Disciplinarum Scientia. Série: Naturais e Tecnológicas, Santa Maria, v. 24, n. 3, p. 57-68, 2023.
Recebido em: 29.01.2024. Aprovado em: 07.02.2024.

ISSN 2176-462X

DOI: 10.37779/nt.v24i3.4800

SYNTHESIS OF ZINC OXIDE MODIFIED MAGNETIC NANO-CHITOSAN

SÍNTESE DE NANOQUITOSANA MAGNÉTICA MODIFICADA COM ÓXIDO DE ZINCO

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ABSTRACT

The global population experienced an unprecedented viral infection known as SARS-CoV-2. Due to it being a novel virus, the range of effective medications was limited. Nevertheless, the occurrence of undesired and often potentially toxic effects further restricted the choices of medications. Considering this, nanotechnology, through manipulating matter, allows many compounds to exhibit nanoscale properties, reducing toxicity, increasing selectivity, and lowering the required dosage. Considering the excellent properties of the nanoparticles mentioned in this study, the objective was to synthesize magnetic nanoparticles doped with zinc oxide, aiming at developing a nanomaterial with antiviral activity. Once the nanoparticles are obtained, subsequent biological assays will be conducted to evaluate their activity against the SARS-CoV-2 virus.

Keywords: *Bionanopolymer, Coronavírus, Magnetization, Nanostructured matetials.*

RESUMO

A população mundial sofreu, entre os anos de 2020-2022, por uma infecção viral extremamente agressiva, denominada como SARS-COV2. Por se tratar de um vírus novo, o escopo de medicamentos que se demonstravam eficientes eram relativamente pequenos. Não obstante, a ocorrência de efeitos indesejados e muitas vezes potencialmente tóxicos, tornavam as escolhas medicamentosas ainda mais restritas. Em vista disso, a nanotecnologia, por meio da manipulação da matéria, permite que muitos compostos apresentem em escala nanométrica, redução da toxicidade, aumento da seletividade e redução da dose. Considerando as excelentes propriedades das nanopartículas mencionadas neste trabalho. Este estudo teve como objetivo sintetizar nanopartículas magnéticas dopadas com óxido de zinco, visando o desenvolvimento de um nanomaterial com atividade antiviral. Comprovada a obtenção das nanopartículas, posteriormente serão desenvolvidos ensaios biológicos para avaliar a atividade contra o vírus da Sars-Cov2.

Palavras-chave: *Bionanopolímero, Coronavírus, Magnetização, materiais nanoestruturados.*

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INTRODUCTION

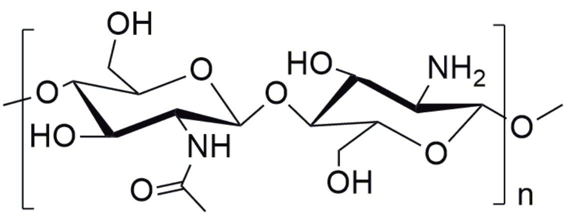
Infections are caused by acellular microorganisms called viruses, which, due to their simplified structure, depend on a host to persist. During the period from 2020 to 2022, the global population experienced an atypical infection caused by a virus called SARS-CoV-2, commonly known as COVID-19 (Zhou *et al*., 2020).

At present, there are still no specified medications for the treatment of the infection, making it necessary to search for pharmacological alternatives that meet the demand for symptom minimization, can act specifically, and have low toxic effects on the individual (Yuan, Yongliang *et al.,*2023)

Considering the presented issue, nanotechnology comes into play, enabling the manipulation of compounds at atomic and molecular levels, often reducing their toxicity (Bruckmann *et al*., 2020), and modifying the reactivity of drug molecules used in the treatment of COVID-19 (Sallem., Hannef., Abbasi., 2018). Polymeric nanoparticles are being widely explored by industry due to their broad applications. Chitosan is a natural polysaccharide derived from the deacetylation of chitin (Nunes, F.B. *et al.* 2023). It stands out for its excellent characteristics such as biocompatibility and biodegradability. (Kyzas; Bikiaris, 2015), antifungal and antibacterial activity (Bruckmann *et al*.,2022) and immunogenic properties (Loutfy *et al*., 2020)

