v. 2, n. 17, 2022

Journal of
Engineering
Research

SYNTHESIS AND CHARACTERIZATION OF FUNCTIONALIZED NANOPARTICLES FOR LACTASE IMMOBILIZATION

Gabriel Cardoso Pinto

Victor Hugo Pitoni de Queiroz

Guilherme Nunes Lucena

Caio Carvalho dos Santos

Rodolfo Debone Piazza

Andréa Maru de Castro Oliveira

Rodrigo Fernando Costa Marques

All content in this magazine is licensed under a Creative Commons Attribution License. Attribution-Non-Commercial-Non-Derivatives 4.0 International (CC BY-NC-ND 4.0).

Abstract: This work focused on the development and characterization of nanoparticles, a branch that is constantly growing and through recent research it has been shown that, no matter how small a particle, it can drive great technological advances. Nanoparticles that have a high surface area cover biotechnological, chemical, physical and even electronic areas due to the great versatility present in their structures. Its functionalization is necessary in order to set desirable properties. As practical objectives, magnetic nanoparticles were synthesized from iron (II) and (III) chlorides and sodium hydroxide and later functionalized with APTS and, submitting the samples to analyzes such as X-Ray Diffractions (XRD), Infrared Spectroscopy (FTIR) and Zeta Potential, in order to confirm the synthesis and functionalization.
Keywords: Nanoparticles, Magnetic Nanoparticles, Functionalization, Nanotechnology.

INTRODUCTION

The concepts of technology and science walk more and more together as the days go by, being strictly linked for better interpretation, adaptation and development of human activities and thus showing the capacity that man has to be in control of new techniques that make it possible to see and understand. the world through new eyes and often bringing positive change (Vurro et al., 2019).

Innovative techniques which nowadays grows more and more as a result of the increase in the search for better products, processes, applications, cost reductions, more efficient results, among others, which are driven by the constant dispute of the Almejo trade for the best merchandise to be offered to the final consumer. Thus, always increasing the "thirst" for the new and the best. These factors are what contribute to the advancement of research on new technologies not yet studied (T.sriwong & Matsuda, 2022).

Studies on technology can no longer be seen only on a macrometric scale, because with discoveries and/or changes of minimal dimensions, it is possible to completely change the way a certain product is produced and thus alter its characteristics astronomically, not to mention the increase in its added value. Just as nanoparticles are doing, modifying concepts, formulas and structures and, showing that yes, no matter how small, they can generate impactful revolutions in development and improvement in product development (Vijayakumar et al., 2022).

NANOSCIENCE

As already mentioned, the use of new methods are developed through technologies that are modernized every day, nanotechnology is in this context, as it is a technique on the rise, as its potential grows in various human sectors and also for being a relatively new technique. recent compared to the others (Zhang et al., 2022).

It is also seen as the technology that has the greatest development today, among its various applications are nanosensors, nanocapsules, transport of substances, as well as in nanoparticles. Nanotechnology consists of manipulating a material in a very small proportion, in the range of 1 to 100 nanometers, which can be associated with a viral size. The "nano" feature gives a common product a distinctive attribute. This term can be found in materials such as thermal shoes, golf clubs with increased resistance and flexibility, mattresses that are able to prevent dust and sweat, creams that promote muscle relief, among countless others (Mazari et al., 2021).

The potential found in them has also been gaining ground in the agricultural sector due to the varied functions mentioned

above. One of the main nanoproducts on the market and/or under development are nanoparticles, which are highly targeted for their versatility in terms of structure, which may also have beneficial properties related to the environment in which they will be used (Samrot et al., 2021).

Following the immensity that are the possibilities displayed by this new science, together with the question of its magnitude there is a common sense, of the existence of two branches of nanoscience. The first one called "top-down", which is based on the manufacture of manometric conformations using machines and/or equipment, while the "bottom-up" branch produces inorganic, organic structural shapes. or even "piece by piece" hybrids, atomically speaking, also known as molecular nanotechnology (Abid et al., 2022).

NANOPARTICLES

With the high investments behind research involving nanotechnology, several branches have been taking advantage of these studies, such as biotechnological, agricultural, cosmetic, food, electronic, hospital, medical, pharmaceutical and biotechnological. Enhancing consumption and leveraging the world market for processes, products and services (Mazari et al., 2021).

