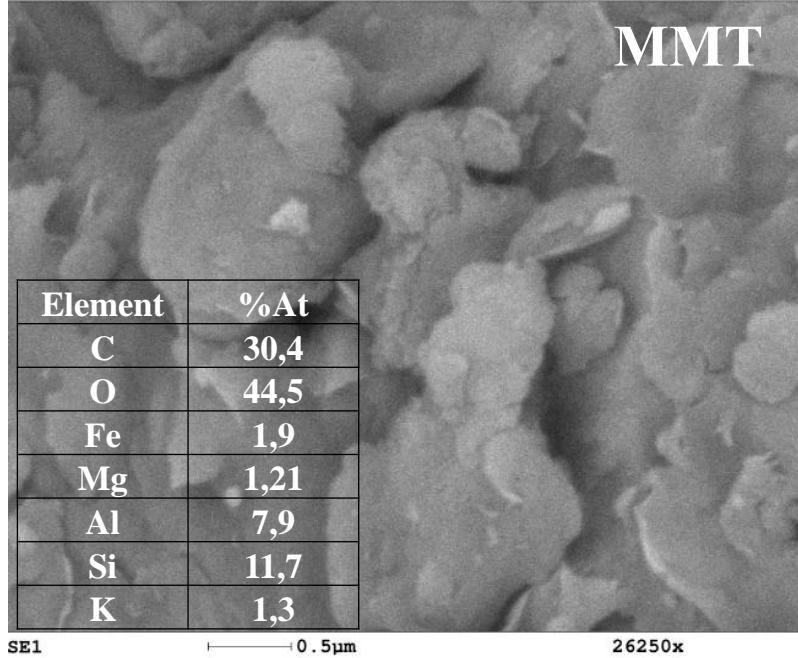


# Scanning Electron Microscopy- EDS

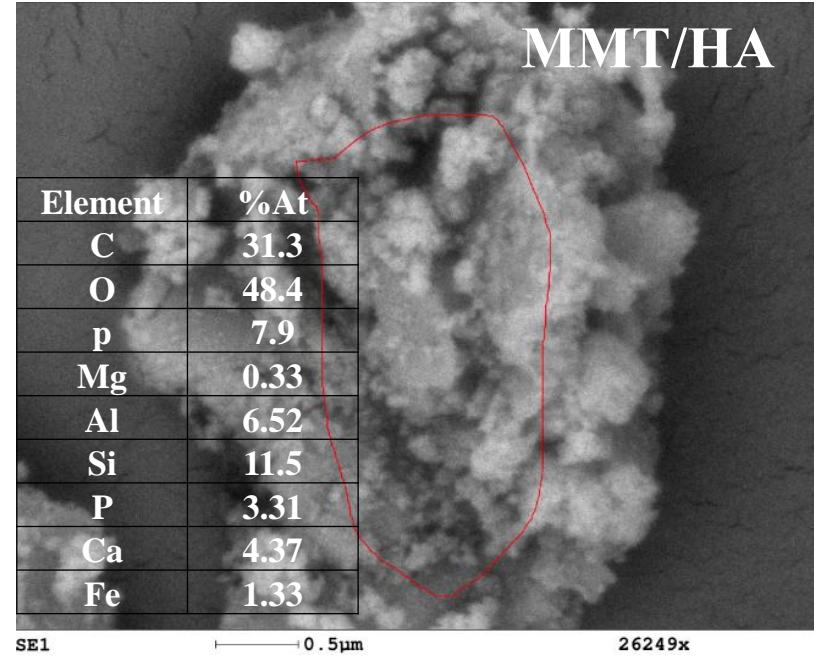
Element	At %
C	31,3
O	48,4
P	7,9
Ca	11,3