Chitosan (Figure 1) is a biodegradable and bioabsorbable biopolymer, and its products are originally non-toxic, non-immunogenic, and non-carcinogenic. Widely used in the biomedical industry, it promotes the physiological reconstitution of the skin. Numerous assessments and studies on potential applications of chitosan as a wound healing coating or as a base for cell growth have been published (Wang; Zhuang, 2022)

Figure 1 - Chitosan structure



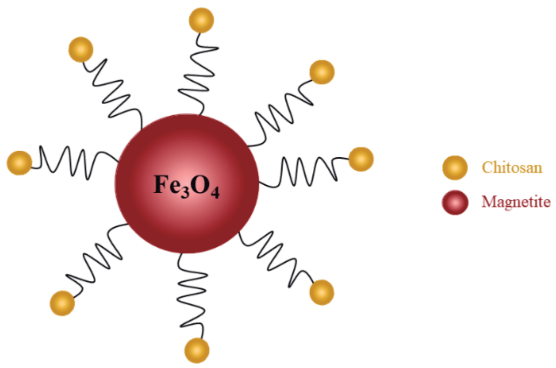
Source: Authors.

The existence of reactive functional groups in its polymeric structure confirms the ability for chemical modification and the development of nanocomposites (Figure 2), such as the incorporation of magnetic nanoparticles (MNPs). (Sureshkumar *et al*., 2016, Rhoden *et al.,* 2020).

Studies on magnetic nanoparticles (MNPs) have been evident since the 1930s, with the possibility that particles of ferromagnetic materials (various magnetic domains), with a structure below the critical size, would have a single magnetic domain, with electrons aligned parallelly, providing a rapid and strong response to a magnetic field, as well as increasing their coercivity (the field required to reduce magnetism to zero). (Frenkel; Doefman, 1930; Harres *et al.*, 2023).

Magnetite (Fe_3O_4) is formed by two iron oxides of valency Fe^{2+} and Fe^{3+}, resulting in a ferromagnetic substance (Vargas *et al.*, 2023). It has a critical diameter for magnetism in the range of 10 to 20 nanometers. In other words, within this size range, magnetite possesses a single magnetic domain, making it a superparamagnetic material. (Whahajuddin, 2012).

Figure 2 - Chitosan functionalized with magnetic nanoparticles.

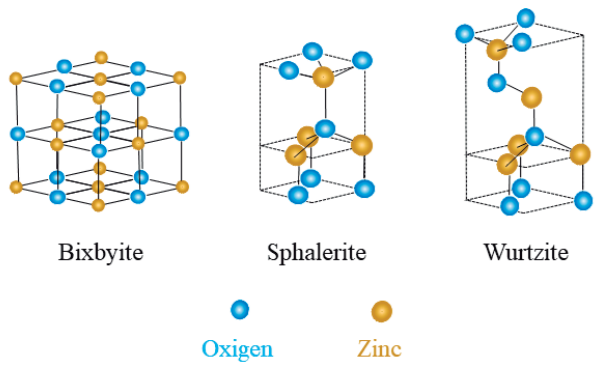


Source: Authors.

Magnetic nanoparticles (MNPs), with an emphasis on those exhibiting superparamagnetic behavior, have proven to be promising in the pharmacological field, as they demonstrate biocompatibility and low toxicity (Cogo *et al.*, 2022). In addition to responding quickly and strongly to a magnetic field, they can be administered into the organism and directed to the target tissue, thereby increasing therapeutic efficiency. (Unsoy *et al.*, Iqbal *et al.*, 2017, Bruckmann *et al.*, 2022).

Among nanoparticulate materials, zinc oxide (Figure 3) is an inorganic material that stands out due to its remarkable characteristics, such as excellent biocompatibility, low toxicity, antioxidant, antibacterial, and antifungal activity (Malaikozhundan, 2018). Recentemente, Ghaffari *et al.,* (2019), They used zinc oxide nanoparticles modified with polyethylene glycol to evaluate activity against the H1N1 virus. Studies demonstrate that the nanoparticles were able to reduce the viral load by approximately 94.6%.