Nanoparticles are compounds with a large surface area, on a scale if possible smaller than 10nm, as shown in figure 1. specific adsorption and advantages such as reduction of working temperature and also and in devices a greater and better performance in the response (Caldorera-Moore et al., 2010).

When not stable, nanoparticles tend to join and form clumps, and because of this instability, it is necessary that they undergo steric or electrostatic protection by means of surfactants or water-soluble polymers, among others. By having a greater interaction with the substrate due to its great kinetic activity, nanoparticles have been attracting the attention of researchers from the most varied areas, for example, the construction of biosensors that would be the junction for synergistic functions of biomolecules with nanoparticles (Ansari et al. al., 2022).

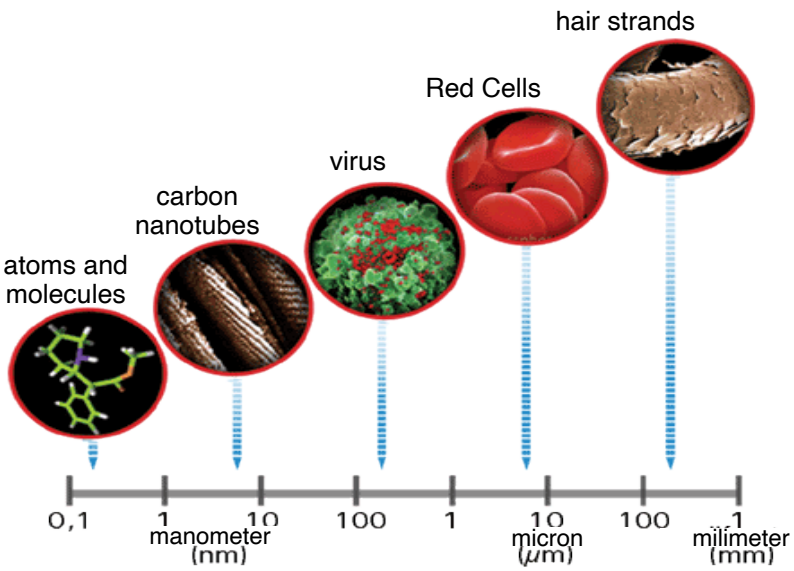

Figure 1- Dimension of nanoparticles.

Source: (Toma, 2009).

FUNCTIONALIZATION

For better performance of the nanoparticles together with the intention of interacting it in the best way with another compound, the functionalization aims to mix different chemical compounds in order to obtain high photostability and luminescence as well as their non-toxicity. The coating through polymers stands out in the expansion of polymeric chains on the surface of the nanoparticles, making them more stable and passive of more severe industrial processes without, however, added element that does not block activities such as colloidal stability and spectroscopic properties, a functionalization well This success allows the addition of new functional groups on the surface of the nanoparticle, creating a link of interaction with compounds of biological origin (Guo et al., 2016).

IMMOBILIZATION OF β-GALACTOSIDASE

Nowadays it is very common to find people with some degree of intolerance to a type of sugar found in milk, lactose. As it is a raw material for numerous industrial activities in the food industry, companies seek a solution to develop products with low, or even free, lactose levels for consumers of different ages, with the enzyme deficiency that would make it possible to break down this sugar. β-galactosidase, known as lactase, is an enzyme derived from various types of microorganisms and has the ability to hydrolyze the disaccharide lactose into the monosaccharides glucose and galactose, but its use on an industrial scale is limited due to its instability and non-reuse, together with its high cost (HERTZ, 2012).

Through an immobilization process, which can be through covalent bonds or encapsulation, it is possible to retain materials with biological activities, such as lactase, in nanometer-scale sensors, in packages where lactose-free is desired. Enzymatic immobilization allows for greater resistance to denaturing agents, along with stability regarding pH, as well as the possibility of controlling the release of the enzyme in the material it is in (Javed et al., 2016).

There are already numerous studies on the development of new types of packaging that involve nanotechnology, called smart packaging, through the technique of nanosensors that use enzymatic immobilization, capable of detecting changes and/or the presence of unwanted compounds in food and correcting them. With the incorporation of these biomolecules, it is possible to obtain more reliable products with a higher added value, not to mention durability during packaging (Cardoso Pinto et al., 2021).

MAIN GOAL

Synthesis and characterization of functionalized magnetic nanoparticles.