SE1 0.5 μm 26250x

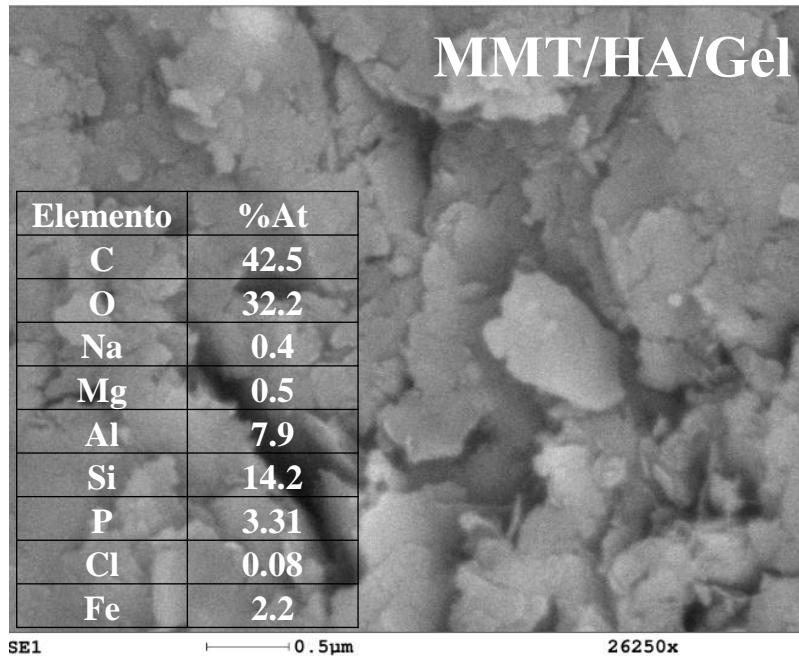
HA



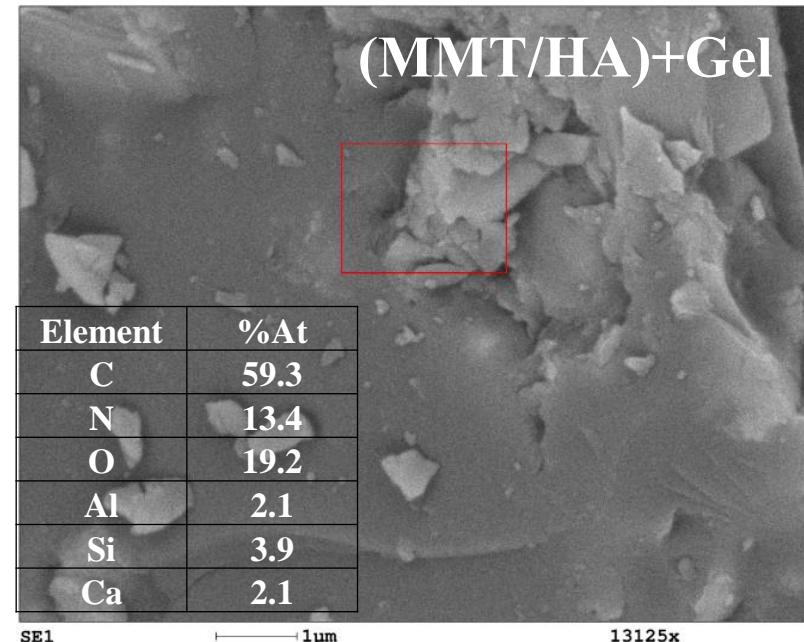
MMT



MMT/HA



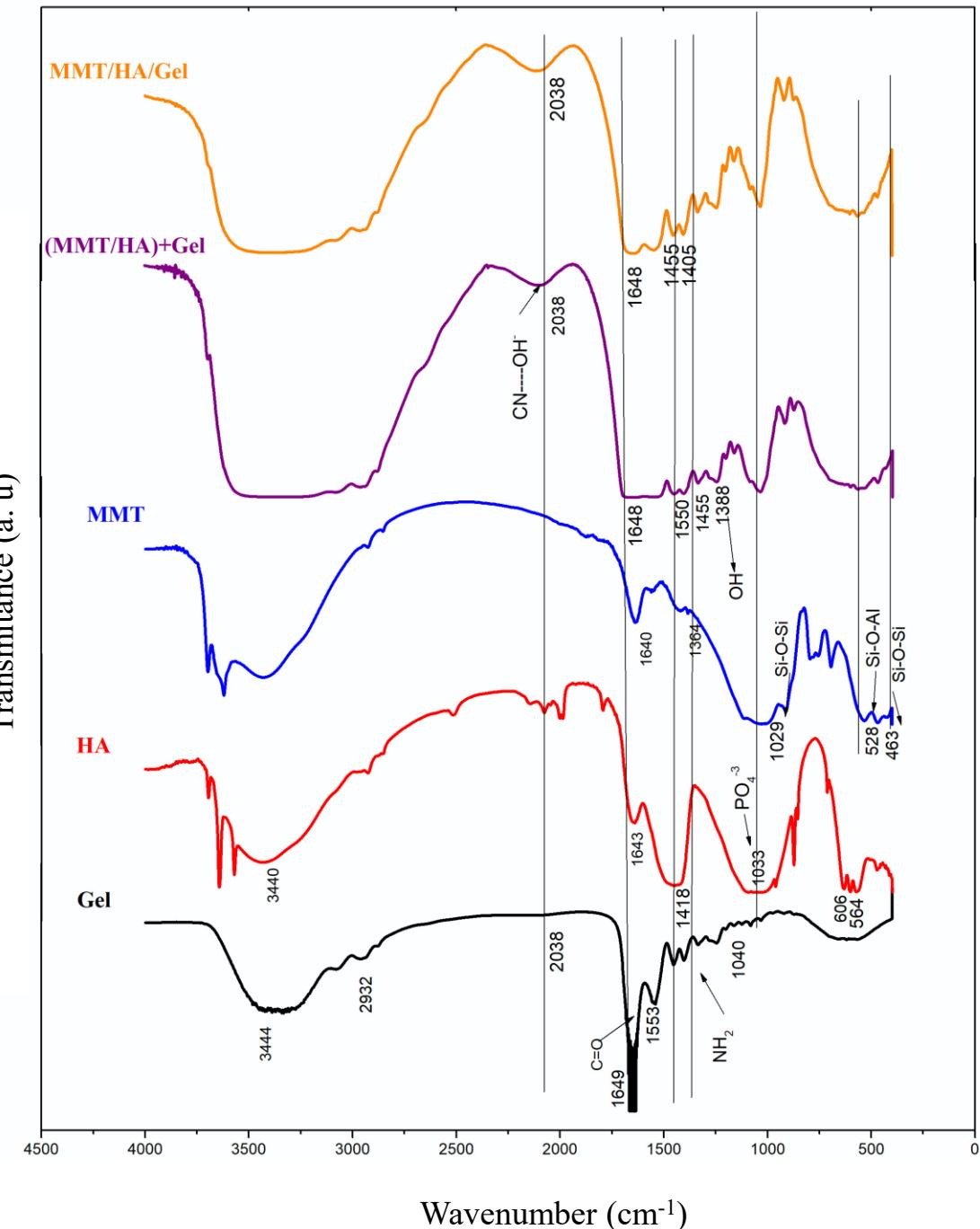
(MMT/HA)+Gel



# Fourier-transformed infrared

Vibration	Material MMT ( $\text{cm}^{-1}$ )	Vibration	Material HA ( $\text{cm}^{-1}$ )	Vibration	Material Gel ( $\text{cm}^{-1}$ )
$\nu_{\text{as}} \text{OH}$	3000-3700	$\nu_{\text{as}} \text{OH}$	3000-3700	$\nu_{\text{as}} \text{OH}$	3000-3700
$V(\text{H-O-H})$	1621	$V(\text{PO}_4^{2-})$	1033	C=O	1649
$V(\text{Si-O-Si})$	1029	$V(\text{PO}_4^{2-})$	564	$V(\text{CN})$	1550
$V(\text{Si-O-Al})$	528	$V(\text{PO}_4^{2-})$	564	$V(\text{CN})$	1455