Figure 3 - Representation of the allotropic structures of ZnO.



Source: Authors.

With the proper presentation of today's issues and considering the excellent properties of the described nanocomposites, the present study aims to synthesize nanoparticles of nano-chitosan with varying amounts of incorporated magnetite and zinc oxide, aiming at the development of an innovative superparamagnetic nanocomposite with potential antiviral activity.

MATERIALS E METHODS

SYNTHESIS OF NANO-CHITOSAN

A synthesis of the nanoparticles was carried out using the ionotropic gelation method, as described by Anitha *et al.,* (2009). A solution of 0.1% chitosan in 1% acetic acid medium was prepared and stirred until complete chitosan solubilization. Subsequently, 10 mL of a 1% sodium tripolyphosphate (TPP) solution was slowly dripped into the chitosan solution, and the reaction was stirred magnetically for 30 minutes. Later, the solution was transferred to microtubes and subjected to centrifugation at 10,000 rpm for 1 hour. The pellet was then resuspended in water, and the pH was neutralized at 0.1 mol. L^{-1} NaOH. Subsequently, the nanoparticles were placed in an oven at 50 ºC for solvent evaporation.

MAGNETIZATION OF NANO-CHITOSAN

The magnetization of nano-chitosan was carried out using the method proposed by Rhoden *et al.,* (2017, 2021). For these reactions, a 250 mL round-bottom flask was used, containing 100 mL of previously deoxygenated ultrapure water. Subsequently, 100 mg of nano-chitosan and iron (II) chloride were added ($FeCl_2$) (100, 500 or 1000 mg) To promote the production of nano-chitosan with varying amounts of Fe_3O_4, the pH was adjusted with ammonium hydroxide until reaching an oxidative pH (pH≈ 9.0). Afterward, the mixture was subjected to ultrasonic radiation, and sequentially, the solution was poured into a beaker. With the assistance of a magnet, the solid was consecutively washed with methanol and acetone. Subsequently, the material was dried in an oven at 50 °C for 20 minutes to completely evaporate the solvents.