METHODOLOGY

Synthesis of magnetic nanoparticles functionalized with APTS

- According to the model illustrated in figure 2, the magnetic nanoparticles were obtained from the dissolution of FeCl2·4H2O and FeCl3·6H2O in the proportion (1:2, respectively) in 10 mL of distilled water. A solution of sodium hydroxide in distilled water was prepared until reaching a pH between 12 and 13. This solution was heated, under stirring, to 80°C and then 1.6 mL of the solution containing Fe2+/Fe3+ and 200 µL of 3 -aminopropyltrimethoxysilane (APTS) dispersed in ultrasound (Cardoso Pinto et al., 2021).

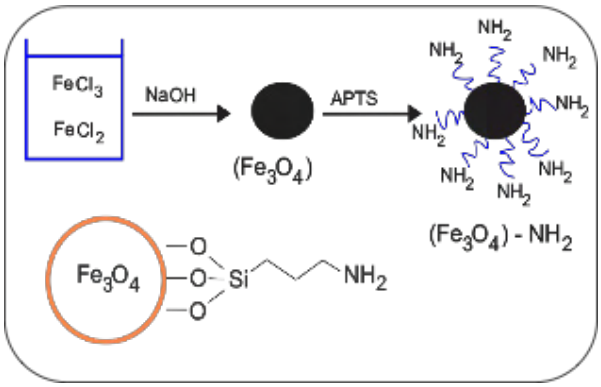

Figure 2- Synthesis of magnetic nanoparticles modified with amine groups

Source: (Rocha, 2016).

IMMOBILIZATION OF LACTASE IN APTS-FUNCTIONALIZED MAGNETIC NANOPARTICLES

After confirming the presence of APTS, an aliquot of 5mL of nanoparticles was removed from the sample and added to a falcon tube containing 18mL of 7,4 phosphate buffer. Subsequently, 2mL of glutaraldehyde (25%) were added; the solution was vortexed and then left to rest for 1 hour at room temperature. Washed with alcohol, water and buffer solution (twice with each solvent) to ensure removal of glutaraldehyde. 100µl of β-galactosidase were pipetted in the first test, then 5ml and 10mL in the following tests, and 0.01 g of polyethylene glycol. Both that were added to the buffer solution with the nanoparticles. Vortexing was performed for 5 minutes. The solution was left to rest for 16 hours at a temperature of 6°C. With the help of a magnet, the solution was washed 5 times with phosphate buffer (pH 7.4) to remove free Lactase.

TECHNIQUES USED FOR SAMPLE CHARACTERIZATION

X-RAY DIFFRACTION (DRX)

The samples were characterized in a Siemens diffractometer, model D5005. Using Kα radiation, with wavelength (λ) equal to 0.15418nm. Measurements were made in a 2θ scan range from 20° to 80°, with a scan speed of 0.02° per second. All samples were analyzed in powder form.

INFRARED SPECTROSCOPY (FTIR)

The FTIR spectra were obtained in reflectance mode in a PerkinElmer equipment, Frontier model, to identify the composition of the nanoparticles. The samples were macerated, dispersed in KBr and pressed into tablets. The spectra were obtained in the range of4000 cm^{-1} at 400 cm^{-1}.

POTENTIAL: ZETA

Zeta potential was determined on the Zetasizer Nano ZS equipment, Malvern. The equipment calculates the Zeta potential from electrophoretic mobility values, applying the Henry equation shown below (equation 1). Electrophoretic mobility is measured by applying potential to each electrode in the cuvette. Charged particles migrate to the oppositely charged electrode when their velocity is determined.

$$U_e = \frac{2\varepsilon\zeta f(ka)}{3n}$$

Where, Ue = electrophoretic mobility, ζ = Zeta Potential, ε = Dielectric Constant, n = viscosity and f(ka) = Henry's function.

RESULTS AND DISCUSSIONS

Through the literature it is possible to find numerous ways to synthesize iron magnetic nanoparticles. In the present work, the method of synthesis by coprecipitation was chosen to obtain magnetic nanoparticles (NP). Through this method, the nanoparticles were obtained by adding Fe2+ and Fe3+ in an aqueous solution of sodium hydroxide in the proportion 1:2 respectively, with the addition of APTS resulting in black precipitates,

between pH 12 and 13, already indicating the presence of magnetite.