Vibration	Material MMT/HA/Gel ( $\text{cm}^{-1}$ )	Material (MMT/HA) + Gel ( $\text{cm}^{-1}$ )
$\nu_{\text{as}} \text{OH}$	3000-3700	3000-3700
OH en MMT/HA	1386	1386
OH ----CN	2038	2038
MMT/HA	1405	1406
MMT/HA	914-840	914-840



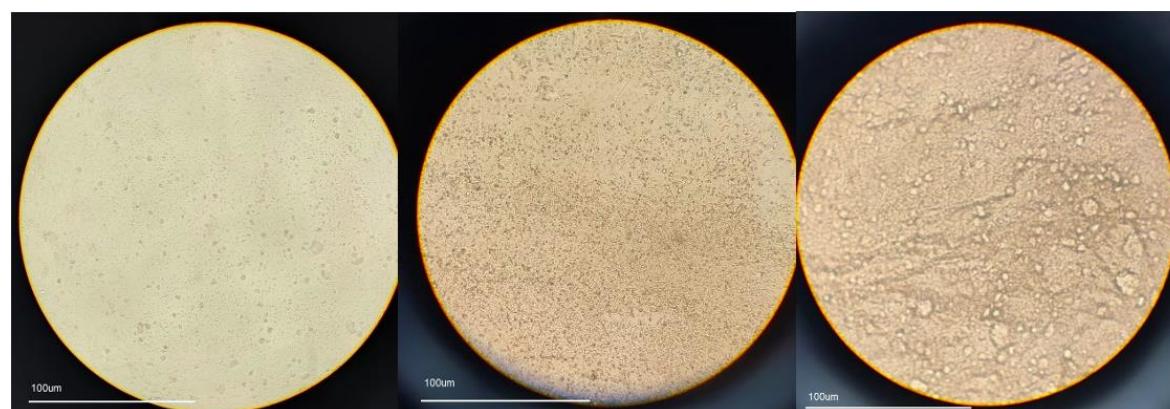
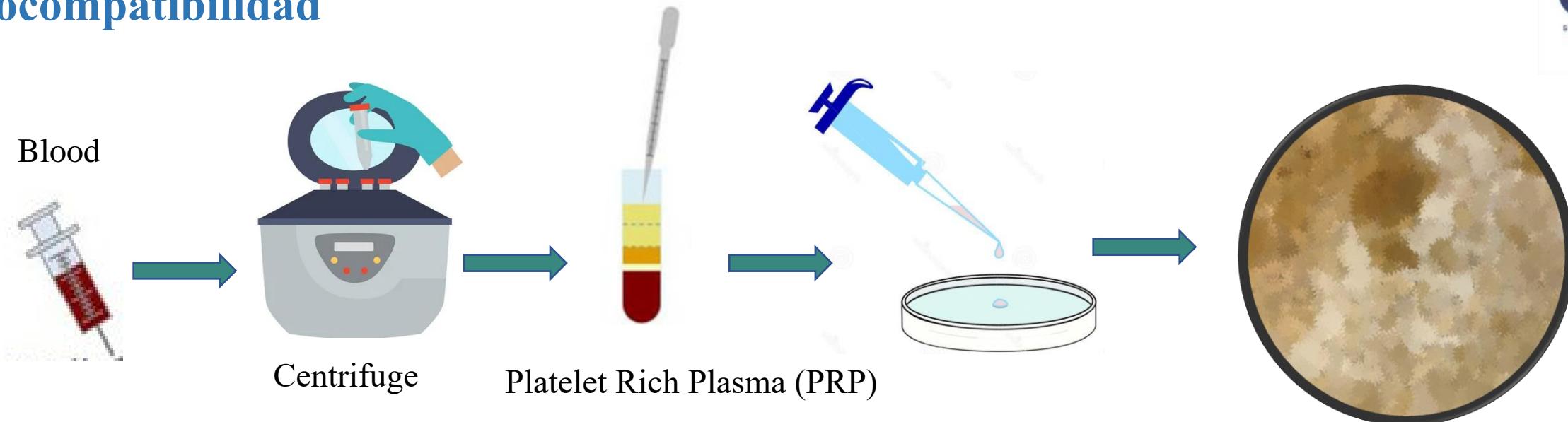
Chaibi *et al.*, *Colloid and Polymer Science*. 2015.