OBTAINING MAGNETIC NANOPARTICLES MODIFIED WITH ZINC OXIDE

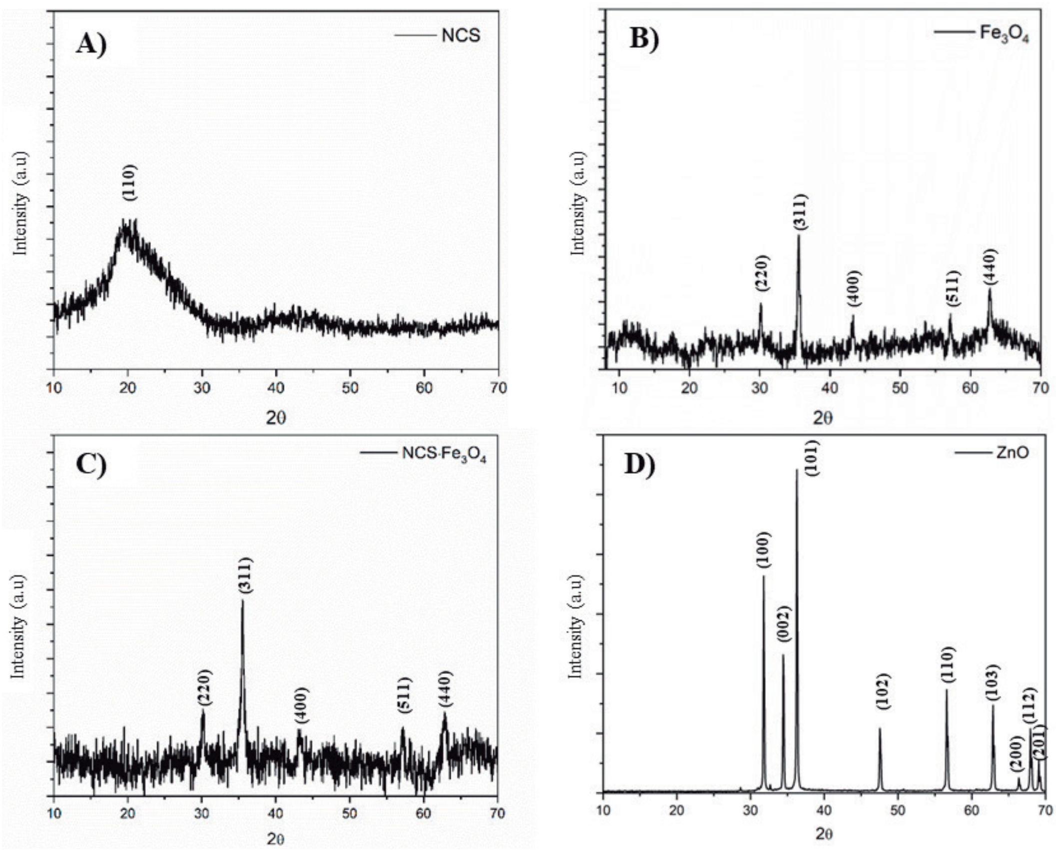
Zinc-doped nanoparticles were obtained by adapting the methodology proposed by Neves (2013). The experiment was conducted using an amount of ZnO equivalent to 20 mg of Zn^{2+}. In a 250 mL beaker, 150 mL of water, 50 mg of magnetic nanoparticles, and the equivalent of 20 mg of Zn^{2+} were added. The mixture was then placed in a shaker incubator, stirring for 8 hours at room temperature. After this period, with the aid of a magnet, the composite was washed with acetone and subsequently dried in an oven at 50 ºC for 12 hours to evaporate the solvent.

RESULTS E DISCUSSIONS

X-RAY DIFFRACTOGRAM (XRD)

The X-ray diffraction (XRD) pattern in Figure A shows peaks at 2**θ ≈ 20°, 23°, 25°, and 27°, characteristic of nano-chitosan, corroborating with the study obtained by** Gokila, S. *et al.,* (2017). The X-ray diffraction (XRD) pattern in Figure B shows peaks at 2θ ≈ 31°, 36°, 44°, 57°, and 63°, characteristic of magnetite, like the results reported by (Salles *et al.,* 2023).

Figure 4 - XRD, A) nano-chitosan, B) magnetite, C) magnetic nano-chitosan and D) zinc oxide.