The analyzes performed to determine the hydrodynamic diameter of the nanoparticles were obtained with the dispersion, with the aid of an ultrasound device, of the same in water. All analyzes were performed in triplicate and the results presented in Table 1 are their averages. The hydrodynamic diameter of the nanoparticles grew after their functionalization with APTS, thus confirming their addition to the surface of the nanoparticles. On the other hand, the polydispersity index (PDI) that determines the homogeneity of a sample, the smaller the PDI, the better the uniformity of the size of the NPs. The work presented very satisfactory results regarding its homogeneity with the first syntheses.

	T ºC	Size (nm)	PDI
NP/without APTS	25	213,1	0,301 +/- 0,023
NP/with APTS	25	1135	0,452 +/- 0,05

Table 1 - Mean hydrodynamic diameter and polydispersity index.

The X-ray Diffraction (XRD) technique was used to verify the formation of the desired phase, magnetite (Fe3O4). According to the diffractograms in figure 3, it is possible to observe the X-ray diffraction profile of the sample that was compared with the X-ray diffraction pattern of magnetite from the crystalline structure database, PDF, number 49-419 (Fe_3O_4).

As shown in figure 4, in all samples bands were observed at 489, 438, 445 cm-1 that could be attributed to magnetite, which according to the literature varies from 600 to 400cm-1. Those at 675, 625 and 628 cm-1 are believed to represent the presence of maghemite (Fe-O), the oxidized form of magnetite.

At 3368 cm-1 there is a band that would be indicative of a primary amine. The band presented at around 1620 cm-1 comes from the O-H bands of water, at 1394 cm-1 it is a reference to the symmetrical deformation of the C-H chain, the band at 2318 cm-1 is possibly attributed to CO2 from the atmospheric air.

In NP@27/06 and NP@08/08 there is a secondary amine shown in the range of 1564 to 1504 cm-1. On the other hand, the sample with APTS can possibly notice the presence of the aliphatic primary amine around 669 cm-1, and the broad band that extends (Cardoso Pinto et al., 2021) from 998 to 898 cm-1 is indicative of primary amine, both serving as a positive indication that the sample contains APTS (Gu & Peng, 2018).

After confirming the presence of APTS on the surface of the NPs, lactase immobilization was performed on them. According to figure 5, which shows the results of the analysis of the spectrum in the infrared region of the sample that must have interacted with these enzymes. It is not possible to detect the presence of some of the functional groups present in the enzyme, for example, amide groups that would appear if there was a successful immobilization. The work obtained an IR spectrum graph very similar to the NP spectrum that had only APTS in its structure and, compared to the pure Lactase spectrum, the same differs, mainly because it does not present such bands to be associated with the amide group.

With the change in the volume of enzymes to be added to the nanoparticles, being 5 and 10mL, the IV graph (Figure 6) shows ranges ranging from 1750 to 1000 cm-1, very similar to those of pure Lactase, unlike the sample with 100µL. Besides presenting a strong band between 1700 – 1630 cm-1, indicative of the C=O bond of an N-substituted amide, together with a weak band between