Wang *et al.*, *Chem. Eng. J.* 2020.

Olad *et al.*, *Ceramics International*. 2020

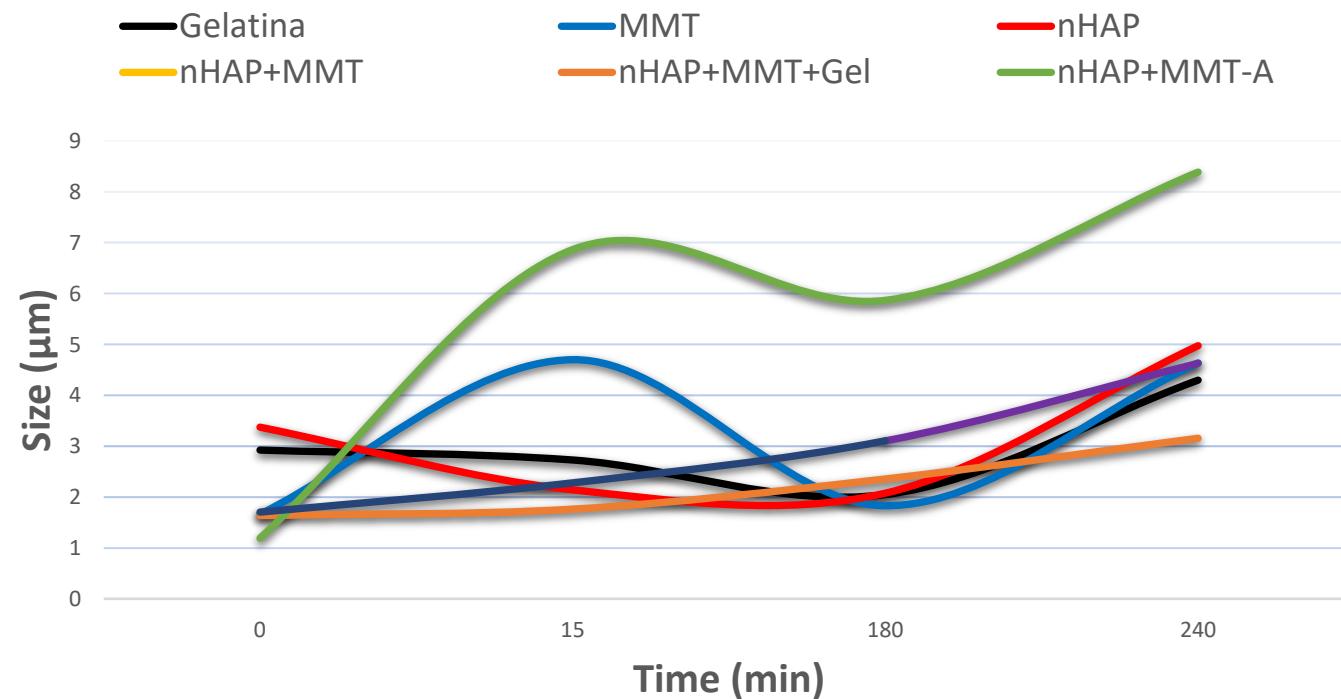
## Biocompatibilidad

### *In vitro* tests with platelet count



Morphological changes of platelets upon treatment with nanohydroxyapatite. (a) 0 min, (b) 180 min and (c) 240min

### Tests de biocompatibilidad



Platelets between 1.7 and 4.6  $\mu\text{m}$  were observed with the material (MMT/HAP)+Gel, while platelets between 1.7 and 3, 1  $\mu\text{m}$  were observed with the MMT/HAP/Gel material.