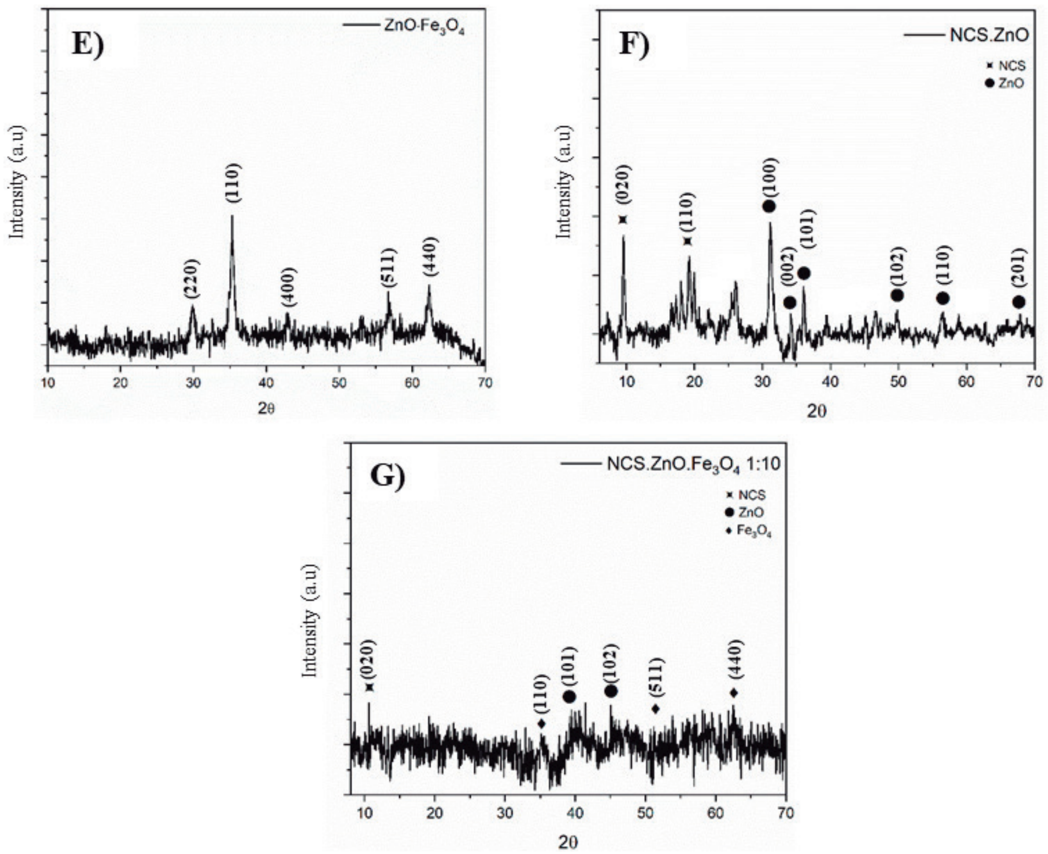
Source: Authors.

The X-ray diffraction (XRD) pattern in Figure D shows peaks at 2**θ ≈ 32°, 34°, 36°, 48°, 56°, 64°, 66°, 68°, 69°, characteristic of zinc oxide, with the peaks confirmed compared to the study by** ZAK, A. *et al.,* (2011). In Figure C, the X-ray diffraction (XRD) pattern shows peaks at 2**θ ≈ 31°, 36°, and 62°, characteristic of magnetite, as confirmed in Figure B, and peaks at 2θ ≈ 20° and 23°, characteristic of nano-chitosan, as demonstrated in Figure A**.

The X-ray diffraction (XRD) pattern in Figure E shows peaks at 2θ ≈ 48°, 56°, and 63°, characteristic of zinc oxide, as demonstrated in Figure D, and peaks at 2θ ≈ 31°, 44°, and 63°, characteristic of magnetite, shown in Figure B, corroborating with the results obtained by Safari, Mojtaba *et al.,* (2014).

In Figure F, the X-ray diffraction (XRD) pattern shows peaks at 2θ ≈ 10° and 20°, which are characteristic of nano-chitosan, as confirmed in Figure A. Additionally, peaks at 2θ ≈ 31°, 34°, 36°, 49°, 56°, 63°,66°,67° and 68° are characteristic of zinc oxide, as confirmed in Figure D, and further supported by the results obtained by Palatsingh *et al.,* (2020) which assessed the antibacterial activity and biodegradability of the zinc oxide and chitosan biofilm for food packaging.

Figure 5 - XRD, figure E) magnetic zinc oxide, F) zinc oxide nano-chitosan hybrid material, G) magnetic zinc oxide nano-chitosan material.



Source: Author's creation.

The X-ray diffraction (XRD) pattern in figure G shows peaks at 2θ ≈ 10°, characteristic of nano-chitosan, and peaks at 2θ ≈ 36°, 52°, and 64°, characteristic of magnetite (Rhoden *et al*., 2021), and the signals 2θ ≈ 38° and 45°, the characteristic peaks of zinc oxide, which were previously mentioned, are observed.

CONCLUSIONS

The results obtained from the synthesis of nano-chitosan and the functionalization of the nanomaterial and biopolymers were extremely positive, confirming the complete incorporation of magnetite into the chitosan surface and the doping of zinc oxide in the proposed nanomaterials, Subsequently, *in vitro* safety and efficacy assays will be conducted to assess the toxicity of the obtained nanocomposite for future applications.

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