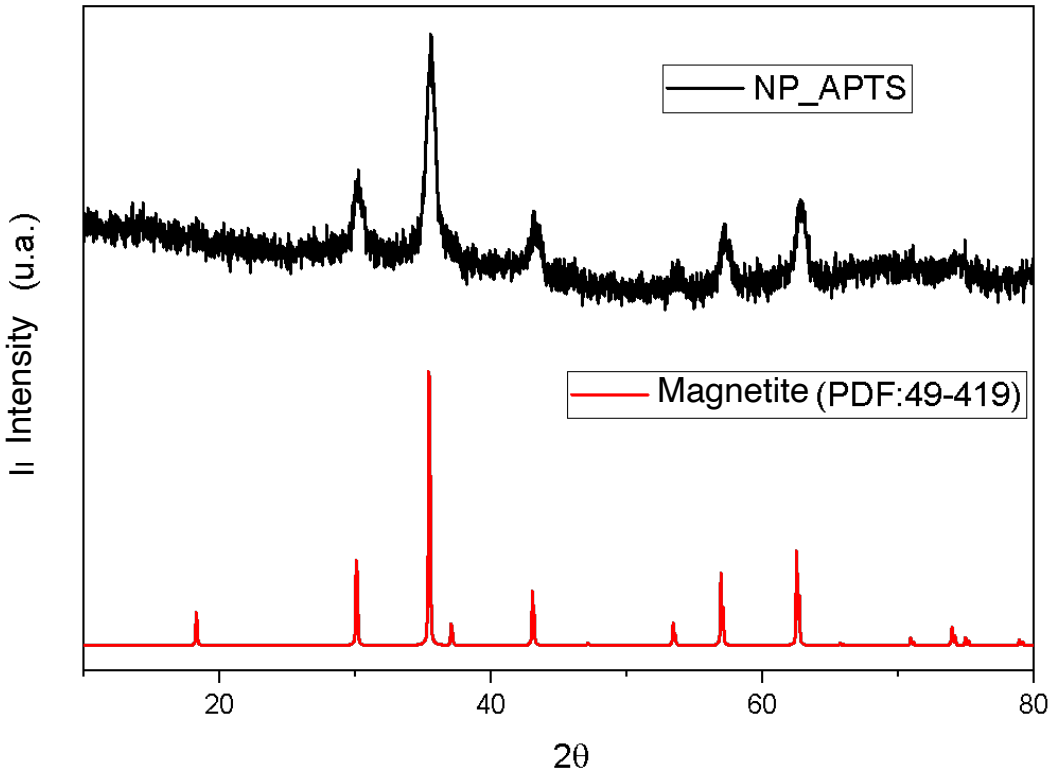

Figure 3- X-ray diffractogram of a sample of magnetic nanoparticles and comparing it to the magnetite pattern.

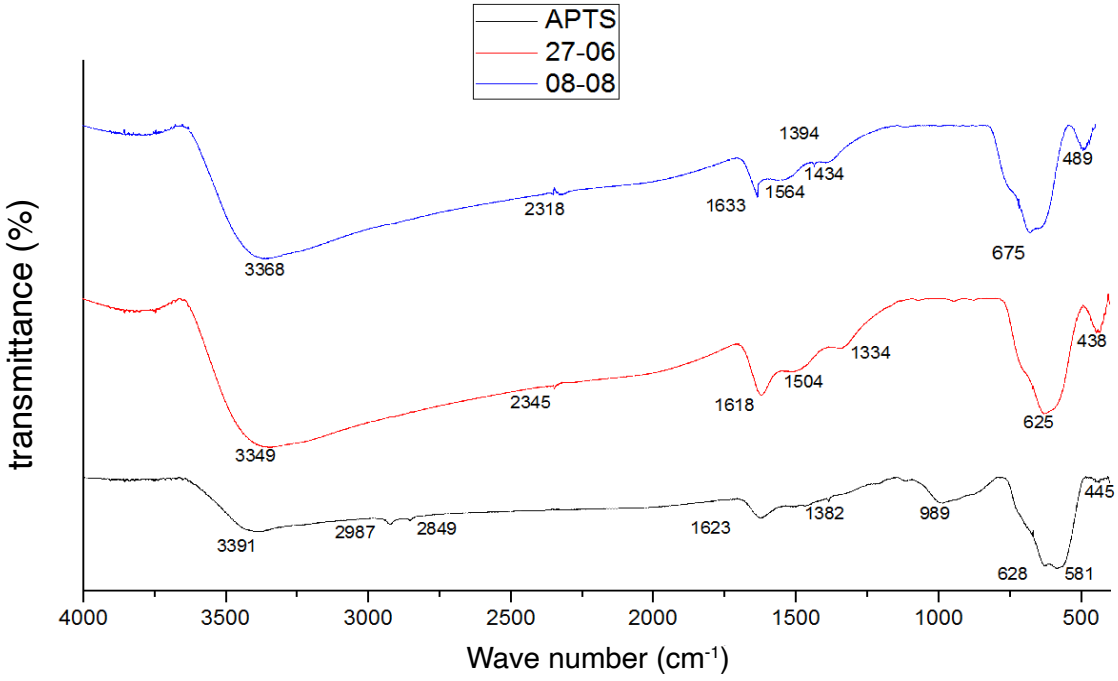

Figure 4- Infrared region (FT-IR) spectra of nanoparticles.