MMT/HAP/Gel material is the one that exhibits a better cellular response.

## Conclusiones

- ❖ The triads were obtained and their preliminary characterization by FTIR suggests a similarity in their chemical composition, but there are also evidences of a new interaction
- ❖ SEM analysis shows important morphological differences in these materials, in agreement with the results obtained in the biocompatibility tests.
- ❖ The MMT/HAP/Gel triad suggests that it is a more promising material in our attempt to promote them as potential tissue regenerating agents.

# Referencias

GBD 2019 Fracture Collaborators. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet Healthy Longev.* 2021 Sep;2(9):e580-e592. doi: 10.1016/S2666-7568(21)00172-0. PMID: 34723233; PMCID: PMC8547262.

Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem.* 2009;78:929-58. doi: 10.1146/annurev.biochem.77.032207.120833. PMID: 19344236; PMCID: PMC2846778.

Sahlin-Platt A. Bone tissue regeneration in dento-alveolar surgery Clinical and experimental studies on biomaterials and bone graft substitutes. *Odontological Dissertations, Series No 119.* 2011

Mousa, Mohamed, Nicholas D. Evans, Richard O.C. Oreffo, and Jonathan I. Dawson. (2018). “Clay Nanoparticles for Regenerative Medicine and Biomaterial Design: A Review of Clay Bioactivity.” *Biomaterials*, 159: 204–14. <https://doi.org/10.1016/j.biomaterials.2017.12.024>.

Nacional Autónoma de México México Vargas Rodríguez U, Marina Y, Vidales G, Labastida V, Bórquez G, Sahagún A, et al. Caracterización espectroscópica, química y morfológica y propiedades superficiales de una montmorillonita. *Rev. mex. cienc. geol* vol.25 no.1 Ciudad de México. 2008

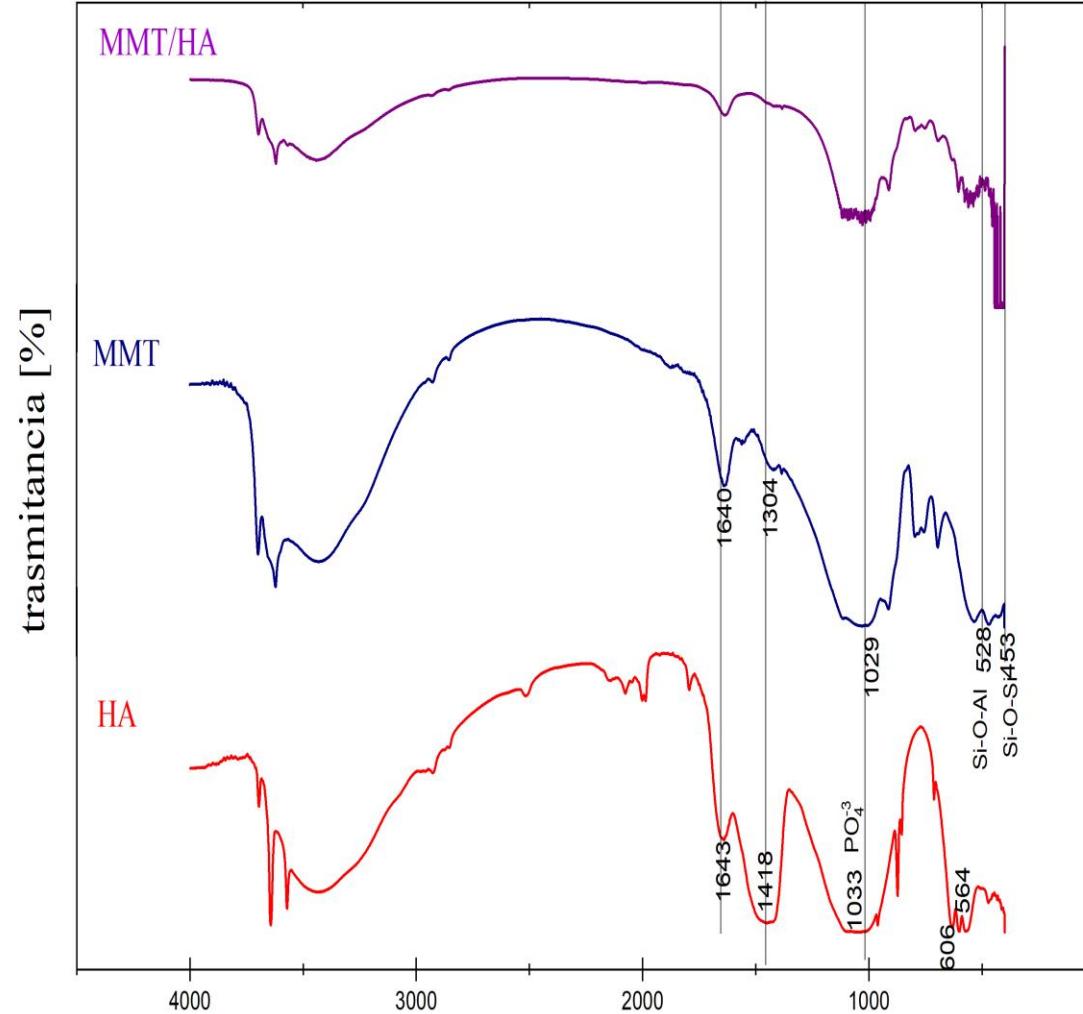
Olad, Ali, and Fahimeh Farshi Azhar. (2014). “The Synergetic Effect of Bioactive Ceramic and Nanoclay on the Properties of Chitosan-Gelatin/Nanohydroxyapatite-Montmorillonite Scaffold for Bone Tissue Engineering.” *Ceramics International*, 40 (7 PART A): 10061–72. <https://doi.org/10.1016/j.ceramint.2014.04.010>.

Chaibi, S.; Benachour, D.; Merbah, M.; Cagiao, M.; Baltá, C. F. J. The role of crosslinking on the physical properties of gelatin based films. *Colloid and Polymer Science*, n. 293, 2741- 2752, 2015.

Wang, Y. Wang, L. Yu, R. Wang, X. Zhang, Highly effective microwave induced catalytic degradation of Bisphenol A in aqueous solution using double-perovskite intercalated montmorillonite nanocomposite, *Chem. Eng. J.* n. 390, 2020.

Olad, A., Azhar, F. F. The synergistic effect of bioactive ceramic and nanoclay on the properties of chitosan-gelatin/nanohydroxyapatite-montmorillonite scaffold for bone tissue engineering. *Ceramics International*, n. 40, 100061- 10072, 2014.

Rajan, T. S, Mitun DAS, Arockiarajan, A. Biocompatibility and corrosion evaluation of niobium oxide coated AZ31B alloy for biodegradable implants, *Colloids and Surfaces B: Biointerfaces*, n. 212, 112342, 2022.



## Characterization



(a)



(b)



(c)

(a) X Ray Difraction, (b) Fourier-transformed infrared (c) Scanning electron microscopy

# Synthesis and characterization of gelatin/montmorillonite/hydroxyapatite nanocomposites: preliminary biocompatibility tests



Química Teórica y Bioinformática  
Grupo de investigación en Cromatografía y técnicas afines  
Grupo de investigación en Física y Matemáticas con Énfasis en la Formación de Ingenieros

# Synthesis and characterization of gelatin/montmorillonite/hydroxyapatite nanocomposites: preliminary biocompatibility tests



Química Teórica y Bioinformática  
Grupo de investigación en Cromatografía y técnicas afines  
Grupo de investigación en Física y Matemáticas con Énfasis en la Formación de Ingenieros