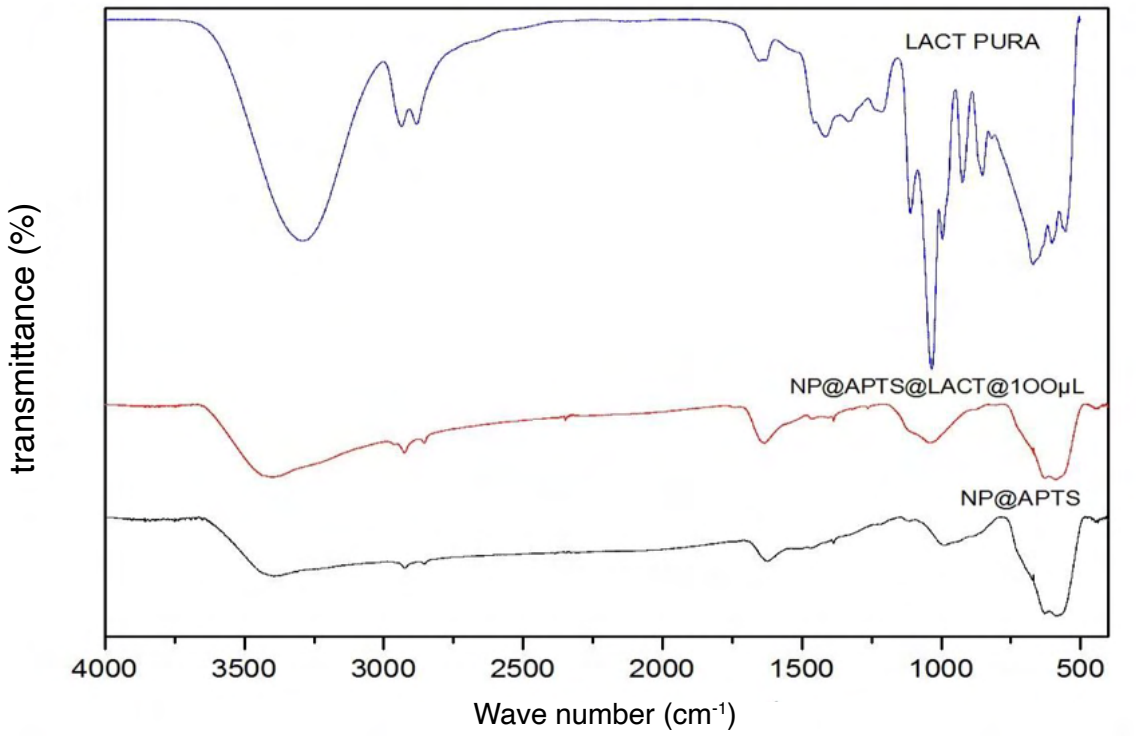

Figure 5- Infrared region spectra (FT-IR) of Nanoparticles Functionalized with APTS, after the addition of 100 µL of pure Lactase.

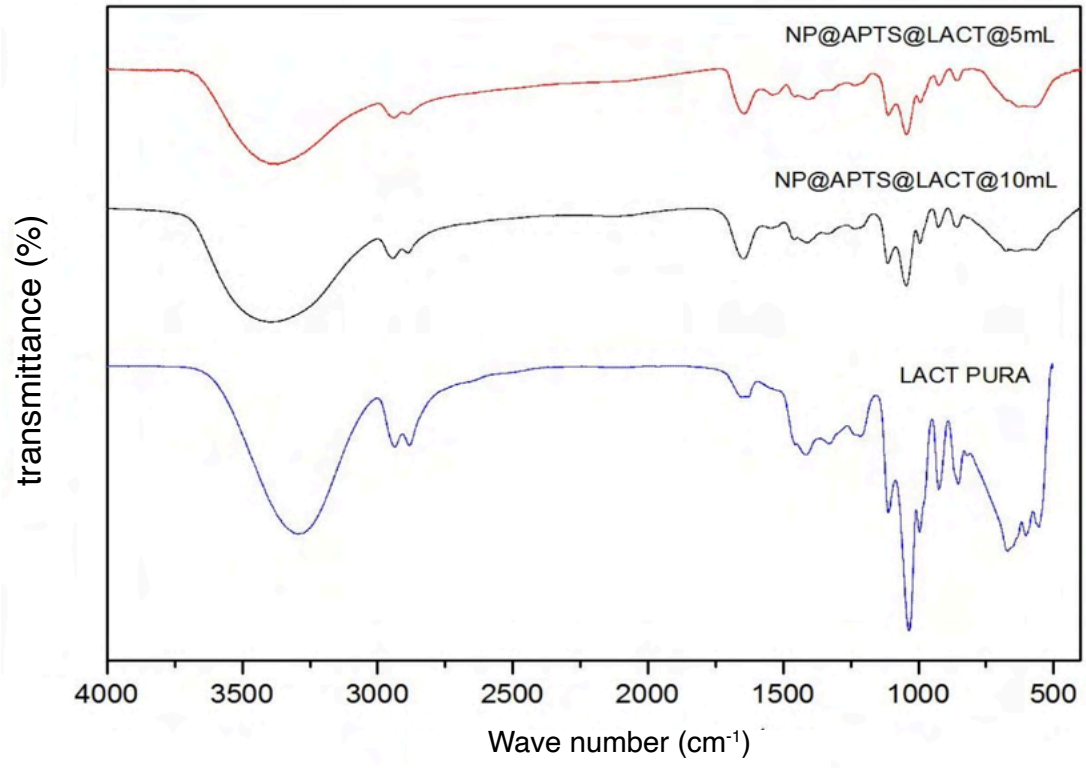

Figure 6- Infrared region spectra (FT-IR) of Nanoparticles Functionalized with APTS, after the addition of 5mL and 10mL and pure Lactase.

1580 to 1495 cm-1 that can be used for characterization of secondary amides. The range that extends from 3650 to 3000 cm-1, with the largest band at 3385 cm-1, would be indicative of secondary amide because it is a medium stretch and fits in the range of N-H bonds that go from 3500 to 3070 cm -1. On the other hand, in the 10mL graph, the Fe-O band (bands 675, 625 and 628 cm-1) were deformed, possibly caused by excess enzyme on the surface (Lucena et al., 2019).

CONCLUSION

It was possible, through the coprecipitation method, the synthesis of magnetic iron nanoparticles and their use as support for the adhesion of other molecules in their structure. As they are initial tests, the samples showed very good PDI, which can be improved over the course of new syntheses in order to develop greater homogeneity regarding the size of the nanoparticles produced.

There was also confirmation of the existence of magnetite (Fe3O4) by XRD, even with some noise in the diffractogram created, compared to the pattern, there was the possibility of perception of peaks characteristic of the magnetite.

The technique of functionalization of the nanoparticles was also successful, presenting, therefore, the presence of APTS on its surface so that the interaction between them and biomolecules could occur. However, the work was not able to detect the presence of lactase according to the analysis of the spectrum in Infrared with the addition of 100μL of enzyme, concluding that there was not an efficient immobilization, possibly referring to the low concentration of the enzyme used in the reaction.

The results with 5 and 10mL, with a much higher concentration of lactase than in the first test, resulted in the presence of lactase in the nanoparticles, showing that immobilization required a concentration of at least 1:1 of enzymes for the nanoparticles, but the 10mL sample modified the IV graph in the band that refers to maghemite, referring to a very high value of added enzyme. The result with the addition of 5mL was the best found, thus showing an ideal volume of enzymes to be added for that volume of nanoparticles used, which was also 5mL.

REFERENCES

Abid, N., Khan, A. M., Shujait, S., Chaudhary, K., Ikram, M., Imran, M., Haider, J., Khan, M., Khan, Q., & Maqbool, M. (2022). Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and disadvantages: A review. *Advances in Colloid and Interface Science*, *300*(December 2021), 102597. https://doi.org/10.1016/j.cis.2021.102597

Ansari, M. J., Kadhim, M. M., Hussein, B. A., Lafta, H. A., & Kianfar, E. (2022). Synthesis and Stability of Magnetic Nanoparticles. *BioNanoScience*, *12*(2), 627–638. https://doi.org/10.1007/s12668-022-00947-5

Caldorera-Moore, M., Guimard, N., Shi, L., & Roy, K. (2010). Designer nanoparticles: Incorporating size, shape and triggered release into nanoscale drug carriers. *Expert Opinion on Drug Delivery*, *7*(4), 479–495. https://doi.org/10.1517/17425240903579971

Cardoso Pinto, G., Brandt, J. V., Piazza, R. D., dos Santos, C. C., Nunes Lucena, G., de Paula, A. V., Jafelicci Junior, M., & Costa Marques, R. F. (2021). Magnetic Graphene Oxide as a Carrier for Lipases Immobilization: An Approach for Hydrolysis of Olive Oil Emulsion. *ECS Journal of Solid State Science and Technology*, *10*(6), 065008. https://doi.org/10.1149/2162-8777/ac054a

Gu, X., & Peng, J. (2018). Preparation of NH_2-Functionalized Graphene Oxide Nanosheets for Immobilization of NADH Oxidase. *Journal of Nanoscience and Nanotechnology*, *18*(6), 3888–3892. https://doi.org/10.1166/jnn.2018.15212

Guo, H., Tang, Y., Yu, Y., Xue, L., & Qian, J. Q. (2016). Covalent immobilization of α-amylase on magnetic particles as catalyst for hydrolysis of high-amylose starch. *International Journal of Biological Macromolecules*, *87*, 537–544. https://doi.org/10.1016/j.ijbiomac.2016.02.080

HERTZ, L. P. F. M. P. K. P. F. (2012). Desenvolvimento de um biorreator com B-galactosidase imobilizada para a produção de leite com baixo teor de lactose. *XXI FEIRA DE INICIAÇÃO À INOVAÇÃO E AO DESENVOLVIMENTO TECNOLÓGICO – FINOVA / 2012 Porto Alegre - RS*.

Javed, M. R., Buthe, A., Rashid, M. H., & Wang, P. (2016). Cost-efficient entrapment of β-glucosidase in nanoscale latex and silicone polymeric thin films for use as stable biocatalysts. *Food Chemistry*, *190*, 1078–1085. https://doi.org/10.1016/j.foodchem.2015.06.040

Lucena, G. N., dos Santos, C. C., Pinto, G. C., da Rocha, C. O., Brandt, J. V., de Paula, A. V., Jafelicci Júnior, M., & Marques, R. F. C. (2019). Magnetic cross-linked enzyme aggregates (MCLEAs) applied to biomass conversion. *Journal of Solid State Chemistry*, *270*(November 2018), 58–70. https://doi.org/10.1016/j.jssc.2018.11.003

Mazari, S. A., Ali, E., Abro, R., Khan, F. S. A., Ahmed, I., Ahmed, M., Nizamuddin, S., Siddiqui, T. H., Hossain, N., Mubarak, N. M., & Shah, A. (2021). Nanomaterials: Applications, waste-handling, environmental toxicities, and future challenges - A review. *Journal of Environmental Chemical Engineering*, *9*(2), 105028. https://doi.org/10.1016/j.jece.2021.105028

Rocha, C. O. da. (2016). *Síntese de nanopartículas magnéticas modificadas com grupos aminas*.

Samrot, A. V., Sahithya, C. S., Selvarani A, J., Purayil, S. K., & Ponnaiah, P. (2021). A review on synthesis, characterization and potential biological applications of superparamagnetic iron oxide nanoparticles. *Current Research in Green and Sustainable Chemistry*, *4*(November 2020), 100042. https://doi.org/10.1016/j.crgsc.2020.100042

T.sriwong, K., & Matsuda, T. (2022). Recent Advances in Enzyme Immobilization Utilizing Nanotechnology for Biocatalysis. *Organic Process Research & Development*. https://doi.org/10.1021/acs.oprd.1c00404

Toma, H. E. (2009). *O mundo nanométrico: a dimensão do novo século.* Oficina de textos.

Vijayakumar, M. D., Surendhar, G. J., Natrayan, L., Patil, P. P., Ram, P. M. B., & Paramasivam, P. (2022). Evolution and Recent Scenario of Nanotechnology in Agriculture and Food Industries. *Journal of Nanomaterials*, *2022*, 17.

Vurro, M., Miguel-Rojas, C., & Pérez-de-Luque, A. (2019). Safe nanotechnologies for increasing the effectiveness of environmentally friendly natural agrochemicals. *Pest Management Science*, *75*(9), 2403–2412. https://doi.org/10.1002/ps.5348

Zhang, Q., Ying, Y., & Ping, J. (2022). Recent Advances in Plant Nanoscience. *Advanced Science*, *9*(2), 6–10. https://doi.org/10.1002/advs.202103414